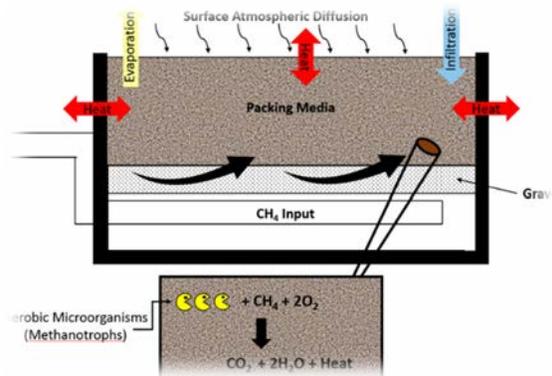


FINAL REPORT

CONTROL OF POINT-SOURCE LOW-VOLUME METHANE EMISSIONS USING METHANE BIOFILTRATION TECHNOLOGY



PROJECT CONDUCTED BY: PATRICK HETTIARATCHI'S TEAM
UNIVERSITY OF CALGARY, CANADA
MAY 2013 TO DECEMBER 2016

SUBMITTED TO: SUSAN WOOD-BOHM
EMISSION REDUCTION ALBERTA (ERA)
MARCH 2017

Table of Contents

EXECUTIVE SUMMARY	2
1. Introduction and Project Overview	3
2. Project Objectives	4
3. Key Activities	4
4. Project Team	5
5. Summary of Progress against Agreed Milestones/Activities	6
6. Scientific Achievements	10
7. Greenhouse Gas Impacts	13
8. Conclusions	14
9. Supplementary Documents	14
9.1 Report: Market Feasibility of Methane Bio-Filtration Technology in Alberta to Control Point Source Low-Volume Methane Emissions	
9.2 Report: GAP Analysis	
9.3 Thesis: Performance of Actively-Aerated Biofilters Using a Multiple-Level Air Injection System to Enhance Biological Treatment of Methane Emissions	
9.4 Thesis: Development of Alternative Medium to Sustain Methanotrophs in Methane Biofilters	
9.5 Report: Monitoring Protocol Development	
9.6 Journal Paper: Methane Biofiltration in the Presence of Non-Methane Organic Compounds in Solution Gas	
9.7 Report: Field Work	
9.8 Progress Report: Methane Biofiltration by Methanotrophic Bacteria Using Biologically Stable Filter Materials	
9.9 Summary: International Workshop on Greenhouse Gas Emission Reductions Using Biological Method	
9.10 Thesis: Investigating the Inhibitory Effect of Acidic pHs on Methane Biofiltration Technology	

EXECUTIVE SUMMARY

This is the final report of the project titled “Control of point-source low-volume methane emissions using methane biofiltration technology”. Although the use of renewable energy sources is increasing, there is still a high dependence on crude oil and natural gas for energy: the production of which contributes to the release of large amounts of methane, a key greenhouse gas (GHG). When the quantities released at individual locations are relatively small, this methane cannot be used as an energy source; therefore, environmentally acceptable methods are needed for their control. Recent research has shown that methanotrophic bacteria are capable of converting methane to carbon dioxide without producing toxic by-products. We have conducted extensive research to develop methanobiofiltration (MBF) technology, utilizing this capability of methanotrophic bacteria. The field application of MBF technology, supported by concurrent laboratory and theoretical investigations is the focus of this project and we have successfully achieve the objectives.

All the milestones in the project proposal: milestone 1 to 4, were completed successfully. The summary of the progress of the project during the project span (i.e May 2013 to December 2016) is included in the first section of this report and the detailed reports of each milestone prepared by the corresponding researchers are attached in the second section of the report.

This report has been produced independently by researchers at the University of Calgary through funding provided, in part, by Emissions Reduction Alberta (ERA). The views expressed in this report are not necessarily the views of the Emissions Reduction Alberta (ERA).

1. Introduction and Project Overview

In Alberta, atmospheric methane emissions are associated with a variety of industry sectors: sanitary landfills, wood waste landfills associated with pulp and paper industry, wastewater treatment plants and the oil and gas sector, including the subsectors of conventional oil production, natural gas production and oil sands operation. Methane (CH₄) is a key greenhouse gas (GHG) with 34 times the global warming potential (GWP) of carbon dioxide (CO₂). When the quantities of methane emissions at individual locations are large, it is economically feasible to capture this gas and use it in a productive manner, such as for space heating or producing electricity. For example, landfill biogas use in power plants is only technically and economically suitable for a CH₄ content superior to 30-40% v/v (which typically occurs during the first 25 years of the landfill) and a total biogas production rate of about 30-50 m³/h. However, when the quantities released at individual locations are relatively small and the quality is low, conventional practices are the venting of the gas into the atmosphere or low-temperature combustion of the gas (flaring). These practices are fraught with unacceptable consequences. First, methane has a high GWP; therefore, direct venting may contribute immensely to climate change. Second, flaring is known to produce gaseous by-products that are highly toxic.

Currently, there is no cost-effective established technology other than flaring to control low-volume methane emissions, hence there is a need to develop and deploy environmentally friendly methods of managing this problem. This research is aimed at demonstrating the application of a biological process based technology to control such emissions. This technology has minimal environmental impacts.

Methanotrophic bacteria, when residing in granular media, such as soil and compost, are capable of converting methane to CO₂, thus serving as an important methane sink and reducing the overall amount of GHGs released to the atmosphere. Over the last few years, our research group from University of Calgary (led by Dr Patrick Hettiaratchi, the Principle Investigator of the current project) have investigated and developed a technology known as methane biofiltration (MBF) to control low-volume methane emissions. MBF is a promising biological process to attenuate point-source low-volume CH₄ emissions. We found that a properly designed, constructed and operated methane biofilter could be used to control emissions of at oil well sites as well as at landfill sites.

The MBF technology generally does not require on-site operator presence and is thus well-suited for oil well sites, small landfills and other remote facilities.

The project included three stages, commercial feasibility study, technical feasibility study and the monitoring protocol development study. We have successfully completed all the stages as promised in the proposal, with the application of this technology under several field situations (eg: oil well sites and landfills) and this report presents all the findings in details.

2. Project Objectives

The key objectives of the proposed project are:

1. Assessment of the commercial feasibility (CF) of MBF technology by undertaking a market analysis to identify the opportunities for the application of this technology in various industrial sectors including economic feasibility and carbon offset analyses.
2. Evaluation of the technical feasibility (TF) of the MBF technology under various field conditions, including the implementation of several full-scale methane biofilters associated with landfills and the oil and gas sector. This includes the development of design and construction guidelines, such as the optimal sizing of the methane biofilters and the granular medium characteristics for various applications.
3. Development of a monitoring protocol (MP) to accurately determine the methane oxidation efficiency of methane biofilters throughout the year under field conditions.

3. Key Activities

The project objectives were achieved by conducting a research program that included laboratory and field-based experimentation and mathematical modeling. Furthermore, this research program also included the design, construction, installation and monitoring of few field-scale pilot MBFs to treat CH₄-rich waste gas streams from several sources such as oil and gas well sites and sanitary landfills. Summary of the completed milestones/activities versus agreed milestones/activities are included in Table 2 and the detailed reports from each research activity are included in supplementary document section.

4. Project Team

The project team consisted of researchers from various backgrounds such as engineering, microbiology, biology, finance and business. A group of HQPs (highly qualified professionals) were trained as a part of this project including undergraduate students, masters student (full time thesis based and course based), doctoral students, post-doctoral researchers and research assistants. While some of them were funded directly through the funding received from CCEMC to undertake this project, some of them also received additional funding through Mitacs accelerate and Mitacs elevate and university of Calgary's eyes high initiative. The list of researchers involved in this project is shown in the Table 1.

Table 1: Project team

Team Member	Role	Time
Patrick Hettiaratchi	Principal Investigator/Project Leader	2013-2016
Peter Dunfield	Co-Investigator	2013-2016
Poornima Jayasinghe	PDF (co-funded by Mitacs)	2013-2016
Eranda Bartholameuz	PDF	2015-2016
Hasti Farrokhzadeh	MSc Student (co-funded by Mitacs)	2014-2016
Roshan Khadka	PhD Student (co-funded by Mitacs)	2014-2016
Samadhi Gunasekara	PhD Student (co-funded by Mitacs)	2015-2016
Sonya Barzegar	MSc Student (co-funded by Mitacs)	2015-2016
Helen La	PhD Student (co-funded by Mitacs)	2015-2016
Matt Steele	Research Associate	2015-2016
Eamonn Irvine	Summer Student	Summer 2016
Hasna Nazir	Summer Student	Summer 2016
Andrew Bustard	Summer Student	Summer 2016
Corry Phu	Summer Student	Summer 2016
Eamonn Irvine	Summer Student	Summer 2015
Shreya Ghimri	Summer Student	Summer 2015
Sheyhan Abeysinghe	Summer Student	Summer 2015
Jesica Goya	MSc Student (co-funded by Mitacs)	2014-2015
JoongJae Kim	PDF	2013-2014
Santosh Yadev	PDF (Funded by Eyes High –UofC)	2014-2015
Blanca Elizabeth	Research Assistant	2014
Rosalie Olivera Encina	Research Assistant	2014
Brett Olson	Summer Student	Summer 2014
Eamonn Irvine	Summer Student	Summer 2014
Kemi Omole	MSc Student – part time on this project	Fall 2013
Seema Jindal	MSc Student – part time on this project	Fall 2013
Shoshi Dharain	MSc Student – part time on this project	Fall 2013

5. Summary of Progress against Agreed Milestones/Activities

This section of the report summarizes the progress of the milestones/activities at the end of the project completion date (i.e. December 2016).

Table 2: Agreed VS Completed Milestones

Milestone /Year	AGREED MILESTONES/ ACTIVITIES	PROGRESS
1 (2013)	<p>Formation of stakeholder group</p> <p>Stakeholder meetings – initial meetings</p> <p>Formation of steering committee</p> <p>Commercial feasibility study</p> <ul style="list-style-type: none"> - GAP analysis - SWOT analysis - PESTEL analysis - Preliminary review of technological aspects of methane biofilters - Preliminary market characterization - Preliminary financial evaluation - Preliminary Carbon offset evaluation <p>Technical Feasibility Study</p> <ul style="list-style-type: none"> - Feasibility study before identifying sites for installation of pilot methane biofilters - Evaluation of preliminary conceptual designs - Preliminary laboratory work 	<p>Stakeholder group - formed and annual meetings were conducted. The identified collaborators/ stakeholders were; Bering Exploration Co ULC, Devon Canada, Tetra Tech EBA, Sperling Hansen Associates Inc., and Canadian Standards Association (CSA).</p> <p>Advisory committee members: Patrick Hettiaratchi (UofC), Susan Wood-Bohm (CCEMC), Matt Rahimi (CSA Group), Ali Abedini (Sperling Hansen Associates Inc.)</p> <p>A market study was completed. The report included; a review of technological aspects of MBFs and the relevant environmental issues, analysis of economic and regulatory aspects, a description of how the methane biofilters (MBFs) could be applied in various industry sectors as well as the feasibility of introducing the technology into industry,PESTEL, SWOT and Five Forces analysis of MBF technology as applicable in Alberta. (Detailed report is attached as a supplementary document: section 9.1)</p> <p>A GAP analysis was conducted separately with a comprehensive literature review to identify the research gaps (Detailed report is attached as a supplementary document: section 9.2)</p> <p>Laboratory Experiments were conducted with conceptual design preparation. Lab experiments to monitor the type of aeration with two different modes; active and passive aeration and experiments to optimize the filter media were conducted. (Final thesis on actively aerated biofilters is attached in section 9.3)</p>

	<ul style="list-style-type: none"> - Setting up passive vs active aeration experiments - filter material identification studies <p>Monitoring Protocol Development Study</p> <ul style="list-style-type: none"> - Preliminary laboratory experimentation to identify methanotrophic populations in mixed media - Preliminary field protocol development -concept development 	<p>(Final thesis on identifying optimal filter media is attached in section 9.4)</p> <p>Laboratory experiments to identify the methanotrophic population were conducted as initial concept/experiments in protocol development process. (Final report on monitoring protocol development is attached in section 9.5)</p>
<p>2 (2014)</p>	<p>Stakeholder meetings /Workshop I</p> <p>Steering committee meeting</p> <p>Technical feasibility study - Stage I</p> <ul style="list-style-type: none"> - Complete active vs passive MBF studies - Preliminary lab work on effect of hydrogen sulphide, other VOCs - Design, construction and operation of field MBFs – stage 1 - Field MBF data collection – Stage 1 - Preliminary studies on optimal media for MBFs - Preliminary studies on optimal MBF configurations <p>Monitoring Protocol Development Study</p> <ul style="list-style-type: none"> - Laboratory experimentation – to determine methanotrophic populations in MBF media from operating systems 	<p>Stakeholder meetings, plan for workshop and the steering committee meetings were conducted as planned.</p> <ul style="list-style-type: none"> ✓ Active vs passive MBF studies were completed (Detailed thesis is attached in section 9.3) ✓ Lab experiments on effect of trace gases were conducted (A published journal paper on this is included in section 9.6) ✓ Design of MBF system and studies (stage 1) on optimal MBF configurations were completed. (Final thesis on actively aerated biofilters is attached in section 9.3) ✓ The stage 1 of Field data collection and field MBF modifications was completed. (Final report on field work is included in section 9.7) ✓ Experiments on optimal filter media were completed. (Final thesis on identifying optimal filter media (fibrous based media) is attached in section 9.4) (Report on identifying filter media (biochar based) is attached in section 9.8) <p>Laboratory, theoretical, and field study on monitoring protocol development study were conducted. (Final report on monitoring protocol development is attached in section 9.5)</p>

	<ul style="list-style-type: none"> - Field protocol development - mathematical model development - Field protocol development – lab data generation - Field protocol development – field data collection and analysis 	
<p>3 (2015)</p>	<p>Commercial Feasibility Study</p> <ul style="list-style-type: none"> - Steering committee meetings - comprehensive market analysis <p>Technical Feasibility Study - Stage II</p> <ul style="list-style-type: none"> - Methane biofilter media development – column experiments - A novel design of an actively-aerated biofilter - Novel biofilter design based on flow patterns - Optimum thickness of biofilters for various flow orientations - Bioreactor configuration for developing a value-added product - Test the developed filter media in field scale MBFs - Design, construction and operation of field MBFs – stage 2: utilizing optimal media and configurations 	<p>Steering committee meetings were conducted.</p> <p>'International Workshop on Greenhouse Gas Emission Reductions Using Biological Methods' was conducted over 2 full days to gather leading researchers and industry professionals across the world to provide a common platform to share knowledge and bridge the gap between university research and industry applications. (http://www.ucalgary.ca/mbf/workshop-2015) (A summary of the output from the workshop is attached in section 9.9). Parallel to the workshop, discussion sessions were conducted to enhance the SWOT analysis and the market analysis of the methane biofiltration technology.</p> <p>Methane biofilter media development:</p> <ul style="list-style-type: none"> ✓ Few potential materials were successfully tested in laboratory biofilter columns - fiber based media (flax) and compost based media (in combination with lava rock, wood shavings and the biochar). (Final thesis on identifying optimal filter media (fibrous based media) is attached in section 9.4) (Report on identifying filter media (biochar based) is attached in section 9.8) <p>Biofilter design:</p> <ul style="list-style-type: none"> ✓ Actively aerated biofilter columns experiments were conducted. MBF performances were compared to different flow patterns, different air delivery methods, and various flow orientations. (Final thesis on actively aerated biofilters is attached in section 9.3) <p>Field work:</p> <ul style="list-style-type: none"> ✓ Design of MBFs to suite different operational parameters were done in collaboration with Devon Energy. ✓ Data collection and field data analysis for existing MBFs were conducted more frequently

	<ul style="list-style-type: none"> - Data collection – field optimal MBFs - Field data analysis <p>Monitoring Protocol Development Study-Phase 1</p> <ul style="list-style-type: none"> - Field protocol implementation (testing and verification) 	<p>during summer and periodically during the rest of the year. (Final report on field work component is included in section 9.7)</p> <p>Based on laboratory experiments, MOM (methylotrophs over methanotrophs) monitoring protocol has been developed. (Final report on monitoring protocol development is attached in section 9.5)</p>
<p>4 (2016)</p>	<p>Commercial Feasibility Study</p> <ul style="list-style-type: none"> - Stakeholder Workshop II - Financial evaluation/ draft report completion - Final Report <p>Technical Feasibility Study- Stage III</p> <ul style="list-style-type: none"> - Completion of laboratory experiments - Design, construction and operation of field MBFs – stage 3: utilizing optimal media and configurations - Data collection – field optimal MBFs - Field data analysis and draft final report preparation <p>Monitoring Protocol Development Study- Phase 2</p> <ul style="list-style-type: none"> - Field protocol implementation (testing and verification) 	<p>Final report is completed.</p> <p>Laboratory experiments were completed.</p> <p>Methane biofilter media development:</p> <ul style="list-style-type: none"> ✓ Potential materials were successfully tested in laboratory biofilter columns – biochar based medium (Report on identifying filter media (biochar based) is attached in section 9.8) ✓ Effect of presence of sulphide in methane rich gas was monitored (Final thesis on this is attached in section 9.10) <p>Biofilter design:</p> <ul style="list-style-type: none"> ✓ Actively aerated biofilter columns experiments were completed: MBF performances were compared to different flow patterns, different air delivery methods, and various flow orientations (Final thesis on this is attached in section 9.3) <p>Field work:</p> <ul style="list-style-type: none"> ✓ Field biofilters were designed/ constructed and installed ✓ Data collection and field data analysis for existing methane biofilters and newly installed biofilters are still in progress and will continue (Final report on field work component is included in section 9.7) <p>Based on laboratory experiments, monitoring protocol has been developed. (Final report on monitoring protocol development is attached in section 9.5)</p>

6. Scientific Achievements

Several documents, including few theses, workshops, journal papers and conference papers, oral presentations and posters are successfully completed/published as a part of this project. A few theses and journal papers are still in preparation stage as some of the experimental work is still under progress. A completed list is included below.

Theses (Completed)

1. Barzegar, S., 2017. Investigating the Inhibitory Effect of Acidic pHs on Methane Biofiltration Technology, MSc Thesis, University of Calgary.
2. Farrokhzadeh, H., 2016. Performance of Actively-Aerated Biofilters Using a Multiple-Level Air Injection System to Enhance Biological Treatment of Methane Emissions, MSc Thesis, University of Calgary.
3. Goya, J., 2015. Development of an alternative media to sustain methanotrophs in biofilter, MSc Thesis, University of Calgary.

Workshops

1. International Certificate Workshop on Reduction of GHG Emissions from Solid Waste Landfills Using Biological Methods, organized and conducted by Hettiaratchi's team from University of Calgary in collaboration with a team from Center for Sustainability, Department of Forestry and Environmental Science, University of Sri Jayewardenepura, Colombo, Sri Lanka, January 2016. (<http://sustainability.sjp.ac.lk/ghg2016/>)
2. International Workshop on Opportunity for GHG Emission Reduction using Appropriate Technology from Waste Sector in Tropical Climate, organized and conducted by Hettiaratchi's team from University of Calgary in collaboration with a team from Solid and Hazardous Waste Management Division, CSIR- National Environmental Engineering Research Institute (NEERI), Mumbai, India, January 2016.
3. International Workshop on Greenhouse Gas Emission Reductions Using Biological Methods, organized and conducted by Hettiaratchi and team, University of Calgary, August 2015. (<http://www.ucalgary.ca/mbf/workshop-2015>)

Journal Papers

1. Farrokhzadeh, H., Hettiaratchi, J.P.A., Jayasinghe, P.A., Aerated biofilters with multiple-level air injection configurations to enhance biological treatment of methane emissions, Bioresource Technology - submitted and under review, 2017

2. Jayasinghe, P.A., Haththotuwa, C.K., Hettiaratchi, J.P.A., Methane biofiltration in the presence of non-methane organic compounds in solution gas, *Current Environmental Engineering*, 3(2), 161-169, 2016
3. Mancebo, U., Hettiaratchi, J.P.A., Jayasinghe, P.A., and Surampalli, R., Determination of water retention capacity of granular media of methane biofilters: a simplified approach, *Environmental Earth Sciences*, 75:74, 1-8, 2016
4. Porkhrel D., Hettiaratchi J. P. A, Steele M. Development of Methane Oxidation Curves in Soil at Different Organic Contents. *Current Environmental Engineering*, 3(2), 2016
5. Mancebo U, Hettiaratchi JPA. Rapid assessment of methanotrophic capacity of compostbased materials considering the effects of air-filled porosity, water content and dissolved organic carbon. *Bioresource Technology*. 177: 125-133, 2015

Conference Papers + Conference Oral Presentations

1. Gunasekera, S., Hettiaratchi. J. P.A., Bartholameuz, E.M., Irvine. E., Design and Operation of Actively-Aerated Field-Scale Methane Biofilters, paper to be presented at the 15th International Conference on Environmental Engineering, Canadian Society of Civil Engineering (CSCE), Vancouver, May 2017 (Accepted).
2. La, H., Hettiaratchi, J. P. A., Achari, G., Dunfield, F. D., Kim, J., Investigation of biologically stable methane biofilter medium for alternative landfill covers, in: 2017 Geotechnical Frontiers Conference, Life Cycle Assessment & Sustainable Engineering, Orlando, Florida, USA, 2017, (Accepted).
3. La, H., Hettiaratchi, J.P.A., Achari, G., Effect of nutrients on methanotrophic oxidation activity in batch experimental studies, in: CSCE, 14th International Environmental Specialty Conference, Canadian Society for Civil Engineering, London, Ontario, 2016, pp. 1-9 (ENV 610).
4. Khadka R., Dunfield P. F., Phylogenetic history of copper monooxygenase gene family, International Symposium on Microbial Ecology (ISME-16), Montreal August, 2016.
5. Jayasinghe, P.A., Hettiaratchi, J.P.A., Control of Low-Volume Methane Emissions from Oil and Gas Sector, Presented at the '65th Canadian Chemical Engineering Conference', Calgary, October 2015
6. Gunasekara, S.S., Hettiaratchi, J.P.A., Bartholameuz, E.M., Jayasinghe, P.A., Yadev, S. Analysis of the Field Performance of Methane Biofilters, Presented at the '65th Canadian Chemical Engineering Conference', Calgary, October 2015
7. Goya, J, Hettiaratchi, J.P.A. Development of an alternative media to sustain methanotrophs in landfill biofilters, presented at the 65th Canadian Chemical Engineering Conference, Calgary, October 2015

8. Farrokhzadeh, H., Hettiaratchi, J.P.A., Performance of Aerated Methane Biofilters with Innovative Air Delivery Systems, presented at the Canadian Chemical Engineering Conference (CSCChE), Calgary, AB, October 2015
9. Farrokhzadeh, H., 2015. University of Calgary **Three-minute thesis finalist** “Methane Biofiltration : An alternative Cure to Global warming”, Calgary, AB
10. Kim, J.J., Khadka, R., Hettiaratchi J.P., Dunfield, P.F. Control of point-source low-volume methane emissions using methane biofiltration technology: Development of a molecular monitoring protocol. The 6th Annual Bioindustrial Meeting: Biorefining Conversions Network – Alberta Innovates Bio (BCN-AI Bio). November, 2015, Edmonton
11. Dunfield P.F., Kim, J.J., Khadka, R. Can understanding microbial communities help to design better biofilters? Application of Biological Methods to reducing Greenhouse Gases: Schulich School of Engineering International Workshop. August 2015, Calgary.
12. Bartholameuz, E.M., Hettiaratchi, J.P.A., Jayasinghe, P.A., Methane biofilter performance under varying climatic conditions in closed landfills, paper presented at the ‘CSCE annual conference’, Halifax, May 2014

Conference Poster Presentations

1. La, H., Methane oxidation by methanotrophs in biologically-stable materials, oral poster presentation at: Canadian Prairies and Northern Section Annual Conference and General Meeting, Air and Waste Management Association, Calgary, Alberta, 2016.
2. Gunasekara, S., Hettiaratchi, J.P.A., Analysis of the field performance of methane biofilters System, poster presented at the Women in Science and Engineering (WISE) Research Night, University of Calgary, September 2015.
3. Farrokhzadeh, H., Hettiaratchi, J.P.A., Performance of Actively-Aerated Biofilters Using a Multiple-Level Air Injection, poster presented at the Women in Science and Engineering (WISE) Research Night, University of Calgary, September 2015.
4. Jayasinghe, P.A., Hettiaratchi, J.P.A., Dunfield, P., Control of point source low volume methane emission using methanobiofiltration technology, poster (display) presented at the Biological Solutions Forum, Climate Change and Emissions Management (CCEMC) Corporation, Edmonton, October 2014
5. Farrokhzadeh, H., Jayasinghe, P.A., Hettiaratchi, J.P.A., A Novel Design of an Actively-Aerated Biofilter to Enhance Biological Treatment of Methane Emissions, poster presented at the Biological Solutions Forum, Climate Change and Emissions Management (CCEMC) Corporation, Edmonton, October 2014

Won the third place in the student poster competition

6. Jessica Goya, Hettiaratchi J P A., Research on alternative media to sustain methanotrophic activity in biofilters for fugitive emissions. Biological Solutions Forum Climate Change and Emissions Management (CCEMC) Corporation, Edmonton, October 2014.
7. Jayasinghe, P.A., Gunasekara, S., Hettiaratchi, J.P.A., Dunfield, P., Control of point source low volume methane emission using methanobiofiltration technology, poster presented at the Women in Science and Engineering (WISE) Research Night, University of Calgary, November 2014.
8. Jayasinghe, P.A., Hettiaratchi, J.P.A., Dunfield, P., Control of point source low volume methane emission using methanobiofiltration technology, Biological Solutions Forum hosted by Biological GHG Management Program, Alberta Innovates Bio Solutions in collaboration with Climate Change and Emissions Management Corporation (CCEMC), Calgary, October 2013

7. Greenhouse Gas Impacts

Table below represents the expected greenhouse gas (GHG) benefits projected over a ten-year period per ONE methane biofilter, considering only the direct impacts from implementation of the project. The expected reductions in GHG emissions would be in the range of 1 to 2 mega-tonnes of CO₂eqv/yr, if methane biofiltration technology is applied with an efficiency of 30 to 60% throughout Alberta.

Table 3: GHG Benefits per One Methane Biofilter

Biofilter methane inlet load (average) per Day	25 m ³ /day
	18 kg/day
Biofilter methane inlet load (average) per Year	6570 kg/year
	6.6 tonnes/year
GWP CH ₄	34 times CO ₂
CH ₄ inlet load to biofilter- CO ₂ equivalent	223.4 tonnes CO ₂ -Eqv /year
Assuming 80% conversion (practical as observed in field situations)	
CH ₄ + 2O ₂ -----CO ₂ + 2H ₂ O	
CO ₂ generation from methan biofiltration process to the given inlet load	18.1 tonnes/year
CO ₂ Reduction per year from ONE biofilter loaded at 25 m ³ /day of methane	205.3 tonnes CO ₂ Eqv/year/biofilter
CO ₂ reduction over 10 years from one methane biofilter	2053.13 tonnes CO ₂ Eqv/biofilter for 10 years

8. Conclusions

We have successfully completed all the milestones/activities of this project during the given period of time. In addition to the technological innovations and scientific outcomes described in the detailed reports, we have also trained a significant number of HQPs who are now ready to utilize their knowledge and hands-on experience to the advancement of the GHG mitigation technologies. Our next step would be to apply this developed MBF technology under several field situations and field application will continue.

9. Supplementary Documents

This section includes the detailed report/theses prepared by various researchers.

9.1 Report: Market Feasibility of Methane Bio-Filtration Technology in Alberta to Control Point Source Low-Volume Methane Emissions

9.2 Report: GAP Analysis

9.3 Thesis: Performance of Actively-Aerated Biofilters Using a Multiple-Level Air Injection System to Enhance Biological Treatment of Methane Emissions

9.4 Thesis: Development of Alternative Medium to Sustain Methanotrophs in Methane Biofilters

9.5 Report: Monitoring Protocol Development

9.6 Journal Paper: Methane Biofiltration in the Presence of Non-Methane Organic Compounds in Solution Gas

9.7 Report: Field Work

9.8 Progress Report: Methane Biofiltration by Methanotrophic Bacteria Using Biologically Stable Filter Materials

9.9 Summary: International Workshop on Greenhouse Gas Emission Reductions Using Biological Method

9.10 Thesis: Investigating the Inhibitory Effect of Acidic pHs on Methane Biofiltration Technology

9.1 Report: Market Feasibility of Methane Bio-Filtration Technology in Alberta
to Control Point Source Low-Volume Methane Emissions



UNIVERSITY OF
CALGARY
HASKAYNE
School of Business

**Market Feasibility of Methane Bio-Filtration Technology in Alberta to
Control Point Source Low-Volume Methane Emissions**

By

Kemi Omole, Seema Jindal and Shoshi Darain

Calgary, Alberta, Canada

September, 2013

EXECUTIVE SUMMARY

Methane has a global warming potential that is twenty five times more than carbon dioxide as a greenhouse gas. It is naturally occurring in the atmosphere and also generated from various anthropogenic activities such as industrial, commercial, agricultural and waste processes. According to the ERCB 2012 Upstream Petroleum Industry Flaring and Venting Report, 2011, “The combined volume of flared and vented solution gas increased to 785 million cubic metres (10^6 m^3) (27 856 million cubic feet [MMcf]) in 2011”. A look at these numbers indicates that though these are low-volume point sources, the numbers add up exceptionally. The question therefore is how to control these lower volume point source emissions.

There are numerous on-going researches to minimize methane emitted in the atmosphere and mitigate climate change. One of such researches is the testing of bio-filters that convert low volumes of methane into CO_2 with the help of bacteria at the University of Calgary. There are on-going pilot projects to aid in the deployment of the technology into industry. The bio-filters are being designed to address point source low volume methane emission of up to 100m^3 flow per day. Low volume emissions are the focus of the research as the volumes add up quickly and become quite significant. Higher volumes are easier to manage as they are more economically viable to generate electricity.

It is advantageous that the research and testing is taking place in Alberta, as the province has a climate change policy in place to reduce overall emissions. The Climate Change and Emissions Management Corporation (CCEMC) through its Biological Greenhouse Gas Management Program fund such researches to find lasting solutions to reduce greenhouse gases.

The Methane Bio-filter Technology is in its early stages with various field-scale pilot projects in place to continually test the technology. The pilot projects have had a high degree of success. This report will explore Methane Bio-filter Technology as an innovative technology with an aim to offset methane emissions from low point sources and thus increase their energy efficiency. It will involve analyzing why and how by offsetting methane from low point sources using Methane Bio-filter Technology makes sense for the industry.

This paper will also assess how the bio-filters can be applied in various sectors as well as the market feasibility of introducing the technology into industry. The industry analysis will include a PESTEL, SWOT and Five Forces analysis with Alberta's industry sector in mind. Furthermore, it incorporates and discusses technological aspects and relevant environmental issues. In addition, economic and regulatory aspects will also be addressed keeping energy as the focal point.

ACKNOWLEDGEMENT

We would like to thank our supervisor, Prof. Patrick. Hettiaratchi, for supervising our research project- 'Market Feasibility of Methane Bio-Filtration Technology in Alberta to Control Point Source Low-Volume Methane Emissions'. His extensive experience in the area of greenhouse gas emission technology development helped us in understanding and completing the project. Without his guidance this project would not have been possible.

We would also like to thank Dr. Poornima Jayasinghe, who was always available to answer questions when we hit a road block.

TABLE OF CONTENTS

Table of Contents

EXECUTIVE SUMMARY	ii
ACKNOWLEDGEMENT	iv
CHAPTER ONE.....	1
Background	1
Methodology	1
Environment: Methane’s Role in Global Warming	2
Environmental Benefits of Reducing Methane	4
Regulatory Requirements at the Federal Level	5
Emission Reduction Goals in Alberta	7
Potential Role of Methane Bio-filtration Technology.....	8
CHAPTER TWO.....	10
Technology	10
Bio-filtration.....	10
Methane Bio-Filtration Technology.....	12
Advantages of Bio-filters	15
CHAPTER THREE	19
Industry Applications	19
Hydraulic fracturing	22
Methane Emissions	23
Mitigation methods	25
Alberta Regulatory Requirements.....	26
Bio-filter Application.....	26
Solution Gas	29
Fugitive Methane Emissions	29
Mitigation methods	29
Alberta Regulatory Requirements.....	30
Bio-filter Application.....	31

Livestock.....	33
Methane Emissions and mitigation methods.....	33
Mitigation of methane emissions	36
Bio-filter Application.....	37
Alberta Regulatory Requirements.....	37
Landfills	39
Methane Emissions	41
Mitigation methods for low volume methane released	42
Alberta’s Regulatory Requirements	43
Bio-filter Application	43
CHAPTER FOUR	45
Economics: Industry Analysis and Strategy.....	45
PESTEL Evaluation	47
Five Forces Analysis	63
SWOT Analysis.....	66
Project Costs.....	71
GHG Emissions Reductions.....	71
CHAPTER FIVE	72
Recommendations for the market feasibility of MBT.....	72
Technology.....	74
Economic.....	74
Environment.....	74
Regulatory	75
Conclusion.....	77
REFERENCES	79

CHAPTER ONE

Background

Methodology

The focus of this paper is to assess the market feasibility of bio-filters in the control Methane emissions. This will help identify market trends, opportunities and need to develop profiles for the target industries. The market analysis will include a PESTEL analysis examining external factors, which may be of crucial importance to the project, a SWOT analysis of the bio-filter project to determine the strengths, weaknesses, opportunity and threats. An analysis of perceived barriers in industry through assessing external forces will also be conducted using the Five Forces analysis tool will also be applied. The results of which will be evaluated to the address the perceived barriers and capitalize on the opportunities in industry.

Bio-filters have been proven to convert low volumes of methane into carbon dioxide. This report will assess various point sources of low volume emissions in industry as well as the applicability of bio-filters in each sector. This analysis will help in the planning and development on a larger-scale and the industry implementation of the technology.

The technology is still in the pilot stages, therefore some information remained confidential. As a result, cost projections could not be reported of the bio-filter units, as well as costs per tonne of emissions.

The significance of this research stems from the nature of methane and its effects on the environment. Methane is a colorless, odourless gas that is widely distributed in nature and a 75 percent constituent in natural gas. It is also a constituent in swamp gas, sewer gas and is one of the main components of landfill gas (Reinhart & Townsend, 1998). It is the most abundant organic trace gas in the atmosphere, with a global warming potential twenty five (25) times more than carbon dioxide.

Methane is the simplest hydrocarbon—a single carbon atom surrounded by four hydrogen atoms. It usually forms when larger organic molecules are broken down, either by microbes or by heat. There are three main sources of methane production in the atmosphere.

They include:

1. Methane from biological sources i.e. natural origin
2. Methane from abiotic production from lithospheric sources i.e. geological formations over longer time scales
3. Methane from anthropogenic sources i.e. human activities including industry, agriculture etc.

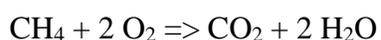
Environment: Methane's Role in Global Warming

Methane is an effective greenhouse gas. It is radiatively active, thus it traps infrared radiation that would otherwise escape into the atmosphere. Methane reacts radiatively similarly to carbon dioxide, but unlike carbon dioxide it traps 25 times as much long wave radiation molecule for molecule; thus the higher global warming potential of 25 times more than carbon dioxide. This increases the incidence of temperature warming in relation to climate change. The increase in

atmospheric temperature also has other effects which include the melting of permafrost which is known to have stored methane for thousands of years (Heikkinen et al., 2002). This also increases methane in the atmosphere and so continues the methane-global warming-climate change cycle. Furthermore methane reacts with the hydroxyl radical (OH) thus producing tropospheric carbon monoxide and ozone (O₃) (Wuebbles & Hayhoe, 2002). It is a greenhouse gas with a short atmospheric lifetime of only 12 years. The chemical reactions constitute a carbon sink for methane in the atmosphere.

A mass balance equation exemplifies the combustion of methane when treated below.

Combustion of CH₄ Reaction:



Methane concentration in the atmosphere is low, usually about 2 parts per million. This concentration has increased by 150 percent between the years 1750 to 1998 from a global mean concentration of 700 parts per billion to 1745 parts per billion (IPCC, 2007). Global anthropogenic methane emissions are projected to increase nearly 20 percent to 8,522 million metric tons of carbon dioxide equivalent (MMTCO₂E) by 2030 (U.S. Environmental Protection Agency, 2011). This reiterates the connection between methane increase and climate change.

Methane from anthropogenic sources, as noted above, includes production by bacteria found in various anaerobic environments, which include landfills, anoxic rumen of cattle, natural wetlands, rice paddies etc. Other anthropogenic activities include production of oil and gas, industrialization and urbanization etc. Accelerated human activities especially in fossil fuel use, waste disposal, enteric fermentation, burning of biomass, landfill etc. have increased the

methane emissions considerably over the years. The current concern over the potentially negative impacts of climate change has resulted in a search for techniques to reduce anthropogenic emissions of methane (CH₄) (Hettiaratchi et. al., 2006).

Environmental Benefits of Reducing Methane

Reducing methane emissions, therefore, can have significant climate change benefits, especially in the short term. In particular, methane reductions can help avoid potential climatic tipping points and reduce environmental impacts, especially in the Arctic (Quinn, et al., 2008). Moreover, methane has a large reduction potential and cost-effective mitigation technologies are available. Achieving a “50-percent reduction in methane emissions from a business-as-usual scenario by 2050 and maintaining those reductions through 2100 could help reduce global temperature on the same scale as similar reductions in CO₂ emissions—about 0.55 degrees Celsius” (IPCC, 2007).

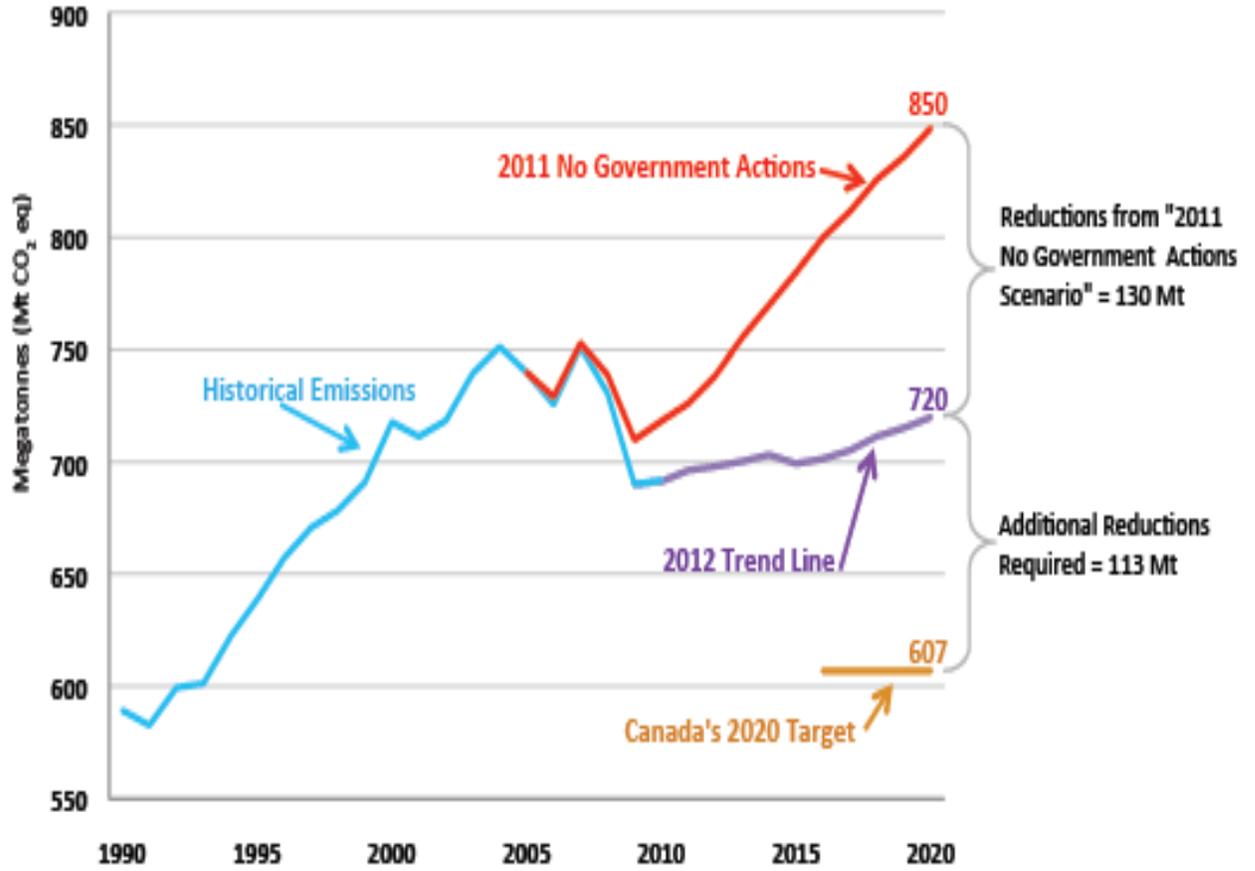
Sources of higher volumes of methane are easier to manage as it can be used for power generation, feedstock in petrochemical plants, carbonising steel, ammonia, ethanol etc. Lower volumes of methane emissions are cumbersome to manage, the volumes emitted also add up rapidly and considerably. Presently there are fewer methods of managing these lower volumes in industry, thus the focus of this project, to assess the feasibility of introducing a technology that can help manage these emissions.

Regulatory Requirements at the Federal Level

Under the Copenhagen Accord, Canada committed to a GHG emission reduction target of being 17 percent below 2005 levels by 2020 which is equal to 607 Mt based on Canada's original 2005 baseline (Environment Canada, 2013). With the combined efforts on both the federal and provincial levels, Canada is working towards passing sector-specific regulations to regulate GHG emissions keeping in mind their circumstances along with environmental and economic goals. This is being done to create incentives for the industry to gear towards clean energy systems. Since 2007, the Government of Canada has been allocating funds through Clean Air Regulatory Agenda funds for efforts to reduce GHGs and air pollutant emissions (Environment Canada, 2013).

According to Environment Canada (2013),

In 2012, the gap between Canada's GHG emissions target of 607 Mt is now projected to be 113 Mt. This means that Canada's 2020 emissions are projected to be about one half of the way to the target.

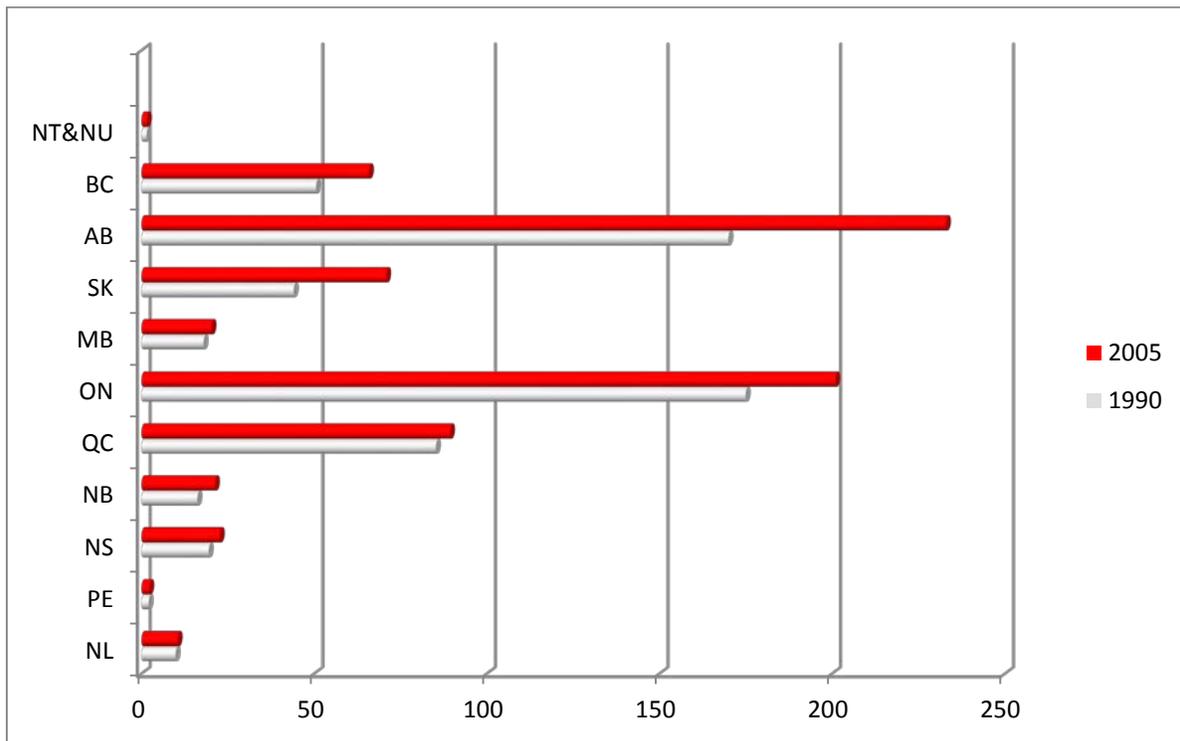


Canada's historical greenhouse gas emissions and projections to 2020

Source: (Environment Canada, 2013)

Emission Reduction Goals in Alberta

Currently, Alberta is under the spotlight when it comes to climate change because its Greenhouse Gas (GHG) emissions, on a per capita basis are extraordinarily high. The figure below shows GHG emissions per capita in Canada. Alberta has the highest emissions per capita as of 2005.



Data Source: (Environment Canada, 2012)

Other regulations that are favourable for adoption of bio-filters are:

- Alberta Environmental Protection and Enhancement Act, formally known as the Clean air Act supports and promotes the protection, enhancement and wise use of the environment. The Clean Air Act allows the Environment Minister to make a

regulations relating to air quality standards, emissions standards, enforcement procedures monitoring methods

- Canadian Environmental Protection Act: "...respecting pollution prevention and the protection of the environment and human health in order to contribute to sustainable development" (CEPA, 2013)
- Climate Change and Emissions Management Act: Alberta first law to impose greenhouse gas cuts on (~100) large industrial facilities
- Energy Utilities Board Directive 60: Regulations to reduce flaring in Alberta. Provides requirements and guidelines for flaring, incinerating, and venting gas
- Alberta Ambient Air Quality Objectives (AAAQO): potential effects on public health from flaring are addressed under Alberta Environment's

Potential Role of Methane Bio-filtration Technology

The controls of low-volume methane emissions are harder to restrict thus, the introduction to Methane Bio-filtration Technology (MBT) is a feasible solution to the problem. The Climate Change and Emissions Management Corporation (CCEMC) estimates,

This project can reduce greenhouse gas emissions by about 2,000 tonnes over 10 years. The estimated ten year (2013-2022) market emission reductions that can be expected from this technology, anticipating market adoption starting in 2016 at a 1 percent adoption rate is: 130,000 tonnes CO₂e.

Possible industry sectors for MBT of low-volume GHG emissions include solution gases from the oil and gas industry, landfills, feedlots etc. These sources contain various GHGs but are predominantly methane. These are small point sources that are not viable for power production. Therefore, the gases are vented or flared. These are both detrimental to the atmosphere as in the case of venting nothing is done to the GHGs, while flaring may release more toxins into the atmosphere including Nitrogen and Sulfur oxides (NO_x and SO_x).

CHAPTER TWO

Technology

Bio-filtration

Bio-filtration is the biological treatment of contaminants using media with living microorganisms. A bio-filter uses moist organic materials to adsorb and biologically degrade contaminants. The typical style bio-filter is a box, which can be customized to the desired size; from one square meter to the size of a basketball court. The knowledge and application of biodegradation of contaminants by active bacteria has been used since the 1950s in soil filters and biological trickling filters. Bio-filtration is a well-known and cost-effective technology for removing environmental pollutants from contaminated gas streams (Yang et al., 2002). The bio-filter brings the pollutants in the air stream in contact with the microorganisms in the system. The polluted air stream is pumped through the bio-filter and is released slowly through perforated pipes. The pollutants are absorbed into the filter media and then the contaminants to be processed are adsorbed onto the biofilm where they are acted upon by the bacteria and degraded into CO₂ and water (Gawande et. al., 2010).

Naturally bioactive media such as soil, peat, compost; which contain micro-organisms such as bacteria, fungi and yeast, and macro-organisms such as protozoa, worms and insect larvae are commonly used in traditional bio-filters (Govind, nd). Media replacement is required every two to five years. Microorganisms present in these media biodegrade the contaminants usually in the gaseous phases, and this has been successfully employed in bioremediation of contaminated sites (Govind, nd). The media is the breeding ground for the microorganisms that grow on a thin moisture layer known as bio-film on the media particles. Bio-filtration is commonly used as a

pollution control technique, typically for odor control in waste water treatment and composting operations.

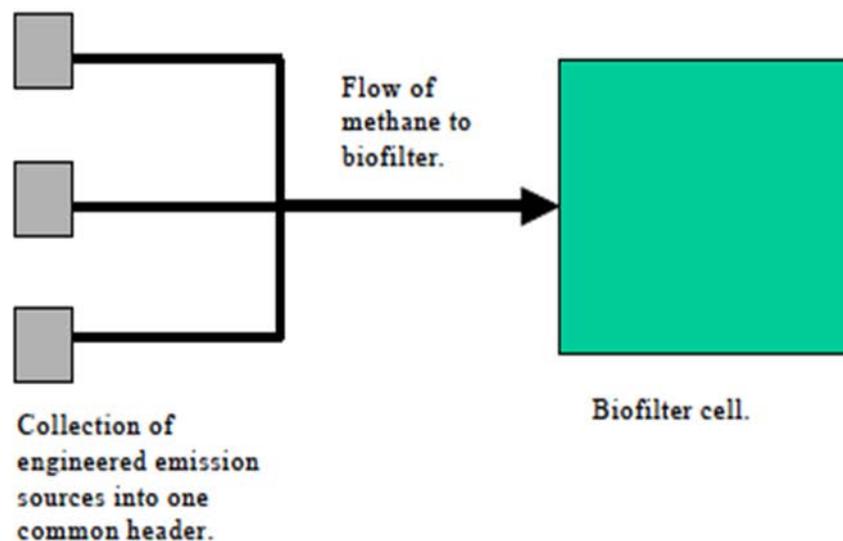
Compost compared to soil has become the more widely used media as it has a higher concentration of microorganisms. The use of compost also has its draw backs such as compost settling, which reduces the amount of nutrients, pH stability and drying of the compost material (Govind, nd). These issues have been addressed through the use of wood chips; coconut husks etc., which help retain moisture and provide additional nutrients to the compost. Lime pellets can also be added to maintain the pH of the system. Nitrogen and phosphorus can also be maintained in the compost system with the addition of fertilizers (Govind, nd). Biologically inert material such as gravel may also be added to maintain adequate porosity. The bed depth which ranges from 1 – 1.5 meters deep is an essential consideration in bio-filter designs. Shallower depths may short-circuit gas flows thus being less effective while maintaining uniform moisture in deeper beds may be difficult (Cornell Waste Management Institute, 2009)

Bio-filters have been effective in treating sulfur, amine and ammonia compounds related to odors especially in composting operations (Cornell Waste Management Institute, 2009). With more research and innovation it is evolving into the treatment of volatile organic compounds (VOCs) and toxic air emissions in industrial and commercial operations. Advancements have also made bio-filtration more economically viable.

Methane Bio-Filtration Technology

Methane, as earlier noted has a global warming potential 25 times more harmful than CO₂. Continuous development and resource extraction continues to increase CH₄ generation increasing the incidence of climate change. Addressing methane generation through reducing the rate of development and anthropologic activities is a herculean task, which may very well be impossible. Looking to other control measures such as increasing operational efficiencies, climate change friendly and cheaper measures, such microbial oxidation of methane to carbon dioxide etc. are more achievable and thus should also be focused on to attain greater reductions.

Microbial oxidation of methane to carbon dioxide involves the use of a bio-filter media e.g. soil or compost in the presence of methanotrophic (methane utilizing) bacteria. “Methanotrophic bacteria are capable of converting methane to carbon dioxide and therefore serve as an important methane sink” (Hettiaratchi et. al., 2006). Methanotrophic bacteria are a unique group that utilize methane as a sole energy source (Hettiaratchi et. al., 2006). These bacteria serve as bio-filters for the oxidation of methane produced in anaerobic environments. Methane bio-filters use a monoculture of methanotrophic bacteria, which have the ability to convert large amounts of methane with concentration of up to 95 percent to carbon dioxide. Atmospheric methane can also be oxidized when oxygen is present in soils (Hanson & Thomas, 1996). Under controlled conditions and proper acclimatization of this strain of bacteria, high rates of methane oxidization can be achieved, creating a solution for low volume point sources (Hettiaratchi, et. al. 2006).



Source: (Hettiaratchi et al., nd)

The figure above shows a basic configuration of a collection system into a bio-filter for processing.

According to Hettiaratchi et al., (nd), laboratory experiments have shown the potential to apply methanotrophic bio-filters to treat low-volume methane from various sources. Field experiments are also being carried out for quantitative and qualitative understanding of these processes to inform and enable development on a commercial and industrial scale.

Typically processed air such as solution gas from oil and gas operations, is injected through a grid of perforated pipes into a bed of filtration media i.e. compost. Migration and processing of gases through the bio-filter media is enabled by advection due to pressure gradients and diffusion due to concentration gradients. The rate of gas migration is influenced by various factors including temperature, moisture (directly affected by precipitation), bio-filter configuration, choice of bio-filter media and its properties. Precipitation can also cause other

operational issues when moisture levels increase. These include leachate production and dissolved CO₂. Dissolved CO₂ changes the composition of the gas thus affecting the flow and decreasing the pH of the bio-filter system. Methanotrophs are mesophilic in nature, thus thrive in temperatures between 10°C – 35°C. Therefore there is a projected increase in microbial activity in summer and a reduction in winter. The increase in temperature in summer will result in a higher microbial activity and oxidation of methane (Hettiaratchi et. al., nd).

A pilot project conducted by Hettiararchi et al., showed indications on the operation of the bio-filters in various configurations to optimize efficiency. The aim of the pilot project was to inform the most efficient configurations for the bio-filter unit and medium for optimum performance. The pilot project was conducted in conjunction with on-going laboratory experiments and models. The pilot project monitored various factors including temperature, moisture content, surface flux and gas concentration. Other factors monitored in both the laboratory models and pilot projects include methane oxidation and transport, heat transfer in the media, atmospheric processes and moisture dynamics. With these parameters established, bio-filters can be customized to fit various industrial applications (Hettiaratchi et. al., nd).

Advantages of Bio-filters

There are numerous advantages in using bio-filters in comparison to traditional methods typically flaring and venting. Some of these advantages are discussed below.

- **Environmental benefits:** Flaring and venting as discussed earlier chapter, are harmful to the environment. Bio-filters are a viable replacement option to substantially reduce the volumes of gas flared in various industries. There are also little to zero NO_x and SO_x which are by-products of incomplete combustion.
- **Low Cost (capital and operating):** Bio-filters operate at average room temperatures, thus eliminating any extra costs of heating the system. Flares require power to operate adding more operational cost; in the case of bio-filters a recirculation pump and a fan to pull the gas stream through the system are the only equipment that requires power. There are also no moving mechanical parts which results in lower maintenance costs.
- **Inherently safe:** Methane is a combustible gas and has been known to spontaneously combust in higher temperature. Bio-filters have a moisture content that regulates the temperature of the system thereby eliminating the possibilities of combustion.
- **Effective and proven technology:** The bio-filter technology has been in existence for a very long time. Methane bio-filters can convert large concentrations up to 95 percent. Though there is a change in its application, its track record of success in varying applications over the years is a major advantage in un-charted territory.
- **No hazardous by-products:** Unlike in incomplete combustion, the process results in the total degradation of pollutants thus avoiding hazardous by-products.

Limitations of bio-filters

Lack of information: According to the (Hettiaratch et. al., 2006) there is information deficiency in the various parameters that inform the operational chemical and physical processes in the bio-filter system. These parameters include optimum bio-filter media configuration, pH, temperature, moisture content, nutrient concentration and environmental factors. Such data is necessary to develop configurations of the methane bio-filters to maximize the methane oxidation efficiency. Methanotrophic bio-filtration does not produce a marketable by-product i.e. carbon dioxide; unlike energy recovery methods in waste to energy operations. Therefore, the bio-filters must be as inexpensive and efficient as possible.

The on-going pilot projects and laboratory experiments have been bridging the information gap in the above parameters.

The figure below shows a picture of a pilot project monitoring and addressing the issues mentioned above. This is a prototype bio-filter, which can be configured to fit any location.



Source: (Hettiaratchi et al., nd)

Other potential issues that could limit bio-filter performance include:

- Replacement of bio-filter media every two to five years
- The replacement of the media requires microbial acclimatization, which requires time. This can take weeks or months, leading to a lag time for bio-filters to become fully operational
- The weather in the location of operation could affect bio-filter efficiency, if not properly managed. Excess moisture from precipitation causes leachate in the compost media. Leachate management can be cumbersome and costly, increasing the overall operating cost of the system. On the other hand, not enough moisture in the media causes it to dry out. Dry media limits the bacteria activity and subsequently system efficiency.

- Carbon dioxide is a gas soluble in water. Excess moisture in the system dissolves the CO₂ by-product which changes the composition of gas, thus affecting the flow and gas concentration. Dissolved CO₂ also decreases the pH of the bio-filter system.
- Limited knowledge on the treatment of other contaminants such as hydrogen sulphide.
- Point sources with fluctuating emission concentrations and volumes may be detrimental to the microbial population and subsequently overall system performance and efficiency.
- Point sources with high volume emissions (still within the low volume threshold of up to 100m³ per day) would require larger units thus larger areas are required.

CHAPTER THREE

Industry Applications

This section includes an assessment of various industries where bio-filters could be applied. The assessment takes into consideration methane production in each industry, current mitigation techniques used as well as relevant regulations that govern methane emissions in each sector.

A substantial percentage of low volume methane emissions are from fugitive emissions from operations which are difficult to control. Fugitive emissions given in the IPCC (2006) Guidelines is “an intentional or unintentional release of gases from anthropogenic activities excluding the combustion of fuels.”

Methane emissions from different industries vary. They depend on the infrastructure of the particular industry, system reliability and volume of waste gas generated. Government regulations and incentives (such as, offset credits) to control these emissions also play an important role in the action an industry takes to comply or benefit by controlling the harmful emissions from its processes.

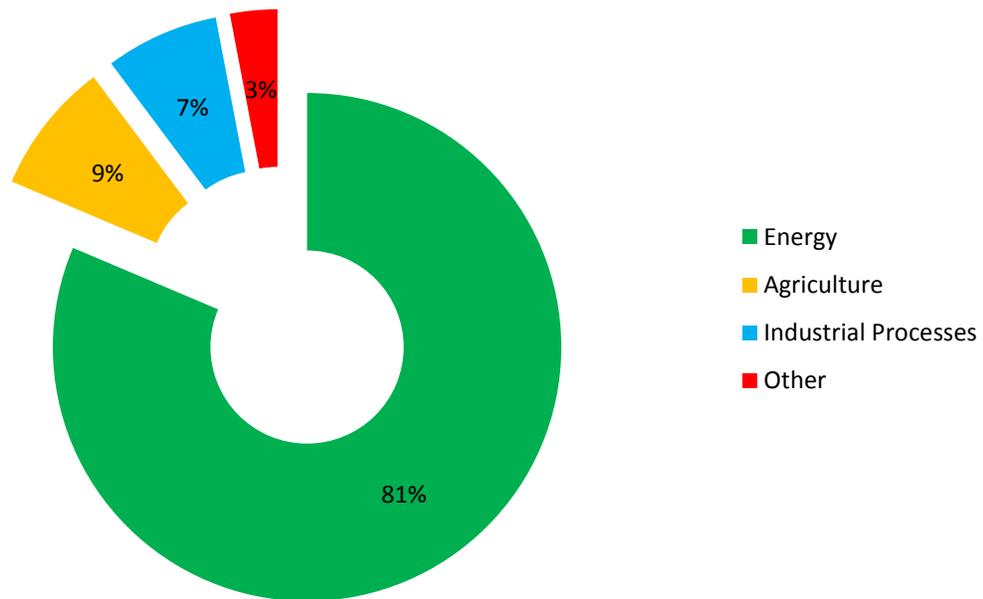
Globally, over 60 percent of total Methane emissions come from anthropogenic activities. According to Alberta Agriculture and Rural Development website, energy sector is the major generator of GHG emissions as compared to agriculture and other industrial processes (Alberta Agriculture and Rural Development, 2013).

According to Environment Canada, in 2008, agricultural emissions were 8.4 percent of the total 2008 GHG emissions for Canada. While energy sector in Canada contributed to about 81 percent of the total amount of GHG emissions (Environment Canada, 2012). In the oil and gas industry, methane is found along with petroleum and may exist as dissolved or solution gas. It releases during upstream operations such as hydraulic fracturing, drilling and production in form of fugitive emissions which are either vented or flared. Some emissions also take place during transportation, distribution and storage of oil and gas.

In the agricultural industry, a large amount of methane is emitted from the normal digestive processes of livestock such as cattle, buffalo, sheep and goats. Moreover, livestock excreta stored in lagoons or holding tanks is also a source of methane. In addition, “livestock use is mainly for human consumption, the resultant emissions are attributed to anthropological activities (USEPA, 2013).” In 2008, the agricultural industry contributed about 70 percent of total Canadian N₂O emissions and about 26 percent of total Canadian methane (Environment Canada, 2012).

In landfills, decomposition of waste produces methane and emissions from Canadian landfills account for 20 percent of national methane emissions (Environment Canada, 2012).

Canada's Greenhouse Gas Emissions by Industry Sector for 2008 (in carbon dioxide equivalents (CO₂e)).



Data Source: (Alberta Agriculture and Rural Development, 2013)

The above figure shows Canada's GHG emissions by sector.

Hydraulic fracturing

Hydraulic fracturing is an unconventional process of extracting natural gas from shale rock formations or coal beds. In this process, pressurized mixture of water, sand and chemicals is injected deep underground into shale rock formations or coal beds to release the trapped natural gas. This pressurized water mixture helps in creating and widening fissures in the rock to help methane gas escape from pores and fractures in the rock (Alberta Energy Regulator, 2013). With release of pressure, the injected water returns to the surface. This water likely contains methane and is a potential source of fugitive methane emissions. Another source of these emissions is leaks in equipment and infrastructure needed for hydraulic fracturing activities (Broomfield & Donovan, 2012).

A 2009 study by Alberta scientists Stephan Bachu and Theresa Watson found that “deviated wells (the same type right angling used for fracturing shale gas and tight oil formations) typically experienced leakage rates as high as 60 percent as they age. Moreover, high pressure fracturing increased the potential to create pathways to other wells, the atmosphere and groundwater.”

Methane Emissions

IPCC (2006) listed detailed sector split for emissions from production and transport of oil and natural gas:

Sector Name	Explanation
Oil and Natural Gas	Comprises fugitive emissions from all oil and natural gas activities. The primary sources of these emissions may include fugitive equipment leaks, evaporation losses, venting, flaring and accidental releases
Oil	Oil Comprises emissions from venting, flaring and all other fugitive sources associated with the exploration, production, transmission, upgrading, and refining of crude oil and distribution of crude oil products.
Venting	Emissions from venting of associated gas and waste gas/vapour streams at oil facilities
Flaring	Emissions from flaring of natural gas and waste gas/vapour streams at oil facilities
All Other	Fugitive emissions at oil facilities from equipment leaks, storage losses, pipeline breaks, well blowouts, land farms, gas migration to the surface around the outside of wellhead casing, surface casing vent bows, biogenic gas formation from tailings ponds and any other gas or vapour releases not specifically accounted for as venting or flaring
Exploration	Fugitive emissions (excluding venting and flaring) from oil well drilling, drill stem testing, and well completions

Source: (IPCC guidelines for National Green House Gas Inventories, 2006)

In oil fields, methane emissions are considered a problem and are therefore, burnt or vented directly into the atmosphere (Lavelle, 2012). Researchers at Cornell University, Robert Howarth and his coworkers are of the opinion that, “shale gas extraction through fracking causes enough emissions to give it a bigger greenhouse gas footprint than conventional gas or oil.” Howarth et. al. (2012) clarifies, "Unfortunately natural gas is mostly methane and methane is an incredibly powerful greenhouse gas...even small venting and small leakages add up hugely to the greenhouse gas footprint of this fuel."

Fugitive methane emissions associated with development of natural gas from conventional wells and from shale formations (expressed as the percentage of methane produced over the lifecycle of a well)		
	Conventional gas	Shale gas
Emissions during well completion	0.01%	1.9%
Routine venting and equipment leaks at well site	0.3 to 1.9%	0.3 to 1.9%
Emissions during liquid unloading	0 to 0.26%	0 to 0.26%
Emissions during gas processing	0 to 0.19%	0 to 0.19%
Emissions during transport, storage, and distribution	1.4 to 3.6%	1.4 to 3.6%
Total emissions	1.7 to 6.0%	3.6 to 7.9%

Source: (Howarth et. al., 2012)

The table on the previous page shows that the Emissions for conventional natural gas wells during completion are lower as compared to shale gas well completion. This could be because conventional wells do not have flow-back and drill out (Howarth, Santoro, & Anthony, 2012).

Natural gas is mostly methane; it emits less carbon dioxide when burnt as compared to other fossil fuels. It therefore, has a potential climate benefit of offsetting emissions from carbon intensive energy production. However, when raw methane leaks from wells, pipelines and storage facilities, it can turn the positive climate impact into a negative one. Methane does not stay in atmosphere for long like CO₂, but is more efficient at trapping radiation than CO₂. Moreover its Global Warming Potential is 25 times that of CO₂. Reducing the amount of fugitive methane emissions could potentially help natural gas to be a cleaner energy source compared to coal intensive energy production (Schueneman, 2013).

Mitigation methods

The most common mitigation methods and options for disposing of unmarketable natural gas include flaring/incineration, venting, underground injection or uses such as pipeline heating and electrical generation.

There are various circumstances where methane volume emissions are too low or if the operations in a remote location may not be economic, practical or safe to conserve natural gas. It may not be economical to build pipelines to process them. Therefore, these low emissions are typically flared or vented.

Flaring is the burning of natural gas in an open flame. However, flaring is a waste of valuable resources and produces emissions that can affect human health, livestock and the environment

(Bott, 2007) by causing local and regional air pollution. Combustion also emits carbon dioxide, a greenhouse gas that contributes to global warming.

Venting is the release of methane directly into the atmosphere. The quantities released may be small from individual sources, but cumulatively they are significant and can have an adverse environmental effect as the GWP of methane is 25 times that of CO₂. More direct methane emissions could play a major role in global warming as explained in the chapter on environment.

Alberta Regulatory Requirements

Alberta's energy regulator has yet to keep track of leaking wells in a rigorous or transparent fashion and agrees that, "While abandoned wells do not place the environment or public at significant risk, small leaks are possible. A well leak can be caused by many things, including corrosion, improper abandonment, and damage incurred during excavation" (AER, 2013). As mentioned by Alberta Energy Regulator in *Abandoned Wells and Potential Risks*, "Gas detection tests are used to identify any leaks and determine if any gases are present. These tests are conducted by the licensee and all results from the test are reported to the AER" (AER, 2013), in Alberta the industry remains largely self-regulated and companies voluntarily disclose to the regulator.

Bio-filter Application

In the past, the oil and gas industry has used bio-filter technology as a cost effective technology for removing environmental pollutants from contaminated gas streams (Yang & Minuth, 2002).

Methane Bio-filter Technology is relatively new; thus, it is difficult to use low volume methane emissions for generating electricity or for incineration. There is potential to apply this simple technology (MBT) to effectively reduce the low volume methane emissions.

It not only reduces the environmental impact but also has the potential to generate carbon offsets for the facilities using them. Since methane bio-filters do not have any movable parts, they are low maintenance and can be customizable for specific sites. One major requirement for them to be functional would be to have a collection system to collect the low volume methane to be passed through the bio-filters for conversion into less potent CO₂.

Hydraulic Fracturing at a glance

Application	Methane released	Alberta Regulation	Current mitigation methods being used
-------------	------------------	--------------------	---------------------------------------

			for CH ₄ emissions
Hydraulic Fracturing	<p>Routine venting and equipment leaks at well sites, liquid unloading, gas processing, transport, storage, and distribution</p> <ul style="list-style-type: none"> • About 5% of Alberta's 300,000 oil and gas wells now leak. • "Deviated wells" typically experienced leakage rates as high as 60 % as they age. 	<ul style="list-style-type: none"> • AER has yet to keep track of leaking wells in a rigorous or transparent fashion • Methane migration are routine items of "high risk noncompliance" that companies voluntary disclose to the regulator • In Alberta the industry remains largely self-regulated. 	Flaring Venting/ leaks

Solution Gas

Solution gas is defined by Alberta Energy Utilities Board (2006), as follows: "gas that is dissolved in the gaseous component of petroleum that is separated from crude oil after the recovery of petroleum from a well." It comes from several different types of petroleum production, ranging from light conventional oil to non-flowing bitumen (Rahim, 2004).

Fugitive Methane Emissions

The major concern for solution gas is how it is treated; the main methods of control today are venting and flaring. "In 2007, 672 million cubic meters (or 4.2%) of all solution gas produced in Alberta was flared or vented, which translates into a significant volume of GHG's released into the atmosphere" (Canadian Natural Resources Limited, 2009). These GHG emissions from flares and vents can cause negative health effects in humans and animals and contribute to rising global levels of greenhouse gas emissions in the earth's atmosphere (Clean Air Strategic Alliance, 2010). Moreover, any gas flared or vented does not provide any economic benefits (such as carbon offsets).

Mitigation methods

In Alberta, most of this gas (roughly 94%) is collected and processed for sale at gas processing plants (Canadian Natural Resources Limited, 2009). When it is not conserved, solution gas from light to medium crude oil production is flared, whereas solution gas from heavy crude oil production is vented (Rahim, 2004). According to CASA (2010), "Flaring is the intentional act of burning natural gas, including solution gas that is not used, captured or sold due to technical or economic limitations, as part of well testing, or in emergencies due to safety concerns". While "Venting is the intentional release of natural gas into the atmosphere. Venting has typically been

used to dispose of quantities of solution gas that are not economic to use and cannot be flared” (Clean Air Strategic Alliance, 2010).

In Alberta, there was a 72 percent reduction in solution gas flaring between 1996 and 2005, and 59 percent reduction in solution gas venting between 2000 and 2005 (Bott, 2007). “Changes in regulations, higher natural gas prices, new technologies and adoption of “best practices” by industry” resulted in these reductions (Bott, 2007). Most of the high volume solution gas that was earlier flared or vented is now conserved, processed and sold to customers or used in industry operations for electrical generation or to be re-injected with produced water (Canadian Centre for Energy Information, 2007).

In case of low volume solution gas emissions, flaring the gas is not economical. It requires equipment and energy to operate. Therefore, low volume methane is generally vented.

Alberta Regulatory Requirements

The Alberta Energy Utilities Board’s (EUB) Directive 060 is the most important regulation with respect to venting and flaring solution gas in Alberta. Through Directive 060, oil and bitumen extraction sites in Alberta are required to evaluate and monthly report their solution gas generation and are required to conserve a part of it (EUB, 2006). Providing emission offsets for solution gas conservation in these cases would provide an incentive to project developers to reduce GHG emissions through the capture and conservation of solution gas (Canadian Natural Resources Limited, 2009).

According to EUB directive 060 (2006), solution gas flaring limit in the Alberta is 670 million cubic metres (10^6 m^3) per year. It is 50 percent of the revised 1996 baseline of $1340 \times 10^6 \text{ m}^3/\text{year}$.

If solution gas flaring exceeds the $670 \times 10^6 \text{ m}^3$ limit in any year, the EUB will impose reductions that will set maximum solution gas flaring limits for individual operating sites on the basis of the most recent annual data to reduce flaring to less than $670 \times 10^6 \text{ m}^3/\text{year}$ (EUB, 2006).

Bio-filter Application

In 2007, 95.8 per cent or 15,328 million cubic meters of solution gas were conserved; the remaining 4.2 per cent (or 672 million cubic meters) of solution gas was flared or vented (Canadian Natural Resources Limited, 2009). If an assumption is made that none of the solution gas vented or flared in 2006 was required to be conserved by regulation, there is potential for mitigation of approximately 5 Mt CO_{2e} through conservation of solution gas that is not required by Directive 060 (Canadian Natural Resources Limited, 2009).

Since the collection systems are in place for solution gas, Methane Bio-filter Technology has a potential to mitigate the low volume emissions that are flared or vented. It is cheaper than flaring, is within the regulations, and provides offsets.

Solution Gas at a glance

Application	Methane released	Alberta Regulation	Current mitigation
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<p>Solution Gas</p>	<ul style="list-style-type: none"> • Major concern: gas flaring and venting • Since 2005, Solution gas flaring was reduced by 72% 	<ul style="list-style-type: none"> • EUB Directive 60: “Upstream Petroleum Industry Flaring, Incinerating, and Venting, the Oil and Gas Conservation Regulation, and the Pipeline Regulation” <p>(Recommendations from CASA in 2002, 2004 and 2005)</p> <p>S.2.1</p> <ul style="list-style-type: none"> • The Alberta solution gas flaring limit is 670 million cubic meters (10^6 m^3) per year (50% of the revised 1996 baseline of $1340 \cdot 10^6 \text{ m}^3/\text{year}$) • If solution gas flaring exceeds the $670 \cdot 10^6 \text{ m}^3$ limit in any year, the AER will impose reductions that will stipulate maximum solution gas flaring limits for individual operating sites 	<ul style="list-style-type: none"> • Venting and flaring <p>Alternative technologies for large volume emissions::</p> <ul style="list-style-type: none"> • Low Pressure Gas Collection • Electrical Generation Using Gas Turbines or Reciprocating Engines • Electrical generation using "mini" gas turbines • Cogeneration • Re-injection with produced water • Oxidation • Collection and Processing
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Livestock

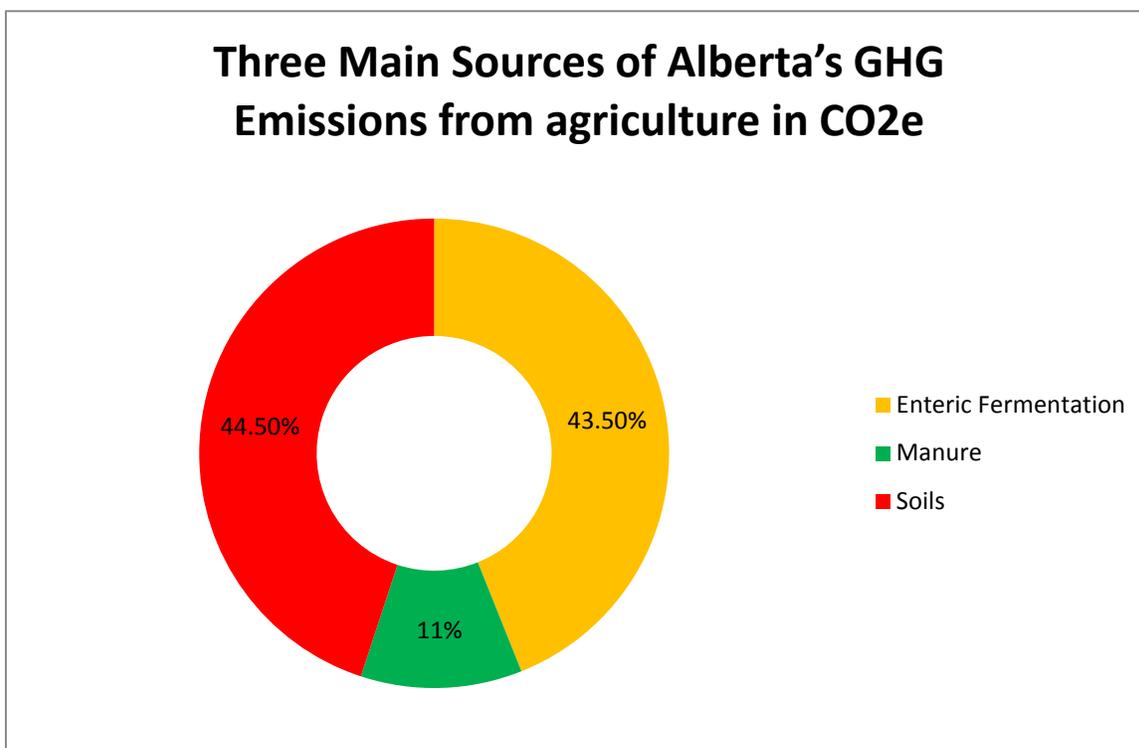
Livestock is defined in the Merriam- Webster (2013) online dictionary as “animals kept or raised for use or pleasure; *especially*: farm animals kept for use and profit.”

Alberta is the largest beef producing province in Canada and as of July 1, 2012, it accounted for 40.0per centof the national herd. In addition “nearly three-quarters of Canadian beef processing happened in Western Canada (72.4%), with the vast majority concentrated in Alberta” (Alberta Agriculture and Rural Development, 2013).

The current trend is that the livestock animals like cattle and pigs are reared in large intensive farms in confined feeding operations (CFOs) in the developed world. When the animals are raised in a controlled environment, it maximizes their growth potential and minimizes production and labor costs. Intensive farming has its own drawbacks when it comes to greenhouse gas emissions. Since these operations are large, even low volume emissions from normal digestion of farm animals such as cattle along with the manure management can leave a notable carbon foot print.

Methane Emissions and mitigation methods

The three main sources of Alberta’s GHG emissions from agriculture as reported by Alberta Agriculture and Rural Development (2013), were 44.5 percent from enteric fermentation 11 percent from manure and 44.5 percent from soil.



Data source: (Alberta Agriculture and Rural Development, 2013)

The above figure shows the breakdown of the emissions from enteric fermentation, manure and soils in Canada.

When ruminants, that is animal with fore-stomach such as cattle, sheep and goats, and digest feed it, it goes through an anaerobic microbial process called enteric fermentation, which results in the formation of methane. The quality and quantity of the feed play an important role in quantity of methane emissions. Diet manipulations can be done by feeding higher quality feeds and balanced rations, feeding ionophores, feeding lipids or adding bacterial supplements to the herd feed (Alberta Agriculture and Rural Development, 2013). These diets would increase the rate of digestion, and the feed will spend less time being digested in the fore-stomach, thus reducing the level of methane produced by enteric fermentation (Alberta Agriculture and Rural Development, 2013).

Another major source of methane from livestock operations is the manure that animals excrete. The increasing density and size of feedlots in North America and Alberta, along with confined feeding operations have resulted in the production of large, concentrated quantities of manure that is not easy to dispose of (Alberta Agriculture and Rural Development, 2013). Since manure is organic in nature, it decomposes fast, converting the carbon in the waste to either carbon dioxide or methane. In the presence of oxygen, decomposition will release the carbon in the manure as carbon dioxide, but in lack of oxygen it generates substantial quantities of methane. Intensive livestock operations with a liquid manure storage system such as swine operations use water to wash the manure from the barns into outdoor lagoons (Langmead, 2003). These swine slurry lagoons are stagnant and because of the lack of available oxygen in the liquid manure, anaerobic decomposition takes place, emitting large quantities of methane. Similarly, in solid manure storage system, such as at cattle feedlot, most of piled manure too decomposes under anaerobic conditions and produces methane (Langmead, 2003).

A study 'Bio-filtration of methane at low concentrations representative of the piggery industry' in the Chemical Engineering Journal, states that

In Canada in 2008, swine slurry management was responsible for the release of 1.3 million metric tons of CO₂ equivalent CH₄. Methane concentrations ([CH₄]) from covered slurry storages with no aeration can reach 425 gm⁻³ (65%, v/v), but storage covers are rarely airtight and concentrations usually vary from 0.1 to 20 gm⁻³ (150–30,600 ppmv).

Mitigation of methane emissions

Methane's global warming potential is 25 times that of carbon dioxide and can therefore cause much more harm to the environment than carbon dioxide. Traditional manure management techniques, such as the direct application of beef cattle manure to agricultural land or the collection of swine manure in open lagoons are no longer applicable due to intensive and confined livestock farming which produce large quantities of manure, which is not easy to manage. Moreover, they have social and environmental problems associated with their use (Langmead, 2003). Many new and advanced technologies such as thermal de-polymerization, ultrasonic plasma resonator systems and up flow bio-filtration are being tested to reduce the environmental impacts of livestock waste. It remains to be seen if they can reduce GHG emissions while enhancing the economic performance of the livestock industry (Langmead, 2003).

To mitigate methane emissions from livestock manure management, it is possible to collect the methane gas and flare it. However, flaring methane may need expensive equipment and skills to operate. Additionally, the methane content of the emitted gas needs to be high enough to allow effective combustion.

The use of bio-digesters is another method to decompose manure under controlled conditions and produce high volume methane, which could be further used as energy to run turbines and produce electricity or heat.

Bio-filter Application

Low volume methane emissions are uneconomical to flare and have adverse environmental effects when simply vented. Methane bio-filters which convert methane to carbon dioxide (CO₂) and water by methanotrophic (methane consuming) bacteria grown in the bio-filter media can successfully mitigate low volume methane emissions from manure management (Huang et.al., 2011). With Alberta's new legislation for agriculturalists to benefit from reducing greenhouse gases, bio-filters could play an important role as simple, low cost and low maintenance technology to earn CO₂e methane offsets.

Alberta Regulatory Requirements

Alberta was the first in North America to introduce a legislation that gives agricultural producers an opportunity to reduce greenhouse gas emissions and benefit from it too. It is called the Alberta Offset System (Alberta Agriculture and Rural Development, 2012). To qualify and earn carbon offsets, producers need to follow a number of rules. These rules include documenting improvements to changes in their livestock practices (Alberta Agriculture and Rural Development, 2012). Documenting “practices for the periods both before and after they adopt emissions-reducing practice changes. This is critical not only to earn offsets, but to protect producers from liability if there is any challenge to the carbon offset credits they are claiming” (Alberta Agriculture and Rural Development, 2012). The offset credits they earn can be sold in the growing carbon offset market. The Alberta Offset system gives producers an incentive to reduce greenhouse gases while benefiting from the emerging carbon offset market (Alberta Agriculture and Rural Development, 2012).

Livestock industry at a glance

Application	Methane released	Alberta Regulation	Current mitigation methods being used for CH ₄ emissions
Livestock	<ul style="list-style-type: none"> - Alberta has 6.6M cattle (43% of the national herd) - The livestock production contributes approximately 11% of GHG emissions in Canada, mainly in the form of CH₄ and N₂O and N₂O - 8% from enteric fermentation - 3% from manure management - Manure is disposed of rather than used as fertilizer for crop production 	<ul style="list-style-type: none"> - Alberta's new legislation in North America gives agricultural producers ways to benefit from helping to reduce greenhouse gas emissions - The Alberta Offset System: includes protocols producers can follow in order to earn carbon offsets - These may be sold in the growing carbon offset market 	<ul style="list-style-type: none"> • Diet manipulation • Animal production management • Manure storage - Composting - Land application - Bio-energy production

Landfills

A landfill is a method of waste management, which is an excavated site that is specifically designed and built to receive and store waste. A landfill in this context does not include open dumps, which are now less prevalent in North America (though this is not the case in developing countries around the world). A landfill could also be specially engineered to enhance waste decomposition through manipulation of specific conditions such as leachate recirculation; this is known as a Bio-cell Reactor.

Waste diversion and recycling is also another method of waste management, which is more prevalent in North America and Europe, and enhances the biodegradation factor in landfills. An example is the waste diversion plan in the City of Calgary looking to go from diverting 20 percent of the waste to 80 percent by 2020 (The City of Calgary, 2013). The removal of non-biodegradable waste increases the bio-degradable content, thus increasing the rate of degradation. The diversion of recyclable materials from landfill will also give an additional advantage of reducing the rate at which landfills are filling up, increasing the life-span of the landfills.

There are different classifications of landfills, which include hazardous waste landfills, municipal solid waste MSW landfills etc. This research focuses on MSW landfills; waste items such as paper, plastic, metal, food waste, yard waste, and other wastes from residential, commercial, construction and some industrial sources can be disposed of in MSW landfills.

A portion of items disposed of in a landfill such as food and yard waste - also known as bio-waste or organic material; are biodegradable with a shorter half-life; though some are broken down at a faster rate than others. The rate of biodegradation is highly dependent on the

composition of waste. The composition of MSW varies with location, and is influenced by various factors including climate (rainfall, temperature etc.) time of year, local socioeconomic conditions etc. A landfill with a higher percentage of bio-waste in a hotter and wetter region will have a faster rate of biodegradation than one in a dry and cold climate.

The rate of degradation in landfills is affected by a number of factors, including:

- Composition of waste (as noted above), which may vary from country to country
- Microbial growth and activity which is influenced by factors such as moisture, available nutrients, pH, temperature
- The design and operation of the landfill (e.g. leachate recirculation) which may impede or enhance waste decomposition (Gwande et al., 2010)

The biodegradation process, involves the breaking down of organic material through chemical processes from complex molecules into simpler molecules. The key environmental threat from biodegradation in MSW is the production of methane during decomposition. The waste sector is a significant contributor to greenhouse gas (GHG) emissions accountable for approximately 5 percent of the global greenhouse budget (IPCC, 2006). It is important to note that approximately equal volumes of Carbon dioxide and Methane are produced during biodegradation. The Carbon dioxide produced is considered biogenic (occurring from natural processes) and therefore it is not considered in this case.

Methane Emissions

The production of methane occurs due to the anaerobic nature of landfills. Waste is stockpiled such that air/oxygen flow is limited. This creates the anaerobic condition that fosters biodegradation of the organic waste and the production of methane. With this, a higher rate of gases will be released during biodegradation. The decaying waste uses up the oxygen within the waste, thus creating anaerobic conditions. The anaerobic bacteria (methanogens) flourish, in turn increasing the rate of biodegradation and subsequently the production of Land Fill Gas (LFG) i.e. methane (Cornell Waste Management Institute, 2009).

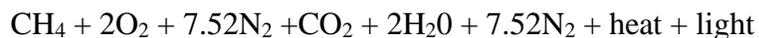
LFG can be used for electricity generation at higher volumes to be economically viable; which makes it an excellent potential marketable resource. Capturing LFG reduces the volume of methane released into the atmosphere and also offsets methane emitted from other sources of electricity generation e.g. fossil fuels. A collection system of wells with an adequate metering/measuring system is required to efficiently extract LFG. An example is the Shepard Landfill Gas Capture and Combustion Offset Project in Calgary, with an LFG flow of between 80 – 189 m³/day with an installed generating capacity of 400 kWh (City of Calgary, 2012).

Mitigation methods for low volume methane released

LFG at lower volumes 0 – 100m³/day are not economically viable for power generation. Presently at lower volumes LFG is vented or flared.

Traditionally, uncovered landfills release LFG into the atmosphere. Venting makes it impossible to measure how much methane is being released into the atmosphere. As climate change issues become more prominent, venting is becoming more socially unacceptable encouraging the evolution of landfill design, operation and management. This also benefits landfill operators in increasing efficiencies and making venting less attractive. Existing landfills are being retrofitted with LFG collection systems, which are capital intensive. New landfills are now being designed and engineered with LFG collection systems. This helps in efficient management and control of LFG and other emissions.

For LFG to be flared, a collection system (which is a series of wells) is required as well as an engine. Flares use LFG as the combustion fuel. For complete (theoretical) combustion, the stoichiometric ratio of air to CH₄ is 9.52:1. The basic combustion reaction is:



Theoretical and actual conditions differ, causing a variation in combustion reactions. Excess air in the flare system causes an oxidizing mixture; not enough air causes a reducing mixture, which results in incomplete combustion of methane and unwanted by-products such as carbon monoxide, non-volatile organic compounds, other contaminants present in air (Cornell Waste Management Institute, 2009).

Open flares were common in landfill operations, but presently European countries such as Ireland has made installing enclosed flares mandatory as a condition of issuing waste licences, to improve emissions control (Cornell Waste Management Institute, 2009). The standard in Clean Air Act in the United States prohibits venting in landfill operations. The methods of compliance are a minimum of a flaring system and up to LFG electricity generation. (US EPA, 2012)

Alberta's Regulatory Requirements

In Alberta, there is presently no regulation for reducing methane emitted from landfills. Landfill operators with waste to energy facilities have done so voluntarily with the aim of selling carbon credits. Unlike in the United States, in Ireland, where venting is prohibited, it is mandatory to install emission management facilities; a minimum of flares up to electricity generation facilities (USEPA, 2013).

Bio-filter Application

Bio-filter application is viable in the landfill sector. According to a case study carried out in the University of New South Wales (2006) "it was found that greater than 90 percent of methane and 97.5 percent of odour can be removed from landfill gas ... recycled materials such as compost can be used successfully in the bio-filtration system" (University of New South Wales and GHD Pty, 2006).

Landfill at a glance

Application	Methane released	Alberta Regulation	Current mitigation methods being used for CH ₄ emissions
Municipal Solid Waste Landfills	Anaerobic nature of landfills produces methane and carbon-dioxide in equal volumes with other trace gases e.g. N ₂ O	<ul style="list-style-type: none"> Climate Change and Emissions Management Act: Specified Gas Reporting Regulation and Specified Gas Emitters Regulation: 100,000 tonnes of CO₂e threshold per year 	<ul style="list-style-type: none"> Routine venting, equipment leaks and flaring Electricity generation is only viable for LFG flows at least 100 m³ per day Voluntary installation for flaring alternatives mainly done for carbon credits Facilities with lower volumes of LFG flare or vent between 50-100m³ per day Only way to flare is with collection systems

CHAPTER FOUR

Economics: Industry Analysis and Strategy

According to Climate Change and Emissions Management (CCEMC) Corporation and Alberta Innovates Bio Solutions estimation, the Bio-filter Technology has the “potential to reduce greenhouse gas emissions by about 2,000 tonnes over 10 years. The estimated ten year (2013-2022) market emission reductions that can be expected from this technology, anticipating market adoption starting in 2016 at a 1 percent adoption rate is: 130,000 tonnes CO₂e.”

An analysis of the broad industry is important to fully understand the likelihood of success for the technology and the project. This section begins with a Political, Economic, Socio-cultural, Technological, Environmental and Legal (PESTEL) analysis that currently impact, and may affect the MBT, and the industry in the future. The PESTEL reveals that regulatory requirements, greater appetites for environmental sustainability, and the need to prove the technology on the commercial scale are important for the success of the project.

The detailed PESTEL analysis is followed by a Porter’s Five Forces evaluation. This examines the industry in which technology and the project exists. Given that the MBT is early in its development and commercial application, the Five Forces analysis concludes that buyers have significant purchasing power, rivalry among existing competitors is low, selling power amongst suppliers is not significant, the threat of substitutes potentially exists, and the threat of new entrants is high as the technology can be replicated in some form.

Following the Porter’s Five Forces examination, a Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis provides a holistic examination from the internal and external perspectives of the project. The project benefits by being small and nimble, with low overheads,

and good personal relationships in the industry; however, the technology has little market presence or industry reputation at this time, which may result in unstable cash flows for funding purposes. From an external perspective, there are many opportunities that can be capitalized. For example, proposed new regulations (e.g. 40/40 plan) may create greater demand to manage and reduce the emission produced from methane.

As the technology is ready to be marketed to the industry it is recommended that the project takes action to create interest in the technology, the project and its key employees. Small success stories with pilot projects will help escalate the technology from proof-of-concept to commercially tested and proven. The MBT is a form of sustainable innovation, which can improve existing industries that emit methane pollution with a more technological, environmental, and economical efficient process.

PESTEL Evaluation

A PESTEL analysis identifies external forces that may hinder or aid with the bio-filtration project at both macro and micro levels. The analysis examines Political, Economic, Socio-cultural, Technological, Environmental and Legal forces that may come into play when looking to penetrate into the treatment of low-volume, point-source methane in Alberta. These forces can create both opportunities and threats for an organization. Thus, the aim of this analysis is to identify the current factors affecting the MBT project, the expected changes in the external environment, and how to exploit those changes or defend against them. The following table presents a summary of the PESTEL evaluation, which is followed by a more detailed explanation of each category examined and their direct impacts on the project.

PESTEL Summary Evaluation

Political	
Heading	Details
Conservative Government	Current government is in support of large oil sands projects and allocating funds towards those operations (e.g. \$1.3 billion for CCS projects over next 15 years). Funds for alternative methods to reduce GHG emissions are not as well funded.
Alberta's Policy for Climate Change	Global pressure is falling on Alberta to reduce GHG emissions, especially from oil sands operations.

Regulatory Requirements	There may be potential increases of taxes/charges/fines on emissions given external pressures and changes in the internal political environment.
40/40 Plan	Potential of the new 40/40 reductions plan to be introduced in 2014. This will give the project significant leverage as companies will need to meet the new standards, and switching to the MBT technology will be more cost efficient.
Economic	
Heading	Details
Taxes/Charges/Fines	Emissions need to be reduced to ensure companies can keep up with changing standards and save on costs. This will be an advantage for the project.
New Technology	There is always an added cost for testing and operating new technology. This may be an issue if enough seed/feasibility funding is not available.
Job Creation	The new technology can potentially create new jobs, as trained people will be required to operating the MBT. Government programs are available that provide job salary subsidization for small companies.

Carbon Credit Trading	This will come to a great selling point for the project as clients will be able to get carbon offset credits through use of the technology.
Socio-Cultural	
Heading	Details
Greater Awareness	Climate change has been brought to public's attention. Public environmental awareness can translate well into business opportunities.
Changing Culture/Attitudes	Many groups are demanding companies to make more environmentally protective decisions when it comes to their operations. This shift in thought will be of great advantage to the project as it can be leveraged as a selling feature for potential clients. Companies are typically willing to change operations if it aligns with the interest of shareholders and general stakeholders alike.
Safety Concerns	Global climate change is becoming a greater safety concern. Impacts of climate change such as shrinking glaciers, and shifting animal ranges have already been observed. NASA scientists predict that future effects like frequent wildfires and longer droughts can have significant, detrimental consequences for the safety of the future global population.

Corporate Social Responsibility (CSR)	As CSR becomes an increasing common topic on corporate agendas, using new technologies to reduce costs and detrimental impacts on the environment will be more commonplace. Specifically, using MBT will allow companies to become more responsible in taking care of their emissions.
Alberta Oil (“Tar”) Sands	There is significant of scrutiny for Alberta being a large emitter of pollutants. Opponents have come from local areas, but increasing are from other areas of the world. While the Alberta government is taking steps to market the Oil Sands to the world (e.g. “remember to breathe” campaign), initiatives like MBT will show the world that Alberta is working to become more environmentally friendly.
Educating the Industry	As MBT is a new technology, effort will need to be allocated to ensure industry understands the uses, effectiveness, costs, and impacts of the technology. Strong marketing is required.
Technological	
Heading	Details
New Technology	As with all new technologies, a series of phases occur as the technologies are accepted and adopted by consumers. These are explained by the Rogers’ Bell Curve and Hype Cycle.

<p>Not Yet Tested on a Commercial Scale</p>	<p>The technology needs to be proved on a commercial scale to be effectively marketed to large consumers. As indicated by the Hype Cycle, as evidence grows of the technology's effectiveness, companies will have greater interest in learning more and potentially purchasing the technology.</p>
<p>Research & Development (R&D)</p>	<p>R&D for the technology needs to continuously occur. This may include adjustments to the process to best accommodate various input sources. A greater understanding is also needed of the actual amount of low-volume methane, as it is currently unaccounted for or recorded.</p>
<p>Industry Acceptance of New Technology</p>	<p>As certain consumers are offered the technology it will gain a reputation of credibility. It is very important that impacts of the technology are measured so consumers can understand the results of their investment.</p>
<p>Environmental</p>	
<p>Heading</p>	<p>Details</p>
<p>Pollution</p>	<p>Significant amounts pollution results from methane such as underground water contamination and air pollution as it has 25 times the global warming potential as compared to carbon dioxide.</p>

Current Treatments	<p>Industry typically uses flaring to deal with methane leaks.</p> <p>Flaring however releases energy, creates pollution, and poses safety concerns. Venting is also used, but is also detrimental to the environment, as methane is not broken down to carbon dioxide.</p>
Legal	
Heading	Details
Alberta Environmental Protection and Enhancement Act (formally Clean Air Act)	<p>Supports and promotes the protection, enhancement and wise use of the environment. The Clean Air Act allowed the Environment Minister to make a regulations relating to air quality standards, emissions standards, enforcement procedures monitoring methods (Environment and Sustainable Resource Development, Government of Alberta, 2013).</p>
Canadian Environmental Protection Act	<p>"...respecting pollution prevention and the protection of the environment and human health in order to contribute to sustainable development" (CEPA, 2012).</p>
Alberta Climate Change and Emissions Management Amendment Act	<p>Alberta first law to impose greenhouse gas cuts on (~100) large industrial facilities.</p>

EUB Directive 060	Provides requirements and guidelines for flaring, incinerating, and venting gas.
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Political

The current political government in Canada plays a big role as to how projects such as the MBT project will succeed. The Conservative Party of Canada, who are in support of expanding and growing the economic benefits of oil sands operations, are willing to invest in the sector and develop accommodative policies that will allow industry to operate. This role of the government can have both positive and negative impacts on the project.

First, the negative impact will be that of policy development and changes in favour of the industry. In this case, emissions standards will not be stringent and the industry may easily be able to vent/flare or continue with current operations without further development of improving current emissions reductions operations. However, the positive role that the government could also play is that of policy development that directs industries onto a course where more stringent regulations are enforced. This will allow for example, small-scale projects with high potential to be financed via technology funds from the industry to develop alternative methods to treat low volume emissions.

Canada's current policies for climate change are also receiving much global pressure for the amount of emissions that are produced, particularly through Alberta and its oil sands operations. This is a great benefit to the project as currently there is a lack of significant regulations in place to reduce emissions in the province. This will be of great benefit to the project as the MBT can leverage this disadvantage in the industry, and market and tailor the technology towards the

industry to help improve Alberta's image by showing that the province is taking measures to reduce emissions.

This project would also be a great marketing avenue for the current government, as it continues to lobby for the United States approval of TransCanada's Key Stone XL pipeline. Should this be applied on a commercial scale as planned, the industry would be able to buy and offset carbon credits if companies are not able to develop mechanisms immediately. This would also protect companies in the industry from any potential or anticipated regulatory changes.

There have also been talks of a new plan to be proposed by the Environment Minister of Alberta, Diana McQueen, to introduce a "40/40" plan, which would increase Alberta's intensity based emissions target and its carbon price in 2014. If this new plan were to go through, this would be very beneficial for the MBT project as many companies would struggle to meet its goals and would need to turn to something that would be able to meet the timeline of the plan. In this case, the MBT project would be a great selling feature as it is developed and could easily be implemented to an existing facility.

"40/40" Plan Proposal

The "40/40" plan, introduced by Alberta's Energy Minister, Diana McQueen, is a new proposal which take a more stringent approach to control Alberta's current emissions. The new "40/40" approach would increase the current target of twelve percent to forty percent and would also raise the technology fund from its current price of \$15 per tonne to \$40 per tonne as early as 2014 (Dyer, 2013). This would exemplify a hefty rise from the current standards. Most climate change policies in Canada are introduced relatively slowly, accompanied by steady increases in stringency over time. As a result, companies would have time to gradually adapt to the changes

and develop efficient methods to adhere to changes. However, with little time to adapt to this new policy, companies would struggle to meet these new changes and have contingency plans to ensure they are on track. Though Alberta has yet to announce the full details of the “40/40” proposal, it would still mean a stronger incentive for companies to cut emissions over the short term horizon.

New climate regulations such as the “40/40” plan would be beneficial for the success of the MBT project. Companies who will surely be unable to make significant changes to meet proposed changes by next year will be enticed with a easy and affordable solution to cut emissions with the MBT.

Economic

The external economic factors play a significant role of the project, as the MBT project is heavily reliant on funding in order to operate and develop further. Currently in place, there are standards set by Climate Change and Emissions Management Corporation (CCEMC) where companies are taxed, charged or fined if they do not adhere to current standards. Thus, emissions need to be reduced to ensure companies can keep up with changing standards, and at the same time reduce costs. This will be of great advantage to the MBT project as the technology is cost efficient and easily constructible with few added costs.

However, as this is still a new technology, there is always room for improvement and added costs when it comes to testing and operating the technology. If enough funding is not available for the project, it will be difficult to follow through on the project at a commercial scale to test the large scale feasibility of the technology.

Should the technology becomes accepted and used by industry, there will likely be potential new job creations as trained and skilled people will be required to build and operate the technology.

Socio-Cultural

The current socio-cultural trend in today's society plays a big role as to how industry will gain acceptance and have social licenses to operate in Alberta's backyard. Recently, there has been a shift in how people are viewing their environmental surrounding and are more aware of how and what needs to be done to protect their surroundings. For example, climate change has been brought to many people's attention and the public is demanding companies to make more environmentally friendly decisions when it comes to their operations. These social values will be of great advantage to the project as it can be leveraged as a marketing feature for potential clients. More and more companies are becoming aware of their emissions footprint and expected to change processes to meet public expectations.

Recent increased safety concerns for global warming and global climate change is of great concern around the world as all large emitting countries are being pressured to make changes to meet future reductions goals. The use of the MBT would be a selling feature to both industry and bring Canada a step closer to meeting its emission reduction goals.

The use of MBT will allow companies to become more responsible in taking care of their emissions and would also deem to be adhering to their corporate social responsibility to their shareholders. There is significant global scrutiny for Alberta being a large emitter. Small steps like MBT will show the world that Alberta is trying to become more environmentally friendly.

However, as MBT is a fairly new method of reducing methane, educating the industry on its use and benefits would be crucial for industry acceptance and the technology's success.

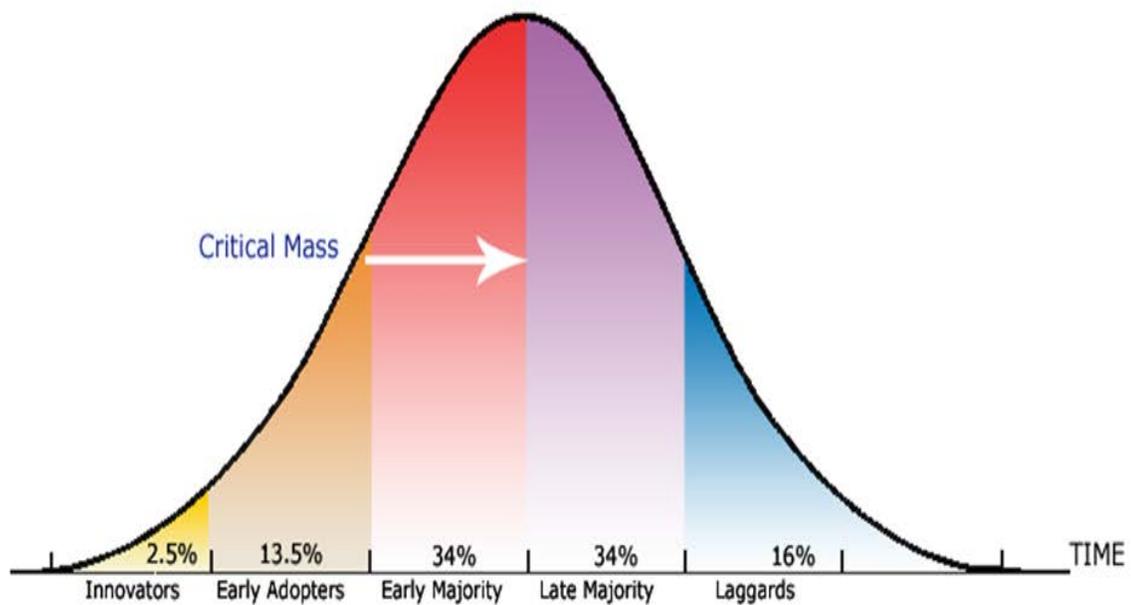
Technological

Technological adoption has been studied at great length by practitioners and academics alike. As the project involves a new technology, it is important to clearly understand the typical path to awareness, acceptance and adoption so that the project can effectively market the technology to target audiences. Two common frameworks are presented below.

Rogers' Bell Curve

Developed in 1957, the Roger's Bell Curve has been used to explain the adoption lifecycle for decades. It begins with "innovators" who are highly risk-orientated and are very willing to try new things. "Early adopters" follow this group. These also have a strong affliction to risk, but have less capital to make purchases. Should the technology be successful, the early and late majority follows these early groups. The highest numbers of consumers fall within these two categories. As illustrated in the following figure, the last group to adopt the new technology is the "laggers", representing approximately sixteen percent of all consumers.

Roger's Bell Curve: Diffusion of Innovation Adopter Categories



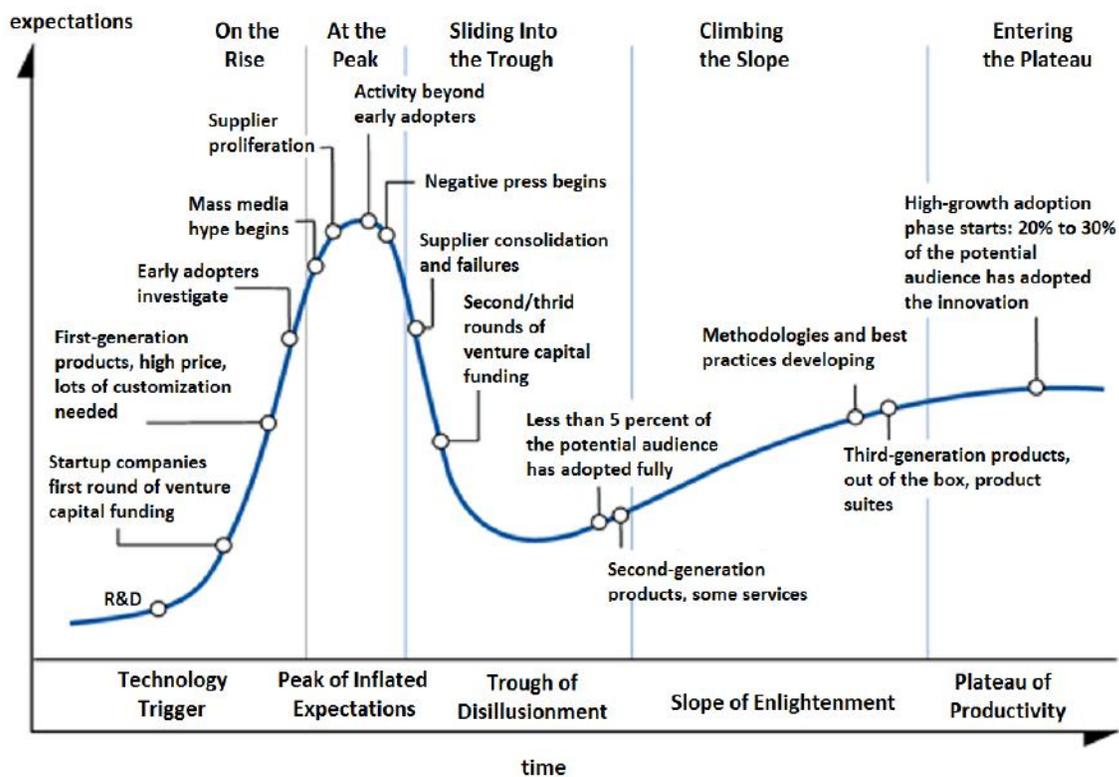
(Kaminski, 2011)

Hype Cycle

Originally created for the Information Technology (IT) by advisory firm, Gartner, the Hype Cycle illustrates the adoption of specific technologies. The Hype Cycle contains five phases, beginning with a proof-of-concept of a new technology, or “technology trigger”. MBT has passed this phase of development. This is followed by expectations of the technology where a number of success stories are produced. At this time, the project’s technology is in this phase.

Some companies may take action to learn more about the technology, but others will not. The next phase, called “trough of disillusionment” is where subsequent rounds of venture funded are invested into the project. At this time, investment continues only if the project improves the technology to the satisfaction of early adopters. The fourth phase, referred to as “slope of enlightenment” includes more cases of how the technology benefits consumers and more consumers fund pilot projects. This phase may include large-scale energy companies trying MBT. The final phase, “plateau of productivity”, is when mainstream adoption starts to take off.

Gartner's Hype Cycle



(Gartner Inc., 2013)

The main component of the project is the effectiveness of the MBT in Alberta's industry. As it is a new form of technology and has yet to be tested on a commercial scale, there are bound to

be barriers on the way to development. One of the main issues the project faces is its reliance on existing collection systems. Technological issues such as this need to be addressed in order to market this technology more reliably and gain industry acceptance.

Another concern is that there is lack of research and development in the industry when it comes to known data. This research and development need to occur to address methane in the industry a significant amount of low-volume methane is unaccounted for or recorded.

Environmental

The MBT project will be of great value to the protection of the environment. A large amount of pollution occurs from methane. This results in underground water contamination and air pollution, as methane has 25 times the global warming potential compared to carbon dioxide. As flaring gas requires energy, and this is what industry is typically doing to reduce methane leaks, the MBT would be a much more effective alternative to flaring and saving on energy costs. Another common method also used is venting, which is also bad for environment as methane is not broken down to carbon dioxide and has a more drastic effect for the increase in global warming. By switching to the MBT, both industry and government can favour environmental protection.

Legal

There are many legislation and regulations, which will help promote MBT to market the uses and meet legal standards or go above and beyond industry standards. This will also help companies to gain intangible statues with their shareholders to support their views of

environmental conservation (for the list of regulations, please refer to PESTEL Summary Evaluation).

CCEMC and Alberta's Emissions Reduction Goals

At the moment, Alberta's industries, including, coal, oil and gas, and other large industrial operators have set a target since 2007 to improve the emissions intensity in the industry. This is measured in emission per unit of production. The target is to reduce emissions by twelve percent in relation to the baseline performance for particular operating facilities (Dyer, 2013). But, if in any case, companies are unable to meet these targets by improving their operational performances, they can pay into a technology and management fund at the rate of fifteen dollars per tonne, or purchase carbon credits to offset emissions (CCEMC, 2012).

The Climate Change and Emissions Management Corporation (CCEMC), created in 2007, is Alberta's main organization for climate change strategy and movement toward a sturdy and more varied lower carbon economy. The CCEMC is an independent organization, which maintains and builds on the strategic course established in Alberta's 2008 Climate Change Strategy (CCEMC, 2012). CCEMC also recognizes the route set by Alberta's Carbon Capture and Storage Development Council and looks to harmonize decisions made on large carbon capture and storage projects. The CCEMC receives money from the Climate Change and Emissions Management Fund and directs it towards innovative projects that will reduce greenhouse gas emissions such as the MBT project.

As of now, Alberta's target is to reduce emissions by 200M, or fifty percent below business as usual, by 2050 (CCEMC, 2012). By doing so, there will be growth in sustainable jobs and an economic shift to green energy production, which in turn will enhance Alberta's competitiveness.

CCEMC has priority areas for funding that are aligned with Alberta's Climate Change Strategy and include:

- Conserving and Using Energy Efficiently (emissions target of 24MT by 2050)
- Implementing Carbon Capture and storage (emissions target of 139MT by 2050)
- Greening Energy Production (emissions target of 37 MT by 2050)

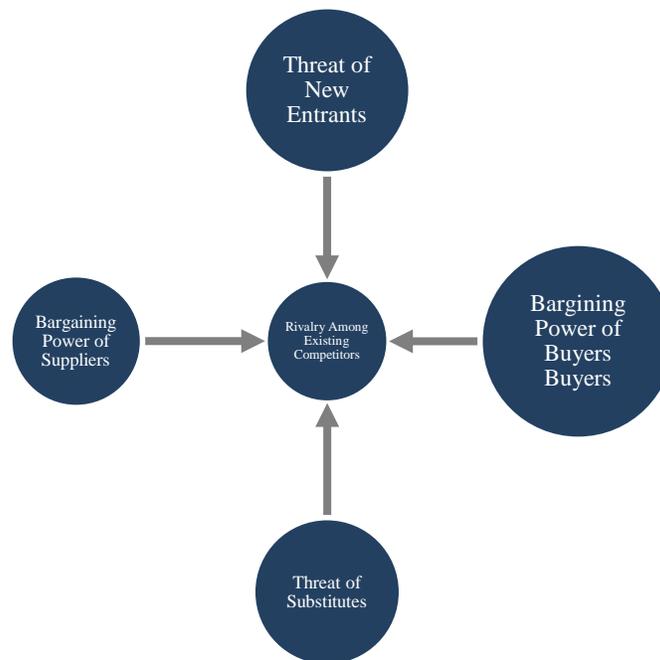
(CCEMC, 2012)

This project will be looking to receive more funding from CCEMC to test the technology in a commercialized scale. Projects submitted for funding consideration are subject to a thorough multi-step processes that will ensure that the funds entrusted to the CCEMC are invested in accordance with its mandate to reduce greenhouse gases and invest in a balanced portfolio of projects that offer transformative solutions (CCEMC, 2012).

Five Forces Analysis

The five forces model of analysis was developed by Michael Porter to analyze the competitive environment in which a product or company operates. There are five forces that act on any product, brand, or company, in this case the Methane Bio-filtration project. The forces, and their relative impacts (represented by their size), are illustrated in the following figure.

Porter's Five Forces for MBT Industry



First, the *threat of entry* examines where competitors can enter from any industry, channel, function, form or marketing activity. For this project, the assumption is that the threat is *potentially low*, as this industry requires a high form of technical knowledge and skills. There

is a collection system that is required for the technology to be easily adapted to a company's operation process. If there are no collection systems available, one will have to be created in order for the application on the technology to be successful. Another reason for this threat to be low is because there is a high capital cost required to start a project like this in the existing industry. If one does have the expertise and skills to make another form of technology, funding must be guaranteed in order for it to succeed, and in this category most fail to come through. The MBT project is already backed with both funding and government support, along with networks on the industries from whom there are significant interests in the project.

Second, *Supplier power* consists of identifying what power suppliers have in this industry. In the case for the project, the assumption again is that it will be *potentially low*, as there are a vast amount of suppliers out there that materials can be bought to create the bio-filter. As there are many suppliers, our client will have the discretionary power to seek out which supplier can provide the lowest cost and the it will be in the project's favour as switching suppliers will not be prohibited. The cost for making the bio-filter is very low as well.

Third, *Buyer power* examines how many players there are in the market to sell the technology to. The assumption is *potentially high* as in the initial stage, there may be few buyers for the product, which could mean that they would drive down prices and dictate business terms. However, this will need to be overcome by educating the industry; As this is a new technology and there has not been much testing done in a commercial scale, companies need to be educated as to what and how the technology can be used. If the commercial testing proves to be economical, as the project is still in its testing phase, there may be more buyer demand changing from a threat to an opportunity.

Fourth, *Threat of substitutes* examines if there is possibility of another substitute to the product. In this case, the threat is *potentially low*, as again, this is a very technical field.

The substitutes which are currently available are all for high volume methane emissions such as low pressure gas collection, electrical generation using gas turbines and reciprocating electrical generation using gas turbines, solution gas reinjection using produced water and oxidation. The only substitutes that companies are currently are doing is flaring, venting or both. These two methods are being criticized and environmental standards are being introduced for the reduction of both these methods.

Lastly, *Competitive rivalry* looks at all the four forces that may come together to produce this force. All the resources at the project's disposal may be put in to maintain market shares and sales as the results of this analysis show that the success of the project is *potentially high*. This is because the threat of entry is low, supplier power is low, and the threat of substitutes is low. Even though buyer power may be potentially high, the other threats outweigh the buyer powers enabling a great forecast for the success of the project in a commercial scale.

SWOT Analysis

A Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis provides a holistic examination of the MBT, the client company and the industry in which it operates. Strengths and weaknesses are analyzed from an internal perspective, while opportunities and threats are examined from the broader, external perspective. The following table provides a summary of the SWOT analysis. This is followed by a more detailed description of each category of the SWOT.

INTERNAL PERSPECTIVE	
Strengths	Weaknesses
<ul style="list-style-type: none"> • Nimble organization so is able to respond very quickly, and change marketing direction if required • Client has a strong reputation in the market and networks • Low overheads results in low prices, and good value to customers • MBT is relatively inexpensive and affordable • MBT process is efficient • Carbon credits can be acquired through use of technology • Little energy required to treat methane (fan for gas distribution), compared to flaring; can also be offset via solar power • Mattress lifecycle of 5 years for MBT 	<ul style="list-style-type: none"> • The project currently has little market presence or reputation • The project is vulnerable to vital staff being unavailable or leaving, as skills and expertise are limited • Cash flow may be unreliable during early stages • MBT is not patented and can be easily imitated • Technology has not been tested on a commercial scale • New application not clearly understood in targeted industry • Process requires a proper collection system for gas collection

<ul style="list-style-type: none"> • Customizable to any operation • Easy to transport • Supported and funded by the Government of Alberta 	<ul style="list-style-type: none"> • Treatment of other contaminants are unknown at the moment • Bio-filter media changes causes lag time for bacteria acclimatization
EXTERNAL PERSPECTIVE	
Opportunities	Threats
<ul style="list-style-type: none"> • The business sector for emissions reduction is expanding, with many future opportunities for success • Local government wants to encourage local businesses and funding is available for new technologies • Competitors may be slow to adopt new technologies • Potential emission reduction regulations may come into play soon • Shift in social mindset of environmental awareness and solutions to global warming • Venting and flaring environmental effects gaining media attention • Not many existing processes available and used for treating methane 	<ul style="list-style-type: none"> • Developments in technology may change this market beyond the project's ability to adapt • A small change in the focus of a large competitor might wipe out any market position the project may achieve • Open markets; any new technology can enter as there are no market barriers • MBT not well known in industry; market reception of unknown technology

Strengths

The MBT project has a strong foundation that will help build the reputation of the project.

Firstly, the lead individual for the project is very well know with high academic standards and developed networks in the industry to pave a path forward to set up a commercial scale project.

The project is able to change direction quickly as there are many experts whom are involved in the development process and thus it is able to respond very quickly to changes, as there is no red tape, and no need for higher management approval.

The overhead costs for the project is significantly low and this allows the technology to offer a great value to customers. Another great feature is that there is no need for additional energy as methane is treated naturally. The technology is proven to be efficient and effective and is suitable for carbon credits for offsetting emissions. The lifecycle for one mattress has an average lifespan of five years, allowing efficient use of technology. Once the full lifecycle is over, the mattress can be easily replaced without the need to rebuild a new system and easily transportable. The technology is customizable to any operation and does not require any process changes for targeted industries. The project is also supported and funded by the Government of Alberta, which is of great benefit for marketing purposes.

Weaknesses

The weaknesses for the project will hinder the feasibility of the project if no solutions are formulated. As the project is new, it has little market presence or reputation in the industry and much work needs to be done in order for industry to be educated on the benefits of the technology. At present stage, the project is also vulnerable to vital staff being sick and leaving as skills and expertise is limited to the confines of the team. Only the members of the larger research and development team of the technology have the skills and expertise to solve any issues that may arise.

The cash flow of the project is dependent on the funding provided by the Government of Alberta. It will be unreliable in the early stages of development in a commercial scale as the project is based on funding and a commercial sized project requires much support from both industries

willing to accept/try the technology and funding for costs.

The MBT is not patented and can be easily imitated by other competitors in the market and thus poses a great risk. Thus, a recommendation would be to patent the technology long enough to get a significant market presence and build a reputation on being the founders and first to the market strategy.

The technology has yet to be tested on a commercial scale and such expansion from pilot projects may have a different, unexpected result in performance. The technology also requires a proper gas collection system, otherwise the technology is obsolete and lastly, as technology is still in its pilot stage, the treatment of other contaminants are unknown, which would be helpful in marketing the effectiveness of the technology.

Opportunities

As the business sector for emissions reduction is expanding, with many future opportunities for success, the MBT has a significant opportunity for success if it is able to penetrate into the market. As Alberta's government wants to encourage local businesses and funding is available for new technologies, the project has high hopes for success. This can also be leveraged with the reputation of the already published research on the technology and the client's networks in the industry.

Competitors may be slow to adopt new technologies, which will be able to compete with the expertise of the MBT. This can also be leveraged as new talks of the "40/40" plan may come into play next year. Also, the shift in social mindset of public and views of environmental awareness and solutions to global warming is in demand. Mass media is focusing of the environmental effects of venting and flaring and industries are being blamed for not taking corrective measures. As there is not much existing competition for alternative methods for

treating methane, MBT technology is the perfect fit for the industry to adopt and make immediate and efficient changes.

Threats

Certain threats that may hinder the project are developments in technology, which may change the market beyond the project's ability to adapt. As companies are looking into internal process efficiencies for emission reductions, it may be hard to input the MBT technology in such cases if it does not compliment the changes for the project itself. A small change in the focus of a large competitor (companies) might wipe out any market position the project may achieve. This would be detrimental to the success of the technology.

Other threats include open markets with no barriers for alternative methods or new technologies to enter the market. As MBT is not well known in the industry, a company with already established reputation and market share can easily wipe out any market share or industry interest in the project.

Industry Conclusions and Recommendations

The project operates in an industry with a tremendous amount of opportunities. As a result of the above analyses, the recommendation to the client is to specialize in rapid response, good value services to local businesses and local governments. This includes the research and development of collection systems that would be easily added to the technology to compliment a company's adaptation to the technology. The marketing of the MBT should be in selected local publications for target industries to get the greatest possible market presence for an advertising budget, and the client should keep up-to-date with changes in technology where

possible as the MBT is not patented and can easily be imitated along with improving on changes based on other competitors. This will ensure that the first mover advantage gained by the commercial application of the technology is preserved.

Project Costs

The project is in pilot stages, thus cost and pricing is yet to be determined. However, as the technology becomes more accepted and adopted by industry, economies of scale will improve unit pricing making it more cost effective to mitigate low volume methane emissions.

Furthermore, there is the potential for the bio-filter units to pay-off through the carbon equivalent offsets generated.

GHG Emissions Reductions

According to CCEMC (2013), the bio-filter “project can reduce greenhouse gas emissions by about 2,000 tonnes over 10 years. The estimated ten year (2013-2022) market emission reductions that can be expected from this technology, anticipating market adoption starting in 2016 at a 1 percent adoption rate is: 130,000 tonnes CO_{2e}.”

With the above projections by the CCEMC, Carbon Credits that can be generated would be:

130 000 CO_{2e} * \$15 = \$1, 950, 000 approximately

CHAPTER FIVE

Recommendations for the market feasibility of MBT

For the success of industry adoption of Methane Bio-filter Technology, some considerations need to be made along with further research. The project requires stakeholder input from the various industries to inform the best strategies on moving forward. Stakeholder input will address specific needs of the various organizations in industry, which will assist in tailoring the bio-filters as specifically as possible. Since the bio-filters are still in pilot stages, which have had a high degree of success, commercial scale testing is still required to solidify its place in industry.

To explore the MBT market viability further and have a sound market strategy, our recommendations are,

- Conduct market characterization focusing on the key industry sectors which should include
 - Surveying and interviewing major market players and other key informants to identify market trends, opportunities and needs to be able to develop target specific profiles for each target sector.
- Conduct a gap and SWOT analysis of each target industry sector.
- Conduct an analysis of market player perceived barriers
 - Identification of key events that have led to the success or failures of similar projects

- Presentation of the key lessons learned and strategies for developing successful methane bio-filters.
- Conduct financial evaluation for the economic projections of developing the markets for methane bio-filters and the cost of project development. This will include:
 - The capital and operational cost estimates to determine economic feasibility.
 - Determining the financial instruments accessible to support the project development.
 - Carbon offset evaluation using the Intergovernmental Panel on Climate Change (IPCC) methodologies to determine CO₂e reductions of methane bio-filter projects.

This would further help to determine the commercial potential and the range of applications for MBT along with forming a basis for business strategy. It would help in the planning and development of a larger-scale and implementation of this technology.

Furthermore, we believe it is important to create awareness about the Methane Bio-filter Technology in the market. This can be done through creating a platform for educating stakeholders such as regulators, shareholders, manufacturers, retailers, purchasers, installers, environmental supporters, maintenance, etc., about this technology and answering their concerns. We have listed some of the issues that could be addressed on this platform. They are some key issues that will need to be studied and disputed at every stage of the project lifecycle. They include:

Technology

- How does the bio-filter work? What is its efficiency?
- What are the results of pilot projects (successes, failures and improvements)?
- What is long term is bio-filter performance?
- What is the relevance of this technology to the industry?
- Review existing technologies for biological oxidation of methane (and considering comments received through stakeholder discussions)
- How will chemical parameters e.g. H₂S be monitored on site for each bio filter?
- Is it sustainable?
- Is the design of bio filter standardized?
- What is the applicability of control measure and its validation and verification?

Economic

- Has this technology been patented?
- What is the manufacturing and market price of per unit bio-filter?
- Is it cost efficient?
- What is MBT Life Cycle Assessment- transportation, installation, operation, maintenance (i.e. bio filter box, mattresses, transportation, availability of raw material, training personnel etc.)?
- What is the decommissioning cost of Methane Bio-filters?

Environment

- What are the environmental factors affecting microbial activity and efficiency? The effect of :
 - Temperature
 - Toxicity- Acidity and alkalinity (pH)
 - Oxygen availability
 - Water availability
 - Nutritional and growth requirements
 - Energy utilization and generation in biological processes
 - Biodegradation
- What is the emission reduction potential for carbon dioxide and other pollutants?

Regulatory

- Have report consultations with stakeholders.
- Does project demonstrates real, quantifiable and verifiable emissions reductions using replicable quantification methodologies for methane reductions that would not otherwise have occurred had the offset project not been implemented?
- Is the project commercially feasible at current carbon prices?

Other basic criteria against which the issues should be analyzed and evaluated should be based on the following:

- Efficiency
- Relevance
- Effectiveness
- Impact

- Sustainability

We believe that addressing these issues would not only educate the industry, but would take away any doubts they have about the Methane Bio-filter Technology. This would increase industry receptivity and acceptance of the technology which is the most important requirement for its commercial success in the market.

Conclusion

This paper has examined the attributes of methane and its effects on the environment. Methane is generated from various sources, especially anthropological sources, and is the reason behind origin of Methane Bio-filter Technology. Various human activities contribute to methane generation, including landfills, agricultural and livestock activities as well as oil and gas production. As seen in the Chapter three, for most industries managing higher volumes of methane is easier as it is economically viable for electricity generation and heating. However, this is not the case for lower volumes up to 100m³ per day. Many point source low volume methane emissions add substantial volumes to overall emissions. The mechanisms available in industry to manage low volume emissions are flaring and venting; which, when considering the detrimental environmental effects of methane are not viable options. With the projected potential offsets that can be gained using MBT, it can fill this gap in industry. Furthermore, the simplicity of the technology facilitates replication and can be customized for different industries on the basis of their circumstance, emissions, location, environmental and economic considerations. Bio-filters provide a cost effective and efficient solution to reducing overall emissions. In comparison to flaring that is energy dependent and produces secondary pollutants such as carbon monoxide, NO_x and SO_x; the operational cost of bio-filters per tonne of treated emissions is considerably lower and the by-products are carbon dioxide and water.

All technologies have their limitations. For the bio-filter to be functional in any industry, collection systems are required and more study is needed in that area.

We have recommended that consultation with the stakeholders such as regulators, shareholders, manufacturers, retailers, purchasers, installers, environmental supporters, maintenance, etc.

about this technology and answering their concerns would further help to determine the commercial potential and the range of applications for MBT along with forming a basis for business strategy. It would also help with the planning and development of a larger-scale and implementation of this technology.

Though the market analysis in the MBT commercial feasibility section of this report, we can conclude that this technology has a potential to be a commercial success. However, for that the industry needs to be educated and made conscious that such an innovative technology exists, which can provide them with sustainable solutions for the low volume, many point source methane emissions from their processes.

REFERENCES

- Alberta Agriculture and Rural Development. (2012, August 2). *Agricultural Carbon Offsets:ARD*. Retrieved from Government Of Alberta Website: [http://www1.agric.gov.ab.ca/\\$Department/deptdocs.nsf/all/cl14131](http://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/cl14131)
- Alberta Agriculture and Rural Development. (2013, March 4). *Alberta Agriculture and Rural Development*. Retrieved July 30, 2013, from Government of Alberta Website: [http://www1.agric.gov.ab.ca/\\$department/deptdocs.nsf/all/cl9706](http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/cl9706)
- Alberta Energy Regulator. (2013). *Alberta Energy Regulator*. Retrieved August 4, 2013, from AER Website: <http://www.aer.ca/about-aer/spotlight-on/unconventional-regulatory-framework/what-is-hydraulic-fracturing>
- Alberta Energy Regulator. (2013). *Why are Wells Abandoned: AER*. Retrieved from Alberta Energy Regulator: <http://www.aer.ca/abandonment-and-reclamation/why-are-wells-abandoned>
- Alberta Energy Utilities Board . (2006). *Alberta Natural Gas Royalty Guidelines*. Alberta Energy Utilities Board .
- Alini, E. (2013, April 10). *Maclean's: Alberta's Carbon Price*. Retrieved from Maclean's Website: <http://www2.macleans.ca/2013/04/10/albertas-carbon-price-if-you-dont-have-an-opinion-on-it-you-should/>
- Bogner, J., Meadows, M., & Czespiel, p. (1997). Fluxes of methane between landfills and the atmosphere: natural and engineered controls. *Soil Use and Management, Vol. 13*, pp. 268-277.
- Bott, R. D. (2007). *Flaring: Questions+ Answers*. Canadian Centre for Energy Information.
- Broomfield, M., & Donovan, B. (2012). *Monitoring and control of fugitive methane from unconventional gas*. Bristol: Environment Agency.
- Canadian Centre for Energy Information. (2007). *Flaring: Questions+ Answers*. Canadian Centre for Energy Information.
- Canadian Natural Resources Limited. (2009). *Technical Protocol Plan Quantification Protocol for Solution Gas Conservation*. Calgary: March.
- CCEMC. (2012). *How does the CCEMC fund projects?: CCEMC*. Retrieved from Climate Change and Emissions Management Corporation (CCEMC) Website: <http://ccemc.ca/funding/>
- Clean Air Strategic Alliance. (2010). *Flaring and Venting Project Team Report*. Edmonton: September.

- Cornell Waste Management Institute. (2009). *Odor Treatment - Biofiltration: Cornell Composting*. Retrieved from Cornell Waste Management Institute Website: <http://compost.css.cornell.edu/odors/odortreat.html>
- Dyer, S. (2013, April 5). *Pembina Institute: What you need to know about Alberta's 40/40 carbon pricing proposal*. Retrieved from Pembina Institute: <http://www.pembina.org/>
- Environment Canada. (2012, August). *Environment Canada*. Retrieved August 4, 2013, from Government of Canada website: <http://www.ec.gc.ca/Publications/default.asp?lang=En&n=3CD345DC-1>
- Environment Canada. (2013, February 15). *Environment Canada: Reducing Canada's greenhouse gas emissions*. Retrieved from Government Of Alberta Website : <http://www.ec.gc.ca/dd-sd/default.asp?lang=En&n=AD1B22FD-1>
- EUB. (2006). *EUB: Directive 060*. Calgary: Alberta Energy Utilities Board.
- Gawande, N. A., Reinhart, D. R., & Yeh, G.-T. (2010). *Modeling microbiological and chemical processes in municipal solid waste bioreactor, part II: Application of numerical model*. Olando: ISSN 0956-053X.
- Girard, M., & Ramirez, A. A. (2011). Biofiltration of methane at low concentrations representative of the piggery industry—Influence of the methane and nitrogen concentrations. *Chemical Engineering Journal*, 151-152.
- Global Methane Initiative. (nd). *About Methane: Global Methane Initiative*. Retrieved from Global Methane Initiative Website: <https://www.globalmethane.org/about/methane.aspx>
- Government of Alberta. (2013). *Environment and Sustainable Resource Development: Air quality management in alberta*. Retrieved from Government of Alberta: <http://environment.alberta.ca/02241.html>
- Govind, R. (nd). *Biofiltration: An innovative Technology for the Future*. Cincinnati: University of Cincinnati .
- Hanson, R., & Thomas, E. (1996). Methanotrophic bacteria. *Microbiol Rev.* 1996 June; 60(2), 439–471.
- Hettiaratchi, J. P., Hettiarachchi, M. C., & Rajbhandar, B. (2006). *CH₄ Biofiltration for Treating Gaseous Engineered Emissions arising from Natural Gas Transport*. Calgary.
- Hettiaratchi, P., Venugopal, S., & Chandrakanthi, M. (nd). *Application of Methanotropic Biofilters (MBFs) in Methane (CH₄) emissions from oil and Gas Indusrty*. Calgary.
- Howarth, R. W., Santoro, R., & Anthony, I. (2012). *Venting and leaking of methane from shale gas development: response to Cathles et al*. Springerlink.com.

- Huang, Q., Zhang, Q., Cicek, N., & Mann, D. (2011). Biofilter: a promising tool for mitigating methane. *JOURNAL OF ARID LAND, VOL. 3, NO. 1, , 67-70.*
- IPCC. (2007). *Summary for Policymakers. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge, United Kingdom and New York: Cambridge University Press.
- IPCC guidelines for National Green House Gas Inventories. (2006). *Chapter 4: Fugitive Emissions.* IPCC .
- Kaminski, J. (2011, Spring). *Canadian Journal of Nursing Informatics: Diffusion of Innovation Theory.* Retrieved from Canadian Journal of Nursing Informatics Website: <http://cjni.ca/journal/?p=1444>
- Langmead, C. (2003). *Manure Management and Greenhouse Gase Mitigation Techniques: Comparative Analysis.* Climate change Central.
- Lavelle, M. (2012, December). Methane. *National Geographic .*
- Merriam-Webster. (2013). *Livestock: Merriam-Webster.* Retrieved from Merriam-Webster Website: <http://www.merriam-webster.com/dictionary/livestock?show=0&t=1378435049>
- Quinn, P., Bates, T., Baum, E., Doubleday, N., Fiore, A., Flanner, M., . . . Warren, S. (2008). *Short-lived pollutants in the Arctic: their climate impact and possible mitigation strategies.* Atmospheric Chemistry and Physics.
- Rahim, S. A. (2004). *Solution Gas Flaring and Venting in Alberta: Volume Trends and Conservation Costs.* CASA Flare/Vent Project Team.
- Richard, T. (2009). *Odor Treatment - Biofiltration: Cornell Composting.* Retrieved from Cornell Waste Management Institute Website: <http://compost.css.cornell.edu/odors/odortreat.html>
- Schueneman, T. (2013, February 2013). *EDF, Chevron Agree Natural Gas Fracking Here to Stay, Part Ways on Fugitive Methane Emissions and Short-Term Impacts of Shale Boom.* Retrieved from Global Warming is Real Website: <http://globalwarmingisreal.com/2013/02/06/edf-chevron-agree-natural-gas-fracking-here-to-stay-part-ways-on-impact-of-methane-emissions/>
- The City of Calgary. (2012). *Offset Project Report for the Shepard Landfill Gas Capture and Combustion Offset Project.* Calgary: The City of Calgary.
- The City of Calgary. (2013). *The City of Calgary Recycling.* Retrieved from City of Calgary Website: <http://www.calgary.ca/UEP/WRS/Pages/Recycling-information/City-of-Calgary-Recycling.aspx>

- Timothy, F. (2013). *Estimates of Methane Recovery in Landfill Gas Flaring and Utilisation: Bio-tech Environmental*. Retrieved from Bio-tech Environmental website: <http://www.biotech.com/resources/what-is-biodegradation.html>
- U.S. Environmental Protection Agency. (2011). *DRAFT: Global Anthropogenic Non CO2 Greenhouse Gas Emissions: 1990*. Washington: U.S. Environmental Protection Agency.
- University of New South Wales and GHD Pty. (2006). *Passive Drainage and Biofiltration of Landfill Gas using Recycled Materials. Project Report for the Department of Environment and Conservation, Sustainability Programs Division*. New South Wales: Department of Environment and Climate Change and NSW.
- USEPA. (2013, July 31). *USEPA*. Retrieved August 2, 2013, from USEPA Website: <http://epa.gov/climatechange/ghgemissions/gases/ch4.html>
- Watson, T., & Bachu, S. (2009). *Evaluation of the Potential for Gas and CO2 Leakage Along Wellbores*. SPE Drill & Compl 24 (1): 115-126. SPE-106817-PA.
- Yang, H. B., & Minuth. (2002). Effects of Nitrogen and Oxygen on Biofilter Performance. *Journals of the Air and Waste Management Association, Vol. 52, No. 3*, 280-286.

9.2Report: GAP Analysis

GAP ANALYSIS REPORT

Control of point source low volume methane emission using methane

biofiltration (MBF) technology:

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Table of Contents

1. Objectives:	3
2. Research Questions to be answered:	3
3. Literature Review:	5
3.1 Inlet capacity applicable to biofilter.....	5
3.2 Applicability in other sectors (apart from landfills).....	9
Agriculture – Pig and Dairy farms	9
Coal mines	10
3.3 Methanotrophs responsible for methane oxidation	11
3.4 Filter media characteristics	12
3.5 Types of filter media.....	13
3.6 Main parameters for methane biofiltration	16
3.6.1 Gas flow rate (GFR) and empty bed residence time (EBRT)	16
3.6.2 Moisture content	17
3.6.3 Temperature	18
3.6.4 pH.....	20
3.6.5 Nutrients.....	21
3.6.6 Oxygen requirements and EPS	24
3.7 Methane oxidation inhibition.....	24
High or low MC:	24
Higher T:.....	24
High or low pH:	25
Presence of trace compounds:.....	25
N–NH ₄ ⁺ :	27
Presence of H ₂ S:.....	28
3.8 Biofilter inoculation	28
3.9 Biofilter configurations	28
4 Models on methane oxidation biofilters.....	30
5 Research areas to be focused in the new project.....	31
References.....	34

1. Objectives:

The main purpose of preparing this report is to review the current knowledge of methane biofilters and hence, to identify the research gaps. This report presents a list of research questions, what have been done by our research group and the other researchers, and what do we have to study furthermore in the new research project.

2. Research Questions to be answered:

1. Where the biofilters would be installed? (site identification)
 - 1.1. What are the characteristics of methane gas in each site? (field testing)
 - 1.2. Can the gas be collected in each site to send through biofilter?
2. What is the maximum flow rate (and concentration) of gas that can be sent through biofilter for optimum efficiency?
3. How would the presence of other gases (ex: H₂S, VOC) affect the oxidation?
4. What combination of material would be used as a media in biofilters? (media identification)
 - 4.1. What are the alternatives for compost? Do they have enough nutrients? Do they have enough methanotrophs (bacteria population)?
 - 4.2. If not, how the nutrients or bacteria be introduced to the biofilter (inoculation)? How cost effective they are?
 - 4.3. What are the fibre materials that can be used to maximize the porosity?
 - 4.4. What are the materials that can be used to maximize water holding capacity?
 - 4.5. Can inorganic material be used? What are the advantages and disadvantages of using these?
 - 4.6. What is the material ratio to obtain optimum methane oxidation efficiency? (methane oxidation efficiency at different ratio)
 - 4.7. Can we enhance the efficiency by inoculation?

- 4.8. What is the optimum particle size (small particles---increases surface area or potential sites for microbial activity----but resists gas flow)?
5. How long a biofilter can work at specific efficiency? How frequent the materials need to be replaced (life of a biofilter)?
6. What is the size of the biofilter? (biofilter design)
 - 6.1. What are the length, width, and thickness? (biofilter configurations)
 - 6.2. What is the aeration method (passive VS active)?
 - 6.3. What are the optimum moisture and nutrient contents?
7. How would be the efficiency changes over a complete year, for example, summer VS winter?
8. What is the methane oxidation efficiency in the field biofilter?
 - 8.1. How would you determine the field efficiency? What is the indicator (eg: temperature, bacterial population)?
9. What is the dominant methanotrophic group of bacteria (important if we want to inoculate)?
 - 9.1. What are the inhibiting factors (ex: EPS formation)? And how to avoid the inhibition?
 - 9.2. How the bacterial activity/nutrient/moisture content in biofilters change over time?
 - 9.3. What are the limiting factors?

3. Literature Review:

3.1 Inlet capacity applicable to biofilter

Some anthropogenic methane emission sources are low volume, and point or diffused (Wilshusen et al., 2004). Examples are sanitary landfills, oil and gas well sites, and natural gas transport sites, where sufficient gas is not available for energy recovery. At most oil well sites, the rate of solution gas emission is less than 300 m³/day per well, and gas utilization would be difficult due to small volumes (Yang et al., 2000). In landfills, the use of biogas is limited to a certain period of time as its calorific value decreases as the concentration of methane decreases. According to Haubrichs and Widmann (2006) and Niekema et al. (2007), biogas use in power plants is only technically and economically suitable for a CH₄ content superior to 30-40% v/v (which occurs during the first 25 years of the landfill) and a total biogas production of about 30 -50 m³/hr.

Flaring is used in cases where no energy recovery is available. According to Haubrichs and Widmann (2006), CH₄ concentrations of 20-25% and a flow rate of 10-15 m³/h must be reached to make flaring economically feasible. Another constraint of this technology is the combustion temperature, which must be equal or superior to 1200 °C in order to prevent the formation of any toxic by-products such as dioxins.

Methane biofiltration is a promising bioprocess to attenuate point source low volume methane emissions, especially from small and old landfills, but also from large and new landfills as a post treatment to energy recovery, when concentrations and flow rates are no longer appropriate (Scheutz et al., 2009). According to Gebert et al. (2008), methane biofilters could receive very high methane fluxes (30-250 gCH₄/m³.h).

The review paper by Nikiema et al. (2007) summarized the various inlet loads and the elimination capacities tested at the laboratory scale biofilters (Table 1). Overall, for IL closer to 300 g/m².d, a conversion of 50% was obtained, as against 100% when the IL was only of 186 g/m².d. Similarly, Scheutz et al. (2009) summarized a similar table.

Table 3 Experiments on the topic of methane biofiltration

Filter bed	Operating conditions	Inlet load	Elimination capacity or conversion	Authors
Compost and soil Clay and landfill cover soil	Aerated at the top Mixture 45% v/v CH ₄ , 45% v/v CO ₂	IL = 202 g m ⁻² d ⁻¹	EC = 80–90 g m ⁻² d ⁻¹ EC = 40–50 g m ⁻² d ⁻¹	Bajic and Zeiss (2001)
Soil and sand Soil	Optimal water content for all experiments		EC = 15–20 g m ⁻² d ⁻¹ EC = 5–7 g m ⁻² d ⁻¹	
Multi-layers: Compost + sand (top) and sand (0.9 m)	Aerated at the top Mixture 50% v/v CH ₄ , 50% v/v CO ₂	IL = 288 g m ⁻² d ⁻¹	EC = 164–283 g m ⁻² d ⁻¹	Berger et al. (2005)
Agricultural soil	Aerated at the top	IL = 214 g m ⁻² d ⁻¹	EC = 171 g m ⁻² d ⁻¹	De Visscher et al. (1999)
Landfill cover soil	Mixture 50% v/v CH ₄ , 50% v/v CO ₂	IL = 368 g m ⁻² d ⁻¹	EC = 240 g m ⁻² d ⁻¹	
Compost of pine bark	Aerated at the bottom	IL < 420 g m ⁻² d ⁻¹ EBRT > 0.85 h	X ≥ 70%	Du Plessis et al. (2003)
Multi-layers(from top to bottom): humic topsoil (0.1 m) + sand (0.02 m) + gravel (0.02 m) + clay (0.67 m) + gravel (0.1–0.3 m).	Pilot-scale open biofilter	IL = 0–6,000 g m ⁻³ d ⁻¹	EC ≤ 1,900 g m ⁻³ d ⁻¹ X = 62% (annual basis)	Gebert and Groengroeft (2006a)
Compost	Aerated at the bottom (3 air input level)	IL = 590 g m ⁻² d ⁻¹	EC = 530–590 g m ⁻² d ⁻¹	Haubrichs and Widmann (2006)
Recycling paper pellets	Mixture 30% v/v CH ₄ , 70% v/v CO ₂	IL = 105 g m ⁻² d ⁻¹	EC = 47 g m ⁻² d ⁻¹	
Compost + recycling paper pellets Compost and wood chips		IL = 105–485 g m ⁻² d ⁻¹ IL = 485 g m ⁻² d ⁻¹	EC = 47 g m ⁻² d ⁻¹ EC = 475 g m ⁻² d ⁻¹	
Compost of leaves	Aerated at the top	IL ~ 500 g m ⁻² d ⁻¹	EC = 325–400 g m ⁻² d ⁻¹	Hettiaratchi and Stein (2001); Wilshusen et al. (2004)
Compost of municipal waste Compost of garden residues Compost of wood chips	Pure CH ₄ , 99% v/v		EC = 200–250 g m ⁻² d ⁻¹ EC = 200–250 g m ⁻² d ⁻¹ EC < 50 g m ⁻² d ⁻¹	

Table 5 continued

Filter bed	Operating conditions	Inlet load	Elimination capacity or conversion	Authors
Peat	Aerated at the top Optimal water content	IL = 160–320 g m ⁻² d ⁻¹	EC < 186 g m ⁻² d ⁻¹	Hettiaratchi et al. (2000)
Soil 1 (Sand 70%, clay 15%, silica 15% wt/wt)	Aerated at the top Mixture 60% v/v CH ₄ , 40% v/v CO ₂	IL = 95 g m ⁻² d ⁻¹	EC = 62 g m ⁻² d ⁻¹	Hettiaratchi et al. (2000)
Soil 2 (Sand 70%, clay 25%, silica 5% wt/wt)		IL = 345 g m ⁻² d ⁻¹ IL = 95 g m ⁻² d ⁻¹ IL = 435 g m ⁻² d ⁻¹	EC = 121 g m ⁻² d ⁻¹ EC = 49 g m ⁻² d ⁻¹ EC = 87 g m ⁻² d ⁻¹	
Landfill cover soil	Aerated at the top Water content: 15% wt/wt Mixture: 50% v/v CH ₄ , 50% v/v CO ₂	IL = 281 g m ⁻² d ⁻¹	EC = 125–140 g m ⁻² d ⁻¹ (Peak) EC = 42–55 g m ⁻² d ⁻¹ (Steady operation)	Hilger et al. (2000a, b)
Fresh compost	Aerated at the top Temperature: 18°C	IL = 135–170 g m ⁻² d ⁻¹	X = 60% from day 25 to day 50	Humer and Lechner (1999b)
Mature compost Soil		Q = 4–5 ml min ⁻¹	X = 100% after 55 days X = 100% after 15 days X = 30–40% from day 10 to day 50	
Compost of municipal waste	Aerated at the top Temperature: 18–20°C	IL ~ 235 g m ⁻² d ⁻¹	EC = ~188 g m ⁻² d ⁻¹	Humer and Lechner (2001)
Compost of clarification sludge		Q = 4–7 ml min ⁻¹	EC = ~175–190 g m ⁻² d ⁻¹	
Soil			EC = ~82 g m ⁻² d ⁻¹ EC = ~95 g m ⁻² d ⁻¹ EC = ~175 g m ⁻² d ⁻¹	
Landfill cover soil			X = 100%	Hupe et al. (1998)
Garden soil			EC = ~700 g m ⁻² d ⁻¹ EC = ~300 g m ⁻² d ⁻¹	Nikiema et al. (2004b)
Mature compost	In situ	2,400 L biogas m ⁻² d ⁻¹	EC = 435 g m ⁻² d ⁻¹	Park et al. (2002)
Inorganic material Compost	Aerated at the bottom 7,000–7,500 ppmv CH ₄	IL ~ 1,700 g m ⁻² d ⁻¹		
Soil	Aerated at the top Pure CH ₄ , Optimal conditions	IL = 525 g CH ₄ m ⁻² d ⁻¹		
Glass tubes	Aerated at the bottom Percolation 0.25–1.0% v/v CH ₄	IL = 200 g CH ₄ m ⁻² d ⁻¹ IL = 750 g CH ₄ m ⁻² d ⁻¹	X = 96–98% X = 27–28%	Sly et al. (1993)

Filter bed	Operating conditions	Inlet load	Elimination capacity or conversion	Authors
Peat	Aerated at the top	IL = 320 g m ⁻² d ⁻¹	EC = 96–160 g m ⁻² d ⁻¹	Stein and Hettriarachi (2001)
Landfill cover soil	Pure CH ₄ 99% v/v	IL = 320 g m ⁻² d ⁻¹	EC = 64–130 g m ⁻² d ⁻¹	
Agricultural soil	Water content: 10% wt/wt	IL = 310 g m ⁻² d ⁻¹	EC = 93–155 g m ⁻² d ⁻¹	
Compost + landfill cover material	In situ open biofilter	IL = 18,500–42,800 g m ⁻³ d ⁻¹	X ≥ 90%	Straka et al. (1999)
Compost	Bench-scale open biofilter	IL = 288–3120 g m ⁻³ d ⁻¹	EC = 1,500 g m ⁻³ d ^{-1a}	Streese and Stegmann (2003)
Compost + peat + wood fibers			EC = 960 g m ⁻³ d ⁻¹	
Multi-layers			EC = 720 g m ⁻³ d ⁻¹	
Compost	Large-scale open biofilter	IL = 288–3120 g m ⁻³ d ⁻¹	EC = 960 g m ⁻³ d ^{-1a}	Streese and Stegmann (2003)
Compost + peat + wood fibers			EC = 480 g m ⁻³ d ⁻¹	
Soil 1	Aerated at the top	–	EC = 40–100 g m ⁻² d ⁻¹	Visvanathan et al. (1999)
Soil 2	Mixture 60% v/v CH ₄ , 40% v/v CO ₂	–	EC = 75–100 g m ⁻² d ⁻¹	

^a This EC was the maximal value obtained. After 5 months of operation, the CH₄ oxidation rate in the biofilter decreased.

Table 2 Performance parameters used in biofiltration

IL: Surfacic inlet load (g m ⁻² d ⁻¹)	$IL = \frac{C_{(CH_4)in} \times Q}{S}$
IL: Volumetric inlet load (g m ⁻³ d ⁻¹)	$IL = \frac{C_{(CH_4)in} \times Q}{V}$
X: Conversion (%)	$X = \frac{C_{(CH_4)in} - C_{(CH_4)out}}{C_{(CH_4)in}} \times 100$
EC: Elimination capacity (g m ⁻² d ⁻¹ or g m ⁻³ d ⁻¹)	$EC = IL \times \frac{X}{100}$

Where C_(CH₄): Methane concentration in g m⁻³; Q: Volumetric flow rate of gases in m³ d⁻¹; S: Biofilter bed cross-section in m²; V: Biofilter bed volume in m³

3.2 Applicability in other sectors (apart from landfills)

Up to date most of the past studies related to methane biofiltration were related to landfills (refer Table 1). However, there are few studies focused on agriculture sector such as piggery industry, and dairy farm (Canadian pork councils, 2006, Girard et al., 2009, Melse and van der Werf, 2005) and coal mines (Apel et al, 1991; Sly et al, 1993).

Agriculture – Pig and Dairy farms

The agriculture sector in Canada contributes to 8.4% (in 2005) of Canadian emissions, which is an increase of 24% since 1990. Agricultural GHG do not come from energy requirements, but rather from livestock production: 55% from enteric fermentation, 24% from agricultural land, and 21% from manure management (Huang et al, 2011)). Enteric fermentation only occurs in ruminants such as cattle; GHG from the piggery industry are therefore associated with manure management and land-based sources. The two most important GHG found on a pig farm are CH₄ and N₂O with, respectively, 49% and 51% of emissions (Jaques 2007). Methane production in pig farms occurs predominantly (65%–70%) during slurry storage (Monteny et al. 2006; Haeussermann et al. 2006). Depending on storage conditions, CH₄ concentrations emitted from covered liquid manure storage vary between 0.1 to 20 g/m³ (0.015 to 3% v/v) while it takes CH₄ concentrations of between 5 and 15% (v/v) to maintain a flame for CH₄ thermal oxidation (Veillette et al., 2012), so thermal oxidation is not effective.

In 2006, the Canadian Pork Council worked on the biofiltration of CH₄ from a 3800 m³ slurry storage reservoir (on 3000 head pig farm) equipped with a floating cover. The evacuated gases have been forwarded to a biofilter which is built in a trailer (4m*2.5m*2m). Four different organic packing materials were tested without inoculation: mixtures of compost, wood chips, soil, and peat moss. After a start-up period of 3 months, the results showed the removal efficiency up to 80%. CH₄ concentrations ranged between 2000 to 35000 ppmv and average IL was 30 g/m³.h.

Study by Girard et al., (2009) was related to piggery industry swine slurry, which is a mixture of pig feces and urine with wastewater. Girard et al. (2008) presented some preliminary results on the biofiltration of CH₄ at concentrations from 100 to 2000 ppmv using an inorganic filter bed. These authors obtained a maximal removal efficiency of 87% for an IL up to 20 g/m³.h. Using a

biofilter packed with a mixture of compost and perlite (60:40 v/v) (inoculation with activated sludge), Melse and van der Werf (2005) treated CH₄ from a 6 m³ pilot-scale slurry storage unit. With concentrations no higher than 5.5 g/m³ (8500 ppmv), up to 85% of the CH₄ was removed.

Pratt et al. (2012) did a field scale study on dairy farm effluent pond in New Zealand; it was the first study on dairy farm methane biofiltration reported in literature. The 70 L filter comprised a 1:1 volumetric mixture of volcanic soil from a landfill and perlite. Biogas collected in a floating cover on a 4 m² section of the pond was directed through the biofilter's base. The filter's maximum CH₄ removal rate was 16 g /m³ h (or 53 microg /g.h), which is high compared with literature landfill soil oxidation rates (typically <1 to 40 microg/ g.h). The results showed that a 50 m³ filter would be needed to offset CH₄ emissions (approximately 720 g /h) from a typical 1000 m² dairy effluent pond. As this calculation is based on the efficiency of a single experimental filter, field testing of replicate biofilters is needed to accurately establish full-scale filter sizing.

Coal mines

By 2008, it was estimated that there were 826,001 million tons of coal reserves worldwide (Jiang et al., 2010). Coal mine gas is also a complicated mixture with high concentrations of methane. Emissions of methane from coal mines, estimated at around 5–30 Tg/year, result mainly from desorption of methane during mining, crushing, and inefficient combustion. Besides the greenhouse effect, coal mine gas can also cause severe explosions. The accidental ignition of methane at concentrations below 5.53% may initiate coal dust explosion (Apel et al, 1991).

Compared to seam gas drainage and air ventilation, which are currently the most effective methods for coal mine gas control, a biological method using methanotrophs would be more economic and efficient (Jiang et al., 2010). The potential of using *Methylomonas methanica* (the growth media – polypropylene bio rings, inoculated with *Methylomonas methanica* grown in serum) to remove methane from coal mine atmospheres was investigated in a bench-scale bioreactor, and 90.4% of the methane in a 35% methane/air mixture was removed in 24 h (Apel et al., 1991). Another strain, *Methylomonas fodinarum* (isolated from coal mine water samples, filter media - glass beads) was used in a continuous biofilter (Sly et al., 1993). In the range of 0.25–1.0% methane (v/v) in air,

which is common in coal mine atmospheres, more than 70% of the methane was removed with a residence time of 15min and with a 90% reduction over 20 min residence time.

3.3 Methanotrophs responsible for methane oxidation

The main bacteria responsible for CH₄ biodegradation are known as methanotrophs; a part of the physiological group of methylotrophs. These are unique in their ability to degrade one carbon compound under aerobic conditions. They produce MMO (methane mono oxygenase) enzyme, which catalyze the oxidation of methane. The phospholipids fatty acid signature, DNA or the diagnostic microarray methods were used to distinguish the two types of methanotrophs, type I, type II, and type X (Menard et al., 2012). Studies found that the areas with low O₂ and high CH₄ molar mixing ratios (1.57 -1.63) are dominated by type II; the opposite conditions, low CH₄ and high O₂ mixing ratios (1.79-1.97), increased the number of type I bacteria (Amaral and Knowles, 1995). In another experiment, both type I and type II were active and both contributed to CH₄ oxidation at high CH₄ concentrations (10,000 ppmv), however type I dominated at a CH₄ concentration of 1000 ppmv (Henckel et al., 2000). The study conducted by Gebert et al (2008) at two field scale landfill biofilters, concluded that they are strongly dominated by type II organisms, as a result of high methane loads, low copper concentration and low nitrogen availability. In biofilters, the analysis of methanotrophs has shown the predominance of *Methylocystis* from type II in either a passively vented or biofilter continuously supplied with air (Gebert et al, 2008, Nikiema et al., 2005).

An overview of the methanotrophic groups identified are as below (Scheutz et al., 2009).

Table 1: Overview of the characteristics of the eleven methanotrophic genera as known to date.

Genus	Phylogenetic position	ICM-arrangement (type)	Dominant PLFA	C-assimilation	Type of MMO	N ₂ -fixation
<i>Methylobacter</i>	γ-Proteobact.	stacks (I)	16:ω1	RuMP	pMMO	no
<i>Methylocaldum</i>	γ-Proteobact.	stacks (I)	16:ω1	RuMP	pMMO	no
<i>Methylocapsa</i> *	α-Proteobact.	Typ III	18:ω1	Serine	pMMO	yes
<i>Methylocella</i>	α-Proteobact.	peripheral (II)	18:ω1	Serine	sMMO	yes
<i>Methylococcus</i>	γ-Proteobact.	stacks (I)	16:ω1	RuMP	pMMO	yes
<i>Methylocystis</i>	α-Proteobact.	peripheral (II)	18:ω1	Serine	sMMO/pMMO	yes
<i>Methylomicrobium</i>	γ-Proteobact.	stacks (I)	16:ω1	RuMP	pMMO	no
<i>Methylomonas</i>	γ-Proteobact.	stacks (I)	16:ω1	RuMP	pMMO	no
<i>Methylosinus</i>	α-Proteobact.	peripheral (II)	18:ω1	Serine	sMMO/pMMO	yes
<i>Methylosphaera</i>	γ-Proteobact.	stacks (I)	16:ω1	RuMP	pMMO	no
<i>Methylothermus</i> *	γ-Proteobact.	n.d.	n.d.	n.d.	pMMO	n.d.

ICM, intracytoplasmic membranes; RuMP, ribulose monophosphate path; Serin, serine path; pMMO, particulate methane monooxygenase; sMMO, soluble methane monooxygenase; *genus so far represented by only one species; Proteobact., Proteobacteria; n.d., not determined.

3.4 Filter media characteristics

The filter bed is the solid phase on which the biofilm containing the microorganisms is to be formed. There are several important characteristics that a good media needs to possess (Akdeniz et al, 2011). Desirable biofilter media properties include suitable environment for microorganisms (nutrients, moisture, pH, and temperature), large surface area to maximize sorption capacity, high pore space to maximize empty bed contact time (EBCT), minimal pressure drops to reduce operating energy requirements, and low bulk density to decrease media compaction. The pressure drop versus airflow rate relation is an important media characteristic.

Low pressure drop is preferred to minimize energy requirement for passing target air through the media. Low cost, local availability and sufficiently long lifespan are additional desirable properties (Williams and Miller, 1992; Swanson and Loehr, 1997; Chen and Hoff, 2009). Wood chips, bark mulch, compost and the mixtures of these are commonly used media. Wood chips and bark mulch increases surface area and porosity while compost provides microorganisms and micronutrients. These common organic materials degrade which causes a decrease in porosity and increased compaction over time (Nicolai and Janni, 2001; Chen and Hoff, 2009). Media replacement every 2–5 years is a significant investment in materials, time and labor. Also there is an operational downtime as the media is replaced with new media and lag time until the new media acclimates and removal efficiencies reach previous levels (Langolf and Kleinheinz, 2006).

The sizing of the particle size is an important characteristic. According to Hettiaratchi & Stein (2000), the mean size of the soil particles must preferably lie between 0.5 and 2 mm. When particle sizes are too small (<0.02 mm), this sizing provides for large specific surface areas, available for essential gas/biolyer exchanges, but it also creates some resistance to gas flow, while if it is too large, it favors gaseous fows but reduces the number of potential sites for the microbial activity (Delhomenie et al., 2002). Contrast to Hettiaratchi & Stein (2000), Eitner and Gethke (1987) and Leson and Winer (1991) have suggested a minimal pellet size of 4 mm (for compost) in order to minimize pressure drop through the bed.

Having a higher porosity is also an important factor in CH₄ oxidation, 85% being the optimum (Gebert et al., 2003). High gas permeability is warranted by a high share of pores > 50 micrometers (Scheutz et al. 2009)

The filter material should have high specific surface area > 300 m²/m³, in order to facilitate the pollutant and O₂ transfer and the development of the biofilm (Nikiema and Heitz, 2005). However, as shown in the following table, as the surface area (of inorganic material) increases, the EC was increased at IL of 90 gCH₄/m³.h (Nikiema et al, 2010).

Surface area m ² /m ³	EC gCH ₄ /m ³ .h
470	17
1250	38
1360	50

3.5 Types of filter media

Various experiments, conducted at the laboratory scale, have been performed to test various filter bed structures, using natural organic materials such as soils, composts and peat or synthetic materials (shown in Table 1), because they satisfy most of the required material characteristics. Various researchers showed that among various composts, the mature compost a preferred

framework for the biofiltration of CH₄ (Refer table 1). The most effective soil are those taken directly from the upper layers of landfill covers.

Han et al., (2010) considered aged refuse from an old landfill as a potential natural medium to use in methane biofilters. Generally, after 8–10 years of placement, there are few explosive gases and liquid leachate produced from the refuse, the ratio of biochemical oxygen demand and chemical oxygen demand (BOD₅/COD_{Cr}) descends to 0.3, COD_{Cr} level decreases to 25–50 mg/L, and the content of organic matters is less than 10%, which suggests that the refuse has been stabilized and could be defined as “aged refuse” (Zhao and Shao, 2004). The aged refuse contains a wide spectrum and large quantity of microorganisms which have been proved to have a strong decomposition capability for both biodegradable and refractory organic matter appeared in some wastewaters (Zhao et al., 2007). Han et al., (2010) used bio-columns filled with aged refuse around the exhaust pipeline to oxidize the methane discharged from exhaust pipelines in landfills and methane conversion reached 95% during 37 days.

Streese and Stegmann (2003) put a special emphasis on filter material optimization. Three months after the beginning of the experiment, very high degradation rates of up to 63 g CH₄/(m³h) were observed in the bench-scale plant at mean methane concentrations of 2.5% v/v and with fine-grained compost as biofilter material. However, the degradation rates of the compost biofilter decreased in the fifth month of the experiment, probably due to the accumulation of EPS formed by the microorganisms. A mixture of yard waste compost, peat, and wood fibers (squeezed spruce wood fibers) showed stable and satisfactory degradation rates. Depending on methane concentration and temperature, the degradation rates obtained with this material ranged from 20 to 40 g/m³.h. From day 150 of the experiment onwards, the degradation rates achieved with the material mixture were generally twice as high as the values generated with the pure compost material under the same operational conditions. In this material, the wood fibers served as a structural material and prevented clogging of the biofilter. To provide optimal gas flow distribution, coconut fibre mats of 10 cm thickness are installed at the inlet, outlet, and one in the middle. The characteristics of the materials are as below.

Table 2
 Characteristics of the biofilter materials as measured at the bench scale plant at the start of the experiment

Parameter	Compost	Peat	Wood fibers	Mixture
Bulk density (kg/m ³) ^a	864	351	91	434
Water content (%) ^a	48.9	77.3	48.7	45.6
Water holding capacity (%) ^a	71.2	88.2	80.8	76.7
Ignition loss (%) ^b	28.2	98.67	99.2	52.1
TOC (%) ^b	13.6	51.7	48.6	26.3
NH ₄ -N (mg/kg) ^b	732	2203	39.0	881
NO ₃ -N (mg/kg) ^b	200	74.9	n.d.	166
NO ₂ -N (mg/kg) ^b	51.7	n.d.	n.d.	40.2
SO ₄ ²⁻ (mg/kg) ^b	417	257	38.2	363
PO ₄ ⁻ (mg/kg) ^b	79.6	6.61	4.48	63.2
Total Kjeldahl nitrogen (mg/kg) ^b	7750	8850	440	7370
pH	8.8	4.1	n.a.	5.7
Conductivity (μS/cm)	1040	224	124	961

n.d., not detectible; n.a., not analysed.

^a Related to moist mass.

^b Related to dry mass.

Akdeniz et al (2011) conducted a study with pine nugget and lava rock as a biofilter media as they have larger porosity and longer service lives. These materials showed lower pressure drop than traditional wood chips biofilter media and behaved well in reducing H₂S in addition to CH₄.

In contrast to organic materials, some researchers have used inorganic material as filter material assuming that only the external surface of the packing material is involved in the biodegradation. They included glass beads or synthetic materials (Nikiema et al, 2007). Inorganic filter beds offer many advantages because, the particle size, porosity, specific surface area can be easily adjusted according to the requirement and they offer good mechanical properties (stable or durable) that reduce the risk of compaction. Furthermore, with an inorganic material, Nikiema et al. (2004, 2005) did not observe any temperature gradient in the biofilter.

During Nikiema et al. biofiltration experiments, the inorganic material was compared to mature compost. At similar operating conditions, the EC obtained with the inorganic material was 29 g/(m³.h) for an IL of 75 g/(m³.h), i.e., twice the EC with the organic-compost-based bed, confirming the usefulness of inorganic materials for CH₄ biofiltration (Nikiema et al. 2005). It

was then established that the performance with the inorganic-based biofilter can be maintained stable for more than 1 year (Nikiema 2008).

However, such beds contain no nutrients or the microbes. In order to inoculate the inorganic based filter bed, 50 g of landfill cover soil were mixed with 0.8 L of nitrate minimal salt solution and incubated under 1% (v/v) methane atmosphere at room temperature. After low speed centrifugation, the supernatant liquid was used for the inoculation. For inorganic filter media, nutrients must be added, with a nutrient solution supplied daily to the biofilter.

3.6 Main parameters for methane biofiltration

3.6.1 Gas flow rate (GFR) and empty bed residence time (EBRT)

The GFR affects the transfer of the CH₄ from the gas phase to the biofilm (Perry and Green, 1997). When the GFR is high, the empty bed residence time ($EBRT = V_{bed}/Q$) is correspondingly short. Therefore, the pollutant and the other gaseous elements have less time to be transferred to the biofilm and vice versa, and these aspects have a detrimental effect on the biofilter performance. On the other hand, when the GFR is low, the EBRT is high, resulting generally in either less or almost no limitation, in terms of mass transfer at the gas-liquid interface, but increases the requirement in terms of the biofilter volume (Kim and Deshusses, 2008). A compromise, between the EBRT and the biofilter performance, must therefore be found.

Nikiema and Heitz (2009) study the influence of gas (CH₄) flow rate on EC using inorganic material as filter media. The results obtained from this study have confirmed the view that the GFR is a very important parameter, the optimum values found, leading to methane conversions of >90%, being ≤ 2 L/min (they varied between 1 and 5.5 L/min in their experiments) for inlet loads ≤ 55 g/m³/h. Based on this result, it was then established that the maximum volumetric load (VL) of methane in the biofilter must be estimated at around 0.075 m³ (methane)/m³ (biofilter)/h, that is, 6.8 m³ (pollutedgas)/m³ (biofilter)/h.

According to the review by Delhomenie and Heitz, (2005) on VOC removal biofilters, two physico-chemical mechanisms may limit the overall elimination efficiency of a biofilter; the

pollutants diffusion transfer from gas phase to biofilm, and the biodegradation reaction. Depending on both the flow rate and the VOC concentration, the VOC degradation process is limited by either one of these mechanisms or both simultaneously. The following figure presents the characteristic-time scales of the various physical, chemical and biological processes occurring in a biofilter. From this time-scale chart, it appears that diffusion mechanisms are slower than the biological reactions. Thus, to improve biofiltration performance, the EBRT should be greater than the time required for diffusion processes, which is the case for low operating flow rates. However, the application of long EBRT requires larger filter bed volumes.

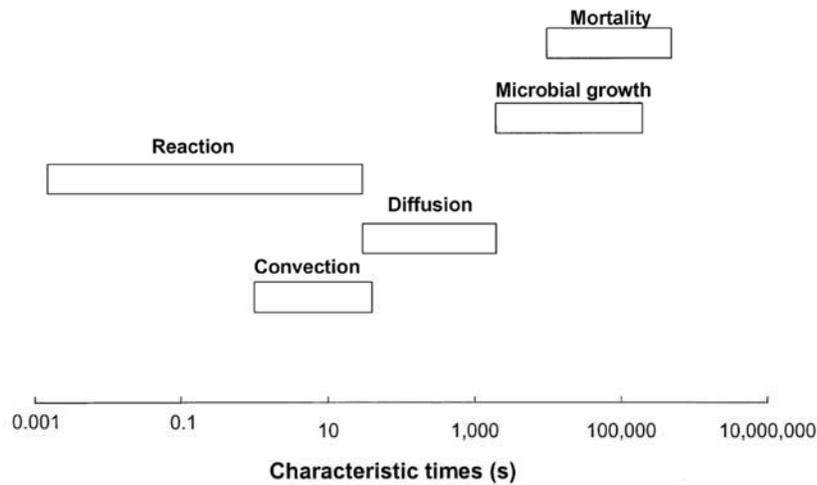


FIGURE 5 The characteristic times of the main mechanisms taking place in a biofilter, based on references of Picioreanu *et al.*, 1999, and Kissel *et al.*, 1989.

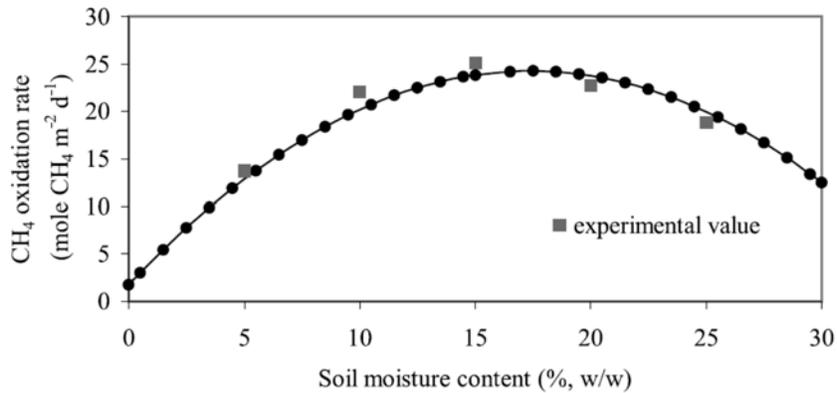
3.6.2 Moisture content

The moisture content of the filter bed is an important parameter as microorganisms require moisture to carry out their metabolic activities. Optimal moisture content of soil materials (from the upper layers of landfills) ideally lies between 13 and 15.5% w/w, on a dry basis (Stein and Hettiaratchi, 2001, Niekiema *et al.*, 2007). It was proven that CH₄ oxidation decreases when the MC of filter bed is less than 13% and that it could be totally inhibited when it reaches 1.5% (Bender and Conrad, 1992; Visvanathan *et al.*, 1999). The optimal filter bed water content depends on both the gas flow rate and the type of filter bed. The following table presents some typical water contents suggested in the literature.

Table 4 Optimal water content for some filter beds for methane elimination

Filter bed	Water content:% wt/wt	Authors
Compost	25–50	Humer and Lechner (1999b)
Landfill cover soil	13–30	Boeckx and Van Cleemput (1996), Park et al. (2002), Stein and Hettiaratchi (2001), Visvanathan et al. (1999), Giani et al. (2002)
Meadow soil	30–50	Mingxing and Jing (2002)
Woodland soil	18–33	Mingxing and Jing (2002)
Various soils	11–35	Bender and Conrad (1995), Christophersen et al. (2000)

Park et al., (2004), developed the following graph for soil.



3.6.3 Temperature

Methane oxidation is exothermic and, theoretically releases about 880 kJ per mole CH₄. In case of bio-oxidation, the larger portion of this energy is used for the anabolic reactions during CH₄ biodegradation. The other portion is transferred to both the filtering material and to the mixture of gases that traverses it. The reaction heat released creates a temperature gradient in the biofilter, between its lower and upper surfaces (Humer and Lechner 1999b; Nikiema et al.2004b; Nikiema et al. 2005). The significance of this thermal gradient depends on the input gas flow rate, the conversion, the type of filtering material and various other influential parameters.

Tests on the influence of temperature during CH₄ biofiltration were conducted with common filter materials, such as soils and composts.

Filter material	Temperature	Authors
Compost	29-30	Dammann et al. 1999; Streese et al. 2001; Mor et al. 2006
Soil	25-36	Stein & Hettiaratchi, 2001; Visvanathan et al. 1999; Cai and Yan1999;

Apart from these intervals, the decrease in the conversion was important. For example, it fell by around 50% when the temperature was reduced from 30 to 20 C (Dammann et al. 1999; Streese et al. 2001). Between -5 and 10 C as the ambient temperature, the biological elimination of CH₄ in an opened biofilter system (landfill cover soil) is considerably decreased, i.e. more than 80% compared to the value at 15 C (Christophersen et al.2000; Le Mer and Roger 2001). Therefore, the influence of temperature on the biological process constitutes the major limit for open biofilters, mainly during the winter season, when temperature falls to values lower than the limit that can be tolerated by the microorganisms consuming the CH₄ (Humer and Lechner 1999b).

The following graph is from a study conducted by Menard et al, (2011), to find the optimum temperature for methane oxidation in an inorganic filter bed (inlet CH₄ concentration of 7000 ppmv and flow rate of 0.25 m³/h).

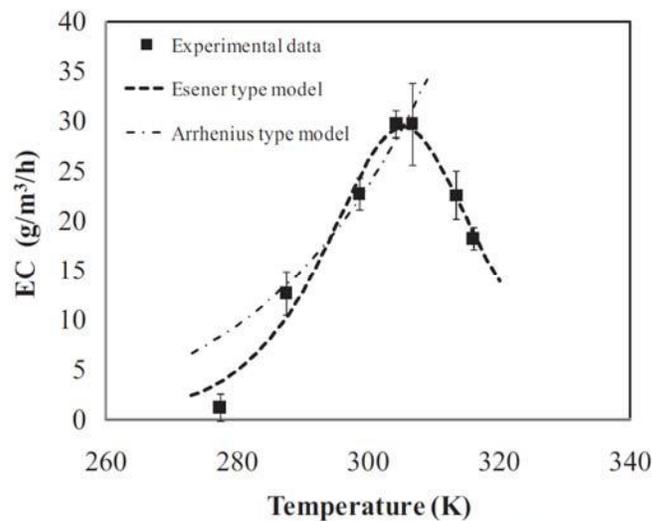


Figure 4: Effect of temperature on elimination capacity (EC) on methane in an inorganic biofilter.

The modified Arrhenius equation is given below:

$$EC = A \cdot e^{(-E_a/RT)} \quad (5)$$

where A is a pre-exponential factor ($\text{g/m}^3/\text{h}$), E_a is the activation energy for CH_4 biodegradation (kJ/mol), R is the universal gas constant (kJ/mol/K) and T is the absolute temperature (K).

The second model is the modified Esener model [30]:

$$EC = \frac{A' \cdot e^{(-E_1/RT)}}{1 + k \cdot e^{(-E_2/RT)}} \quad (6)$$

where A' ($\text{g/m}^3/\text{h}$) and k (dimensionless) are both pre-exponential factors and E_1 , E_2 are the activation energy for CH_4 biodegradation and for the thermal denaturation processes (kJ/mol), respectively.

Table 3: Temperature coefficients for Arrhenius and Esener models.

Coefficients	
A, $\text{g/m}^3/\text{h}$	8.5E + 6
E_a , kJ/mol	32
A' , $\text{g/m}^3/\text{h}$	1.6E + 13
k (-)	1.7E + 25
E_1 , kJ/mol	67
E_2 , kJ/mol	148

3.6.4 pH

From a practical viewpoint, the pH of the filter bed is a parameter of lesser importance because the biodegradation of CH_4 does not generate intermediate or final products capable of influencing significantly the pH. The optimal pH values for the oxidation of CH_4 are in fact the same as those promoting the growth in the majority of methanotrophs bacteria. For example, in soil-based filter beds, the optimum pH ranges between the values of 6.7 and 8.1 (Bender and Conrad 1995) while for peat, the range lies between 5 and 6.5 (Le Mer and Roger 2001).

Overall, Park et al., (2004) build an empirical regression model (R^2 of 0.858) of CH_4 oxidation rate as a function of environmental conditions as below,

$$\begin{aligned} \text{OR}_{\text{CH}_4} = & 1.7751(T_s) - 0.03712(T_s)^2 + 259.2124(\theta_s) - 744.925(\theta_s)^2 \\ & - 0.13519(\text{NH}_4^+\text{-N}) + (-18.2899) \end{aligned}$$

Where:

OR_{CH_4} = CH_4 oxidation rate ($\text{mol CH}_4 \text{ m}^{-2} \text{ d}^{-1}$)

T_s = soil temperature ($^\circ\text{C}$)

θ_s = soil moisture content (percent by weight), and

$\text{NH}_4^+\text{-N}$ = concentration of ammonium nitrogen (mg kg^{-1})

Average monthly soil T and MC

3.6.5 Nutrients

The methanotrophs require both macronutrients such as N, P, K and micronutrients such as Cu, Zn, Fe. Organic filter beds, such as compost usually contain some of them, but they decrease in long term operation. Among them N, P and Cu are strong determining factors for the performance of oxidation; K, Ca, Mg play a minor role (Nikiema et al, 2007). For CH₄ biofiltration, nitrate (NO₃⁻) seems to be the preferred type of inorganic nitrogen while ammonium can be a competitive inhibitor to CH₄ biodegradation (Girard et al., 2011).

The following table presents some studies on the impact of nutrients on methanotrophic bacteria (Veillette et al, 2012).

Table 2 Studies on the influence of nutrients on methane oxidation

Nutrient	Filler bed	[CH ₄] (% v/v)	Variation	Effect on CH ₄ oxidation	Maximum elimination capacity (EC)	Reference
Nitrogen	Inorganic (bio filter)	0.7–0.75	Increase of the NO ₃ ⁻ concentration from 0.14 to 0.75 g N L ⁻¹	Increase of the EC from 5.4 to 29.2 g CH ₄ m ⁻³ h ⁻¹	29.2 g CH ₄ m ⁻³ h ⁻¹	Nikiema et al. (2005)
		0.024–0.42	Increase of the NO ₃ ⁻ concentration from 0 to 0.5 g N L ⁻¹	A [NO ₃ ⁻] of 0.1 gN L ⁻¹ was as efficient to maintain the biofilter performance	14.5 g CH ₄ m ⁻³ h ⁻¹	Girard et al. (2011, 2012)
	0.3	Increase of the NH ₄ ⁺ concentration from 0.05 to 0.5 g N-NH ₄ ⁺ L ⁻¹	A linear decrease of the RE from 70 to 13 % Smaller increments of NH ₄ ⁺ are preferable	14.3 g CH ₄ m ⁻³ h ⁻¹	Veilleux et al. (2011, 2012)	
	Compost (Batch)	0.35–0.39	Increase the NO ₃ ⁻ concentration from 0 to 1.5 g N L ⁻¹ (over 6 months)	Decrease of the maximum uptake rate (<i>V_{max}</i>) from 131 to 109 g CH ₄ g ⁻¹ compost h ⁻¹	131 g CH ₄ g ⁻¹ compost h ⁻¹	Wilkinson et al. (2004)
Carbon	Soil	0.02–0.035	980 mg N kg ⁻¹ of NH ₄ NO added	Decrease of the EC from 3.8 to 1.4 g CH ₄ m ⁻³ h ⁻¹	3.8 g CH ₄ m ⁻³ h ⁻¹	Kightley et al. (1995)
		20	Increase of the NO ₃ ⁻ concentration from 10 to 50 mg N-NH ₄ ⁺ kg ⁻¹ soil	Little effect on the EC	55.8 g CH ₄ m ⁻³ h ⁻¹	Paik et al. (2002)
	0.12	Increase of the NH ₄ ⁺ concentration from 0 to 1 mg N g ⁻¹ soil	Decrease of the EC from 160 to 710 ng CH ₄ g ⁻¹ soil h ⁻¹	710 ng CH ₄ g ⁻¹ soil h ⁻¹	Krawchenko (2002)	
	0.05	Increase of the NH ₄ ⁺ concentration from 0 to 50 µg N-NH ₄ ⁺ g ⁻¹ soil	Decrease of the EC from 115 to 82 ng C g ⁻¹ soil h ⁻¹	163 ng CH ₄ g ⁻¹ soil h ⁻¹	Cai and Mosier (2000)	
Copper	Soil (batch)	0.2		Decrease of the EC from 1,128 to 1,176 ng CH ₄ g ⁻¹ soil h ⁻¹	1,176 ng CH ₄ g ⁻¹ soil h ⁻¹	Weir and Schmidt (1999)
		0.00018	0.4 mg g ⁻¹ soil of added acetate	Increase of the EC from 0 to 1.9 ng CH ₄ g ⁻¹ soil h ⁻¹	1.9 ng CH ₄ g ⁻¹ soil h ⁻¹	
	0.3–0.35	0.4 mg g ⁻¹ soil of added formate	Increase of the EC from 0 to 1.05 ng CH ₄ g ⁻¹ soil h ⁻¹	1.05 ng CH ₄ g ⁻¹ soil h ⁻¹		
	0.08–1	0.1 mg Cu L ⁻¹ of added Cu	Increase of the EC from 0 to 2.1 ng CH ₄ g ⁻¹ soil h ⁻¹ No effect on the CH ₄ oxidation rate	2.1 ng CH ₄ g ⁻¹ soil h ⁻¹	Not available	Henry and Orbic-Galic (1990)
Oxygen	Inorganic (bio filter)	0.08–1	Increase of the Cu concentration from 0 to 0.006 g Ca L ⁻¹	EC stable at 33.3±1.3 g m ⁻³ h ⁻¹	34.6 g CH ₄ m ⁻³ h ⁻¹	Nikiema et al. (2010)
		0.2	Increase of the O ₂ concentration from 30 to 70 % (w/v)	Decrease of the EC from 1.4 to 0.35 µg mL ⁻¹ culture h ⁻¹	1,400 ng CH ₄ mL ⁻¹ culture h ⁻¹	Hutton and Zobell (1949)
	0.35–0.39	Increase of the O ₂ concentration from 1.5 to 10.5 % (w/v)	Increase of the <i>V_{max}</i> from 30 to 178 ng CH ₄ g ⁻¹ compost h ⁻¹	178 ng CH ₄ g ⁻¹ compost h ⁻¹	Wilkinson et al. (2004)	
Phosphorus	Inorganic (bio filter)	0.13–1.2	1.5 g P L ⁻¹ is better than 0.3 g P L ⁻¹	EC up to 45 % higher	60 g CH ₄ m ⁻³ h ⁻¹	Nikiema and Heinz (2009)
		0.08–1	Variation of P concentration from 0 to 6.2 g P L ⁻¹	Highest EC at 3.1 g P L ⁻¹	44.7 g CH ₄ m ⁻³ h ⁻¹	Nikiema et al. (2010)
Potassium			Increase of the K concentration from 0 to 0.076 g K L ⁻¹	EC increased by 19 %	31.3 g CH ₄ m ⁻³ h ⁻¹	
Zinc			Nutrients added in various concentrations	Little effect on the EC	31.3 g CH ₄ m ⁻³ h ⁻¹	

Some of the other studies not listed in the above table are summarized as below (Huang et al, 2012).

Table 2 Studies of investigating the effect of N on the work of CH₄ biofilters (Nikiema *et al.*, 2007)

Sources	Filter beds	N forms & Concentration	Effect
Hettiaratchi <i>et al.</i> (2000)	Soil	25 mg N/kg soil in the form of NH ₄ ⁺ or NO ₃ ⁻	improve CH ₄ elimination by 100%
Chiemchaisri <i>et al.</i> (2001a)	Soil	≥30 mg N/kg soil in the form of NH ₄ ⁺ or NO ₃ ⁻	Inhibit CH ₄ elimination
Bronson and Mosier (1994); Cai and Mosier (2000); Hettiaratchi <i>et al.</i> (2000); Novikov and Stepanov (2002); Park <i>et al.</i> (2002)		10–200 mg N–NH ₄ ⁺ /kg soil	Inhibit CH ₄ elimination, however, its extension depends on the type of soil
Nikiema <i>et al.</i> (2005)	Inorganic filter material	Sodium nitrate, from 0.14 to 0.75 g N/L Sodium nitrate > 0.75 g N/L	5 times increase in the EC (from 130 to 700 g/(m ² -d). Decrease the CH ₄ oxidation
Boeckx and Van Cleemput (1996); Park <i>et al.</i> (2002)	Soil	25–100 mg N–NO ₃ ⁻ /kg soil	No CH ₄ elimination effect
Kumaraswamy <i>et al.</i> (2001)	Soil	2,500 mg N–NO ₃ ⁻ /kg soil	Inhibit CH ₄ elimination

For **inorganic** filter media, nutrients must be added, with a nutrient solution supplied daily to the biofilter (Nikiema et al., 2010). It is mainly composed of nitrogen (variable concentration) in the form of sodium nitrate (NaNO₃), a compound that has been frequently used as a nitrogen source for the preparation of culture media for methanotrophs (Choi et al. 2003). The quantities of the various compounds used to prepare a 1 L solution are presented in following table.

Table 1. Composition of multicomponent nutrient solution 1 (NS1).

Component	Concn. (g/L)
NaNO ₃	0.85–6.07
Na ₂ HPO ₄	0.86
KH ₂ PO ₄	0.53
K ₂ SO ₄	0.17
MnSO ₄ ·7H ₂ O	0.037
CaCl ₂ ·2H ₂ O	0.007
FeSO ₄ ·7H ₂ O	0.00112
ZnSO ₄ ·7H ₂ O	0.000576
MnSO ₄ ·7H ₂ O	0.000466
CuSO ₄ ·5H ₂ O	0.00025
KI	0.000166
H ₃ BO ₃	0.000124
NaMoO ₄ ·2H ₂ O	0.000096
CoCl ₂ ·6H ₂ O	0.000096

Optimum 4.55

3.6.6 Oxygen requirements and EPS

Oxygen plays an important role in regulating methanotrophic bacteria (Wilshushen et al., 2004). 0.75-1.5 % of O₂ was identified as optimal while above 3% of O₂ did not change the CH₄ oxidation efficiency. However a decrease of O₂ concentration from 3 to 1 % causes the fall off of CH₄ oxidation more than 50%. A relationship between the O₂ concentration and exopolysaccharides (EPS) was found by Wilshusen et al. (2004). High O₂ concentrations of 10.5% v/v increased EPS formation up to 250% when compared with low O₂ concentrations of 1.5% v/v. The formation of EPS was responsible for the decline of the CH₄ oxidation rate. This is confirmed by other studies where substantial accumulation of EPS was observed in soil with prolonged landfill gas exposure, more specifically in the most oxygenated area (Hilger et al. 2000). EPS are hydrophilic and they decrease the mass transfer of CH₄ and O₂. Wilshusen et al. (2004) also found that type I methanotrophs to be dominated in EPS formation areas, where type I grow in higher O₂ concentrations.

The study conducted by Hettaratchi et al (2004), confirmed the active aeration to be superior to passive aeration, in terms of methane oxidation rates. According to Haubrichs and Widmaann, (2006), CH₄ oxidation rate increased by a factor of 5.5 in active aeration experiments compared with passive tests. At O₂/CH₄ ratio of 2.5, nearly 100% of the CH₄ load was decomposed. By lowering the ratio from 2.5 to 2, the efficiency fell to values from 88% to 92%.

3.7 Methane oxidation inhibition

High or low MC: CH₄ oxidation could be totally inhibited when MC reaches 1.5% (Bender and Conrad, 1992; Visvanathan et al, 1999). When the moisture is too high, it acts as a rate limiting factor by preventing the flow and transfer of CH₄ and O₂ (Nikiema et al, 2007, Menard et al, 2012).

Higher T: If higher temperatures (>35 C) stimulate the activity of some methanotrophs, it should be noted that in such cases, the biofilter beds dry more quickly; this in turn leading to a decrease in the conversion rate (Visvanathan et al. 1999). Higher temperature above 45 C induces the denaturation of enzymes, which could inhibit the CH₄ oxidation.

High or low pH: A permanent inhibition was noted when the pH of the soil was changed by around 2 units, from 6.8 to 4.7 or from 6.8 to 9.0. This inhibition was partial for a unit variation, from 6.8 to 5.9 or 6.8 to 7.7, over the same operating conditions.

Presence of trace compounds: Menard et al. (2005) reviewed the co-oxidation of CH₄ and various non- methane organic compounds (NMOC) found in landfill biogas.

Table 2. Experiments of CH₄ oxidation in the presence of NMOCS.

References	CH ₄ concentrations (v/v)	Compounds tested	Observed effects (inhibition concentration level in % v/v)	Sample origin	
Chan and Parkin (2000)	1%–3%	Acetylene (0–1% v/v)	Inhibition (0.001% v/v)	Top soil from landfill	
		Ethylene (0–1% v/v)	Inhibition (0.1% v/v)		
		Ethane (0–1% v/v)			
		Methylchloride (0–1% v/v)	Methanogenesis inhibition		
		Methylfluoride (0–1% v/v)	Inhibition (0.1% v/v)		
		Dichloromethane (0–34 ppmv)	Competitive inhibition		
Chiemchaisri et al. (2001)	5%	Trichloroethylene (0–31 ppmv)	Competitive inhibition	Landfill soil biofilter	
		Tetrachloroethylene (0–70 ppmv)	Competitive inhibition		
		Benzene (0–45 ppmv)	Toxicity		
		HCFC-21 (0–380 ppmv)	Toxicity		
		HCFC-22 (0–463 ppmv)	Competitive inhibition and toxicity		
		Tetrachloromethane (9 ppmv)	No visible effect		
Scheutz et al. (2004) Scheutz et al. (2005)	15% 50%	Trichloromethane (10 ppmv)		Landfill cover soil Landfill cover soil	
		Dichloromethane (366 ppmv)			
		Trichloroethylene (19 ppmv)			
		Vinyl Chloride (121 ppmv)			
		Benzene (273 ppmv)			
		Toluene (93 ppmv)			
		Trichloroethane (1 ppmv)			
		Trichloroethylene (3 ppmv)			
		Dichloromethane (14 ppmv)			
		Mixture (18 ppmv)			
					May have toxic effect
					May have toxic effect
		No visible effect			
Alhama et al. (2010)	10%		Uncompetitive inhibition	Compost biofilter	

Note: HCFC-21: dichlorofluoromethane; HCFC-22: chlorodifluoromethane

As seen from the table, chlorinated compound have significantly affect CH₄ oxidation. No significant effect on CH₄ oxidation was detected due to the presence of aromatics such as benzene or toluene. Acetylene is a well-known 'suicide' substrate for enzymes due to its inactivation of the enzyme and its irreversible binding to the enzyme complex.

N-NH₄⁺: Generally, an increase of the N-NH₄⁺ concentration results in a higher percentage of inhibition at constant CH₄ concentration. Conversely, an increase of CH₄ concentration results in a lower percentage of inhibition at constant N-NH₄⁺ content (DeVisscher et al. 1999; Cai and Mosier 2000). Therefore, the inhibitory effect of NH₄⁺ could be minimized if higher CH₄ concentrations were continuously provided to the filter media. **Nitrite (NO₂⁻):** is clearly identified as an inhibitor for methanotrophs (Menard et al, 2012, Nikiema et al., 2005).

Presence of H₂S: The presence of H₂S was observed in the field scale study conducted by Pratt et al (2012) on dairy farm effluent pond. H₂S concentration in inlet biogas was 427 ppmv, and 0 ppmv in exit gas, indicating effective H₂S oxidation by the filter (landfill top soil:perlite). At the trial's conclusion, the filter experienced acid accumulation, due to oxidation of H₂S in the inlet biogas (evidenced by low pH of 3.9 and high sulphate-S 1079 mg/ kg] at the base of the filter compared with the top pH of 4.6, sulphate-S 369 mg/ kg). Eventhough, H₂S oxidation had adverse impact on methanotrophy, the filter's oxidation rate peaked at the end of the experiment indicating negligible H₂S impact on overall performance over the 16-month period.

3.8 Biofilter inoculation

None of the past studies focused on biofilter inoculation to enhance methane oxidation, except inorganic material was inoculated with landfill cover soil.

3.9 Biofilter configurations

The majority of biofilters, as used in lab-scale experiments, are closed systems. The air supply is ensured by a forced ventilation system. In a closed biofilter, maintaining the operational parameters unchanged is also a relatively easy practice, resulting in good performance, with CH₄ conversion values as high as 90% (Streese et al. 2001; Gebert et al. 2001; Nikiema et al. 2005).

The biofilter can also be an open system. Usually, in this case, the flow of the polluted gas in the bed proceeds upwards, while the O₂ diffuses from the ambient air into the bed (passive ventilation). The main disadvantage of this process lies in the difficulty of controlling the operational parameters, such as the temperature and moisture levels.

Based on the results obtained through bench scale (60 L biofilter) and pilot scale biofilter (4 m³), (two filters, one with compost [optimum at middle of the operation] and another with a mixture of

compost, peat and wood fibre [stable over a long period]) the following table is developed by Streese and Stegmann (2003). Assuming first order kinetics, and using empirical results.

Table 2: Calculation of the required biofilter volume for a methane degradation biofilter at a fictitious landfill.

parameter	optimum conditions	stable conditions
landfill gas production rate		50 m ³ /h
methane concentration		20%
dilution to obtain 2.5% of methane:		
⇒ raw gas flow rate		400 m ³ /h
⇒ raw gas concentration		2.5% = 18 g CH ₄ /m ³
given filter efficiency		90%
⇒ methane concentration of treated gas		0.25% = 1.8 g CH ₄ /m ³
kinetic coefficient k ₁	2.22 h ⁻¹	0.98 h ⁻¹
biofilter volume (equation (3))	415 m ³	940 m ³
volumetric load	0.96 m ³ /(m ³ h)	0.43 m ³ /(m ³ h)

$$V = \frac{1}{k} \cdot \dot{V} \cdot \ln \frac{c_m}{c_{out}}$$

Equation 3, \dot{V} – flow rate [m³/h]; V – biofilter volume [m³]

Using the same results, they developed the following chart. It shows the biofilter efficiencies and corresponding residual methane concentrations in the cleaned gas which can be achieved with different biofilter volumes. The required volume to obtain 90% efficiency is 800 m³ at a temperature of 22°C and 370 m³ at a temperature of 30°C, respectively.

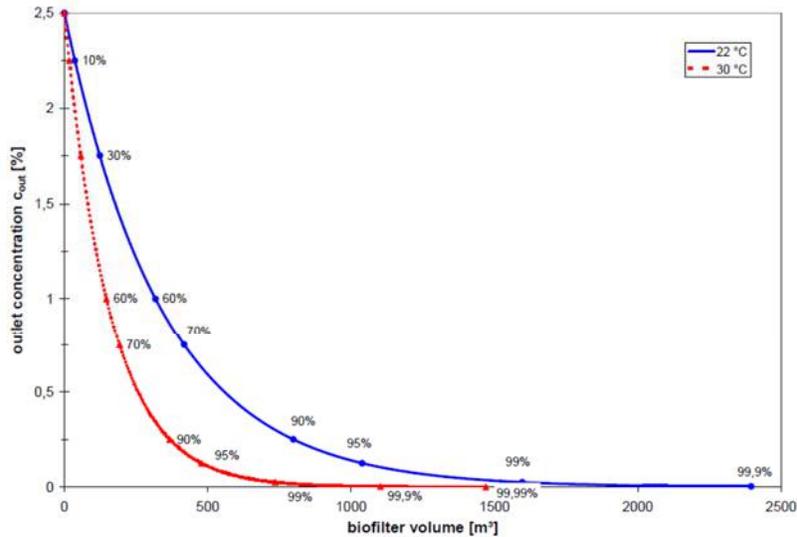


Figure 3. Treatment efficiencies and corresponding residual methane concentrations achievable with different biofilter volumes. Values calculated for the treatment of 400 m³/h of diluted landfill gas with a methane concentration of 2.5% using an optimised biofilter packing material consisting of compost, peat and squeezed wood fibres.

4 Models on methane oxidation biofilters

To gain better quantitative understanding of the biological and physical processes in CH₄ oxidation in biofilters, various models such as reactive-transport model, were developed.

Stein et al (2001) developed a numerical 1D reactive-transport model to determine the output gas concentration profiles, CH₄ oxidation rates, and surface flux rates, based on CH₄ source strength, soil bulk density, moisture content, and biological kinetic as the model inputs. Based on their model validation and Pokhrel (1998), the following relationship was obtained for first-order estimate of V_{max} for sandy loam soils,

$$\text{Average } V_{\text{max}} \text{ (nmol/hr.gdw)} = (500/315) \cdot \text{CH}_4 \text{ Flux (g/m}^2\text{.day)}.$$

Hettiarachchi et al, (2005, 2011) developed a comprehensive 3D numerical model incorporating advection-diffusive flow of gas, biological reactions and heat and moisture flow. It is expected that their numerical model (1D and 3D) could be used to select the most suitable granular medium, MBF bed thickness, and required surface area.

5 Research areas to be focused in the new project

Shorter version of description

The poorly studied areas were identified, so that we can build up new projects on these areas.

1. Filter material optimization or continued assessment of the suitability of the different filter materials and identifying parameters including loading rates, and residence time require for their optimum performance. (Potential materials: coconut fibre or flax seed or aged refuse, combine with compost as a source to bacteria, nutrients,).
2. Inoculation would be a novel approach as none of the past researchers focused on that ; identifying the groups of methanotrophs and hence how to stimulate them
3. How the methane oxidation efficiency is affected by presence of trace amounts of other gases
4. Phenomena impeding the operation, especially EPS formation, require further investigation
 - Knowledge of the specific pathways and mechanism of EPS formation may help to mitigate the inhibition
 - Lack of understanding of which factors control the EPS formation and thus how to avoid it
 - Influence of EPS on the CH₄ oxidation in field scale is unclear
5. Accurate monitoring of methane removal efficiency
 - Quantitative guide to the selection of field measurement methodology or fine tuning of the existing field methods, models, or validate empirical methods

Expanded version of new projects

1. Biofilter media development (conduct experiments to assess various filter media in terms of methane oxidation and long term durability).

A granular media/filter bed is required for the methanotrophs to grow and form a biofilm. There are several important characteristics that a good media needs to possess including suitable environment for microorganisms (nutrients, moisture, pH, and temperature), large surface area to maximize sorption capacity, high pore space to maximize empty bed contact time (EBCT), minimal pressure drops to reduce operating energy requirements, and low bulk density to decrease media compaction. The past applications mainly considered soil and compost based methane biofilter systems. However, due to potential instability of compost based materials, the risk of compaction and clogging of biofilters can occur. On the other hand this instability can shorten the service life of the biofilters. Therefore, in this research project we will put a special emphasis on filter material optimization. We will identify a new stabilized material that can use with coconut fibres and flax fibres. These fibres can serve as a structural material to prevent clogging of the biofilter and to provide optimal gas flow distribution.

2. Determination of limitations of biofilter operation in terms of trace gas toxicity.

The new filter materials also should withstand the other trace gases contain in waste gas streams from oil and gas operations, such as hydrogen sulphide (H_2S) and volatile organic compounds (VOCs).

On the other hand, none of the past researchers focused on the inoculation of biofilter materials that can enhance the methane oxidation. We will test the feasibility of seeding the reactors with methanotrophic species that are not present naturally in the filter material. Of particular interest are acid-tolerant and H_2S -tolerant methanotrophic species that we have recently discovered in geothermal environments and species found in oil sands tailings and natural oil sands outcrops that are adapted to withstand levels of H_2S and VOCs present in petroleum environments.

3. Development of biofilter configuration (vertical, horizontal and/or modular designs). Should be able to perform modeling using one of our numerical models. We may consider aerated biofilter systems as well.

The biofilter (bioreactor) design and construction is another major goal of this project. The design

criteria are based on the gas flow rate, and the other operating parameters of the methane biofilters such as moisture content, temperature, nutrients etc. The methane biofilter configurations that will be designed in this exercise include the size, thickness and the type of aeration (active vs passive aeration) for a given application.

4. Field construction and monitoring of biofilters to apply the monitoring protocol we are developing.

A monitoring method that provides information about the growth trends of methanotrophs and, therefore, the methane biofilter performance trends over a long period could overcome the limitations of the flux chamber method and significantly decrease the cost of methane biofilter monitoring. We plan to develop a method that involves monitoring microbial communities in addition to taking flux measurements to assess methane oxidation efficiencies.

References

1. Akdeniz, N., Janni, K. a., & Salnikov, I. a. (2011). Biofilter performance of pine nuggets and lava rock as media. *Bioresource technology*, 102(8), 4974–80. doi:10.1016/j.biortech.2011.01.058
2. Apel, W. a., Dugan, P. R., & Wiebe, M. R. (1991). Use of methanotrophic bacteria in gas phase bioreactors to abate methane in coal mine atmospheres. *Fuel*, 70(8), 1001–1003. doi:10.1016/0016-2361(91)90058-I
3. De Visscher, A., & Van Cleemput, O. (2003). Simulation model for gas diffusion and methane oxidation in landfill cover soils. *Waste management (New York, N.Y.)*, 23(7), 581–91. doi:10.1016/S0956-053X(03)00096-5
4. Delhoménie, M.-C., & Heitz, M. (2005). Biofiltration of air: a review. *Critical reviews in biotechnology*, 25(1-2), 53–72. doi:10.1080/07388550590935814
5. Delhoménie, M.-C., Nikiema, J., Bibeau, L., & Heitz, M. (2008). A new method to determine the microbial kinetic parameters in biological air filters. *Chemical Engineering Science*, 63(16), 4126–4134. doi:10.1016/j.ces.2008.05.020
6. Gebert, J., Groengroeft, A., & Miehlich, G. (2003). Kinetics of microbial landfill methane oxidation in biofilters. *Waste management (New York, N.Y.)*, 23(7), 609–19. doi:10.1016/S0956-053X(03)00105-3
7. Gebert, J., Stralis-Pavese, N., Alawi, M., & Bodrossy, L. (2008). Analysis of methanotrophic communities in landfill biofilters using diagnostic microarray. *Environmental microbiology*, 10(5), 1175–88. doi:10.1111/j.1462-2920.2007.01534.x
8. Girard, M., Nikiema, J., Brzezinski, R., Buelna, G., & Heitz, M. (2009). A review of the environmental pollution originating from the piggery industry and of the available mitigation technologies: towards the simultaneous biofiltration of swine slurry and methane This article is one of a selection of papers published in this Sp. *Canadian Journal of Civil Engineering*, 36(12), 1946–1957. doi:10.1139/L09-141
9. Han, D., Zhao, Y., Xue, B., & Chai, X. (2010). Effect of bio-column composed of aged refuse on methane abatement – A novel configuration of biological oxidation in refuse landfill. *Journal of Environmental Sciences*, 22(5), 769–776. doi:10.1016/S1001-0742(09)60175-3
10. Haubrichs, R., & Widmann, R. (2006). Evaluation of aerated biofilter systems for microbial methane oxidation of poor landfill gas. *Waste management (New York, N.Y.)*, 26(4), 408–16. doi:10.1016/j.wasman.2005.11.008
11. Hettiarachchi, V., Hettiaratchi, J. P. A., & Mehrotra, A. K. (2007). Comprehensive one-dimensional mathematical model for heat, gas, and moisture transport in methane biofilters. *Practice Periodical of Hazardous, Toxic, And Radioactive Waste Management*, (11), 225–233.
12. Hettiarachchi, V., & Hettiaratchi, P. (2011). Field-scale operation of methane biofiltration systems to mitigate point source methane emissions. *Environmental Pollution*, 159(6), 1715–1720.
13. Jiang, H., Chen, Y., Jiang, P., Zhang, C., Smith, T. J., Murrell, J. C., & Xing, X.-H. (2010). Methanotrophs: Multifunctional bacteria with promising applications in environmental bioengineering. *Biochemical Engineering Journal*, 49(3), 277–288. doi:10.1016/j.bej.2010.01.003

14. Menard, C., Avalos Ramirez, a., Nikiema, J., & Heitz, M. (2011). Analysis of the effects of temperature, the amount of nutrient solution and the carbon dioxide concentration on methane biofiltration. *International Journal of Sustainable Development and Planning*, 6(3), 312–324. doi:10.2495/SDP-V6-N3-312-324
15. Ménard, C., & Ramirez, A. (2012). Biofiltration of methane and trace gases from landfills: A review. ... *Reviews*, 53, 40–53. doi:10.1139/A11-022
16. Ménard, C., Ramirez, A. A., Nikiema, J., & Heitz, M. (2012). Effect of trace gases, toluene and chlorobenzene, on methane biofiltration: An experimental study. *Chemical Engineering Journal*, 204-206, 8–15. doi:10.1016/j.cej.2012.07.070
17. Nikiema, J., Bibeau, L., Lavoie, J., Brzezinski, R., Vigneux, J., & Heitz, M. (2005). Biofiltration of methane: An experimental study. *Chemical Engineering Journal*, 113(2-3), 111–117. doi:10.1016/j.cej.2005.04.005
18. Nikiema, J., Brzezinski, R., & Heitz, M. (2007). Elimination of methane generated from landfills by biofiltration: a review. *Reviews in Environmental Science and Bio/Technology*, 6(4), 261–284. doi:10.1007/s11157-006-9114-z
19. Nikiema, Josiane, Girard, M., Brzezinski, R., & Heitz, M. (2009). Biofiltration of methane using an inorganic filter bed: Influence of inlet load and nitrogen concentration This article is one of a selection of papers published in this Special Issue on Biological Air Treatment. *Canadian Journal of Civil Engineering*, 36(12), 1903–1910. doi:10.1139/L09-144
20. Nikiema, Josiane, & Heitz, M. (2010). The Use of Inorganic Packing Materials during Methane Biofiltration. *International Journal of Chemical Engineering*, 2010, 1–8. doi:10.1155/2010/573149
21. Nikiema, Josiane, Payre, G., & Heitz, M. (2009). A mathematical steady state model for methane bioelimination in a closed biofilter. *Chemical Engineering Journal*, 150(2-3), 418–425. doi:10.1016/j.cej.2009.01.032
22. Park, S. O. Y., Brown, K. W., & Thomas, J. C. (2004). The use of biofilters to reduce atmospheric methane emissions from landfills: Part 1- Biofilter Design. *Water, Air, and Soil Pollution*, 155, 63–85.
23. Pokhrel, D., Hettiaratchi, P., & Kumar, S. (2011). Methane diffusion coefficient in compost and soil–compost mixtures in gas phase biofilter. *Chemical Engineering Journal*, 169(1-3), 200–206.
24. Pratt, C., Walcroft, A. S., Tate, K. R., Ross, D. J., Roy, R., Reid, M. H., & Veiga, P. W. (2012). Biofiltration of methane emissions from a dairy farm effluent pond. *Agriculture, Ecosystems & Environment*, 152, 33–39. doi:10.1016/j.agee.2012.02.011
25. Scheutz, C., Kjeldsen, P., Bogner, J. E., De Visscher, A., Gebert, J., Hilger, H. Spokas, K. (2009). Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions. *Waste management & research*, 27(5), 409–455. doi:10.1177/0734242X09339325
26. Sly, L. I., Bryant, L. J., Cox, J. M., & Anderson, J. M. (1993). Development of a biofilter for the removal of methane from coal mine ventilation atmospheres. *Applied Microbial Biotechnology*, (1), 400–404.
27. Stein, V. B., Hettiaratchi, J. P. A., & Achari, G. (2001). Numerical Model For Biological Oxidation And Migration Of Methane In Soils. *Practice Periodical of Hazardous, Toxic, And Radioactive Waste Management*, (5), 225–234.

28. Streese, J., & Stegman, R. (2003). Design of biofilters for methane oxidation. *Proceedings of Sardinia*, (October). Retrieved from http://www.image.unipd.it/tetrawama/S2003/design_of_biofilters.pdf
29. Streese, J., & Stegmann, R. (2003). Microbial oxidation of methane from old landfills in biofilters. *Waste management (New York, N.Y.)*, 23(7), 573–80. doi:10.1016/S0956-053X(03)00097-7
30. Veillette, M., Avalos Ramirez, A., & Heitz, M. (2012). Biofiltration of air polluted with methane at concentration levels similar to swine slurry emissions: influence of ammonium concentration. *Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering*, 47(7), 1053–64. doi:10.1080/10934529.2012.667327
31. Veillette, M., Girard, M., Viens, P., Brzezinski, R., & Heitz, M. (2012). Function and limits of biofilters for the removal of methane in exhaust gases from the pig industry. *Applied microbiology and biotechnology*, 94(3), 601–11. doi:10.1007/s00253-012-3998-z
32. Visvanathan, C., Pokhrel, D., Cheimchaisri, W., Hettiaratchi, J. P. A., & Wu, J. S. (1999). Methanotrophic activities in tropical landfill cover soils: effects of temperature, moisture content and methane concentration. *Waste Management & Research*, 17(4), 313–323.
33. Wilshusen, J. H., Hettiaratchi, J. P. a, & Stein, V. B. (2004). Long-term behavior of passively aerated compost methanotrophic biofilter columns. *Waste management*, 24(7), 643–653.
34. Wilshusen, J., & Hettiaratchi, J. (2004). Methane oxidation and formation of EPS in compost: effect of oxygen concentration. *Environmental Pollution*, 129(2), 305–314.

9.3 Thesis: Performance of Actively-Aerated Biofilters Using a Multiple-Level Air Injection System to Enhance Biological Treatment of Methane Emissions

UNIVERSITY OF CALGARY

Performance of Actively-Aerated Biofilters Using a Multiple-Level Air Injection System
to Enhance Biological Treatment of Methane Emissions

by

Hasti Farrokhzadeh

A THESIS

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Abstract

The present research is intended to remove methane from a gas stream by converting it into carbon dioxide by means of aerobic methane-oxidizing microorganisms. Such technology can be useful when dealing with biogas from landfills, or solution gas from natural gas wells. Taken that methane oxidation is an aerobic process, a major enhancement in efficiency is observed by the active introduction of oxygen throughout the biofilter profile. Thus, with the aim of improving conventional biofilters, in this study a multiple-level aeration biofilter design is proposed.

Laboratory column experiments were run to study three different actively-aerated methane biofilter configurations. Columns were aerated at one, two, or three levels along the bed thickness. Inlet methane loading rates were increased at five stages between 6 mL/min to 18 mL/min. A first set of columns were operated introducing air at flow rates calculated based on the oxidation reaction stoichiometry. The effects of methane feeding rate, levels of aeration, and residence time were evaluated. Based on the results obtained from a mixed Analysis of Variances, the response surface, and laboratory observations, it was suggested that the biofilter column with two aeration levels has the most even performance over time, maintaining an average oxidation efficiency of 85.1% over the 195 days of experiments.

A second set of columns with the same aeration designs were run for varying air to methane flow rates. Air flow rates were changed inlet air flow rates between $\frac{1}{4}$ of stoichiometric levels to 1.5 times higher than stoichiometric values. The performance of columns was recorded for 90 days. With air flow rates set at $\frac{1}{4}$ of the stoichiometric value, an average 13.8% reduction in performance of the biofiltration designs was observed. However, more experiments are required to evaluate the long-term performances of aerated biofilters operated under low air to methane flow rate ratios.

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Table of Contents

Abstract	i
Acknowledgements	ii
List of Tables	vi
List of Figures and Illustrations	viii
List of Symbols, Abbreviations and Nomenclature	xiii
CHAPTER ONE: INTRODUCTION	1
1.1 General	1
1.2 Scope and Objectives	5
1.3 Overall Approach	7
CHAPTER TWO: LITERATURE REVIEW	1
2.1 Overview	1
2.2 Atmospheric Methane Chemistry	1
2.3 Methane Elimination Methods	7
2.3.1 Thermal Treatment	7
2.3.2 Biological Treatment	8
2.3.2.1 Airlifts	9
2.3.2.2 Stirred Tanks	11
2.3.2.3 Bioscrubbers	13
2.3.2.4 Biotrickling filters	14
2.3.2.5 Biofilters	15
2.4 Role of methanotrophs in CH ₄ Oxidation	17
2.5.1 Filter Bed	21
2.5.2 Methane Concentration	22
2.5.3 Oxygen Concentrations	23
2.5.4 Temperature	23
2.5.5 Moisture content	25
2.5.6 Gas contact time	27
2.5.7 pH levels	28
2.5.8 Nutrients	29
2.5.9 Exopolymeric substances	31
2.6 Gas Transport and Biological Reactions	32
2.6.1 Gas Transport Mechanisms	33
2.6.2 Microbiological reactions	37
2.7 Methane Biofilter Configuration	40
2.7.1 Mode of aeration: Active vs. passive	40
2.7.2 Flow direction: up-flow vs. down-flow	45
2.7.3 Setting: closed vs. open	46

CHAPTER THREE: THEORETICAL AND METHODOLOGICAL CONSIDERATIONS	47
3.1 Overview.....	47
3.2 Compost characteristics	47
3.2.1 Organic Carbon Fraction	47
3.2.2 Moisture Content	48
3.2.3 Water Holding Capacity	50
3.2.4 Compost Maturity Index.....	51
3.2.4.1 Oxygen uptake rate	52
3.2.4.2 Carbon dioxide evolution rate.....	53
3.2.4.3 Dewar self-heating test	53
3.2.4.4 Solvita ® test.....	54
3.2.5 Material properties.....	55
3.2.6 Flow rates	58
3.2.7 Gas residence time.....	60
3.2.8 Compaction Procedure	65
CHAPTER FOUR: MATERIALS AND METHODS.....	68
4.1 Overview.....	68
4.2 Variable definition	68
4.3 Operating conditions.....	70
4.3.1 Compost characteristics.....	70
4.3.1.1 Moisture Content	70
4.3.1.2 Water Holding Capacity	70
4.3.1.3 Compost Maturity Index	70
4.3.1.4 Material properties	71
4.3.2 Flow rates	73
4.3.3 Compaction Procedure	73
4.4 Experimental protocol.....	73
4.4.1 Experimental design	75
4.4.2 Response Surface Methodology	83
4.4.3 Analysis of Variances	83
4.5 Laboratory-scale Biofilters	85
4.6 Biofilter Packing Media.....	88
4.7 Gas Sampling System	89
4.8 Gas Chromatography (GC).....	89
4.9 Biofilter Performance Assessment.....	91
4.10 Laboratory batch experiments for determination of reaction kinetics parameters	92
CHAPTER FIVE: EXPERIMENTAL RESULTS AND DISCUSSION	95
5.1 Overview.....	95
5.2 Preliminary Results.....	97
5.3 Methane Oxidation as a function of time: Experimental results	101
5.4 Residence time	107

5.5 Concentration Profiles	110
5.5.1 Concentration Profiles for the first stage.....	110
5.6 Analysis of Variances (ANOVA)	120
5.6.1 Assumptions	120
5.6.2 Results	122
5.6.3 Response Surface.....	129
5.7 Batch experiment Experiments: Operated under stoichiometric aeration rates.....	139
5.7.1 Methane Oxidation Kinetics.....	139
5.7.2 Microbiological analysis	143
5.8 Performance of aerated biofilter columns run at various air to methane flow rate ratios	148
5.8.1 Gas concentration profiles.....	149
5.8.2 Concentration Profiles for the first stage.....	149
5.8.3 Concentration Profiles for the second stage	153
5.8.4 Concentration Profiles for the third stage.....	155
5.8.5 Concentration Profiles for the fourth stage	157
5.8.6 Response surface	159
 CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH.....	161
6.1 Conclusions and Summary	161
6.1.1 Effects of aeration levels on MBF performance.....	161
6.1.2 Effects of gas residence time following changes in inlet flow rates on MBF performance	162
6.1.3 Changes in MBF performance imposed by changing O ₂ flow rates	163
6.2 Recommendations.....	163
 REFERENCES	166
 APPENDIX A : CONCENTRATION PROFILES FOR COLUMNS RUN AT STOICHIOMETRIC AIR FLOW RATES	178

List of Tables

Table 2-1 Gas diffusion models in porous media	37
Table 3-1- EBRT comparisons of EBRT values for lab-scale CH ₄ biofilters (Limbri et al., 2013).	61
Table 4-1 Compost characteristics used for CH ₄ biofilter columns	71
Table 4-2 Laboratory columns material characterization	72
Table 4-3 Star design for three factors.....	76
Table 4-4 Experimental design matrix based on Doehlert approach (two-factor design)	80
Table 4-5 Experimental design matrix based on Doehlert approach (Three-factor design).....	82
Table 4-6 ANOVA table for the least squares fit of a linear model (Ferreira et al., 2007)	84
Table 4-7 Gas chromatography system specifications.....	91
Table 5-1 Varying loading rates in g/m ³ /day for preliminary experiments	97
Table 5-2 Average CH ₄ oxidation rates in g/m ³ /day for preliminary experiments	99
Table 5-3 Average CH ₄ oxidation efficiencies (%) for preliminary experiments	101
Table 5-4 Average oxidation rates and efficiencies of aerated columns over time	102
Table 5-5 Residence times of biofilter columns under varying flow rates and aeration levels ..	108
Table 5-6 SPSS Output 1-Descriptive Statistics.....	123
Table 5-7 SPSS Output 2-Mauchly's Test of Sphericity.....	125
Table 5-8 SPSS Output 3- Tests of Within Subjects Effects	126
Table 5-9 SPSS Output 4- Levene's Test of Equality of Error Variances	127
Table 5-10 SPSS Output 5- Tests of Between Subjects Effects	128
Table 5-11- SPSS Output 6- Pairwise Comparisons	129
Table 5-12 Michaelis-Menten reaction parameters for the three-level aerated biofilter column	140
Table 5-13 Michaelis-Menten reaction parameters for the two-level aerated biofilter column .	141

Table 5-14 Michaelis-Menten reaction parameters for the one-level aerated biofilter column .	142
Table 5-15 Summary of the microbial population in column biofilters	144
Table 5-16 Methanotroph community in the biofilter columns.....	145
Table 5-17 First stage of the Doehlert experimental design for columns run at varying air to CH ₄ ratios.....	150
Table 5-18 Second stage of the Doehlert experimental design for columns run at varying air to CH ₄ ratios.....	153
Table 5-19 Third stage of the Doehlert experimental design for columns run at varying air to CH ₄ ratios.....	155
Table 5-20 Fourth stage of the Doehlert experimental design for columns run at varying air to CH ₄ ratios.....	157

List of Figures and Illustrations

Figure 1-1 Recent trends in global atmospheric methane concentrations (adapted with permission from (Chai et al., 2016).	3
Figure 2-1 Major reactions in CH ₄ oxidation chain in NO _x rich air direct path to CH ₂ O dominates, whereas in NO _x -poor air CH ₂ O is mostly formed through the CH ₃ O ₂ H pathway (Reprinted with permission, Lelieveld, Crutzen, and Dentener 1998).....	6
Figure 2-2 Schematic diagram of a concentric tube airlift (Adapted with permission from (Lopez et al., 2013)).....	10
Figure 2-3 Schematic diagram of a stirred tank (Adapted with permission from (Lopez et al., 2013)).....	11
Figure 2-4 Experimental setup of a lab-scale capillary bioreactor (copied with permission from (Rocha-Rios et al., 2013))......	12
Figure 2-5 Schematic diagram of a bio-washer or bio-scrubber. The adsorption and biodegradation steps are separated (Copied with permission from (Devinny et al., 1999)).	14
Figure 2-6 Schematic diagram of biological trickling filter. Because the packing material is covered by a biofilm, adsorption and biodegradation are achieved in the same reactor (Copied with permission from (Devinny et al., 1999))......	15
Figure 2-7 Internal mechanism of biofiltration. Contaminated air (CG) passes through the filter bed medium with O ₂ and sorbs into a microbial biofilm/liquid phase attached to the filter medium. Microbes convert the contaminant to CO ₂ and water (Copied with permission from (Devinny et al., 1999))......	16
Figure 2-8 Pathways for the oxidation of CH ₄ and assimilation of formaldehyde. Abbreviations: CytC, cytochrome c; FADH, formaldehyde dehydrogenase; FDH, formate dehydrogenase (Copied with permission from (Hanson and Hanson, 1996)).	18
Figure 2-9 Schematic of a passive MBF for landfill gas emission control (Reprinted with permission from (Scheutz et al., 2009))......	41
Figure 2-10 schematic representation of a lab-scale active MBF set-up (Reprinted with permission from (Du Plessis et al., 2003))......	43
Figure 3-1- Methane Oxidation Curves (MOCs) for V _{max} at different Moisture Contents (Adapted with permission from (Pokhrel, 2006))......	50

Figure 3-2 Number of hammer drops vs. compost bulk density (g/cm^3) in the standard proctor test	67
Figure 4-1 Experimental procedure in optimizing O_2 levels for the multiple level air injection MBF.	75
Figure 4-2-Two factor design based on the uniform shell design (starting points of the simplex are marked with an equilateral triangle).	80
Figure 4-3 3D view of the Doehlert experimental domain. The dots represent the experiments of the Table 4-5 (Adapted with permission from Imandi, Bandaru, Somalanka, & Garapati, 2007).	81
Figure 4-4 Aeration probes	86
Figure 4-5- Schematic of laboratory biofilter column	87
Figure 4-6 Photograph of biofilter columns.....	Error! Bookmark not defined.
Figure 4-7 Block diagram of a gas chromatography system Reprinted, with permission, from (Terry et al., 1979)).....	90
Figure 4-8 Graphical representation of different forms of the Michaelis-Menten equation (reprinted with permission from (Pokhrel, 2006))	94
Figure 5-1 Varying loading rates for preliminary experiments	98
Figure 5-2 CH_4 oxidation rate for preliminary experiments	99
Figure 5-3 CH_4 oxidation efficiency for preliminary experiments	100
Figure 5-4 Three-level aerated column oxidation rate ($\text{g}/\text{m}^3/\text{day}$) over time	103
Figure 5-5 Three-level aerated column oxidation efficiency (%) over time	103
Figure 5-6 Two-level aerated column oxidation rate ($\text{g}/\text{m}^3/\text{day}$) over time	104
Figure 5-7 Two-level aerated column oxidation efficiency (%) over time	104
Figure 5-8 One-level aerated column oxidation rate ($\text{g}/\text{m}^3/\text{day}$) over time	105
Figure 5-9 One-level aerated column oxidation efficiency (%) over time	105
Figure 5-10 Gas profiles for day 6 (first stage) for a) Column aerated at three levels b) Column aerated at 2 levels c) Column aerated at one level.....	111

Figure 5-11 Gas profiles for day 58 (second stage) for a) Column aerated at three levels b) Column aerated at 2 levels c) Column aerated at one level	113
Figure 5-12 Gas profiles for day 92 (third stage) for a) Column aerated at three levels b) Column aerated at 2 levels c) Column aerated at one level	115
Figure 5-13 Gas profiles for day 147 (fourth stage) for a) Column aerated at three levels b) Column aerated at 2 levels c) Column aerated at one level	117
Figure 5-14 Gas profiles for day 190 (fifth stage) for a) Column aerated at three levels b) Column aerated at 2 levels c) Column aerated at one level	119
Figure 5-15 3D response Surface for columns run at stoichiometric air flow rates (the x-axis indicates flow rates measured in ml/min, the y-axis is the No of aeration levels (1,2, or 3), and the z-axis is the oxidation rate in g/m ³ /day)	130
Figure 5-16 Droplets formed on the top of the three-level aerated biofilter column.....	131
Figure 5-17 EPS formation in the column aerated at one level	132
Figure 5-18 Contour plot for average oxidation rates over time at varying flow rates and number of aeration levels.....	133
Figure 5-19 Oxidation rate contour plots for constant flow rate of 6 mL/min	134
Figure 5-20 Oxidation rate contour plots for constant flow rate of 9 mL/min	135
Figure 5-21 Oxidation rate contour plots for constant flow rate of 12 mL/min	136
Figure 5-22 Oxidation rate contour plots for constant flow rate of 15 mL/min	137
Figure 5-23 Oxidation rate contour plots for constant flow rate of 18 mL/min	138
Figure 5-24 RuMP pathway in type I methanotrophs (reprinted under the Creative Commons Attribution-Share alike license)	146
Figure 5-25 Serine pathway in type II methanotrophs (reprinted under the Creative Commons Attribution-Share Alike license)	147
Figure 5-26 Gas profiles for day 24 (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	152
Figure 5-27 Gas profiles for day 62 (second stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level	154

Figure 5-28 Gas profiles for day 76 (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	156
Figure 5-29 Gas profiles for day 90 (fourth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	158
Figure 5-30- 3D response surface for biofilter columns operated at different air to CH ₄ flow rate ratios.....	160
Figure 6-1 Conceptual Design of the field-scale multiple-level aerated MBF.....	165
Figure A-1 Gas profiles for August 13th (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	178
Figure A-2 Gas profiles for August 16th (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	179
Figure A-3 Gas profiles for August 19th (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	180
Figure A-4 Gas profiles for September 10th (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	181
Figure A-5 Gas profiles for September 16th (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	182
Figure A-6 Gas profiles for September 25th (second stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	183
Figure A-7 Gas profiles for October 1st (second stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	184
Figure A-8 Gas profiles for October 8th (second stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	185
Figure A-9 Gas profiles for October 13th (second stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	186
Figure A-10 Gas profiles for November 13th (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	187
Figure A-11 Gas profiles for November 18th (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	188
Figure A-12 Gas profiles for November 24th (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	189

Figure A-13 Gas profiles for November 30th (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	190
Figure A-14 Gas profiles for December 4th (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	191
Figure A-15 Gas profiles for December 21st (fourth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	192
Figure A-16 Gas profiles for December 27th (fourth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	193
Figure A-17 Gas profiles for January 18th (fourth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	194
Figure A-18 Gas profiles for January 21st (fourth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.....	195
Figure A-19 Gas profiles for January 26th (fifth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level	196
Figure A-20 Gas profiles for March 26th (fifth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level	197
Figure A-21 Gas profiles for March 28th (fifth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level	198
Figure A-22 Gas profiles for April 1st (fifth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level	199

List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
θ	Porosity
ϕ	Porosity
ε	Air-filled porosity
α	Dispersivity
μ	Gas viscosity
τ	tortuosity
ρ_d	Dry bulk density
ρ_s	Soil particle density
Aq.	Aqueous phase
Atm.	Atmospheres
C	Concentration
$^{\circ}\text{C}$	Celcius
CH_4	Methane
CH_2O	Formaldehyde
Cl	Chlorine
CO	Carbon Monoxide
CO_2	Carbon dioxide
CSt	Centistokes
Cu	Copper
D	Dispersion coefficient
dw	Dry weight
h	hours
H	Henry's law constant
HO_x	Hydrogen Oxides
J	Flux
kg	kilograms
L	Litres
k_z	Intrinsic permeability of the porous medium
K_m	Half-saturation constant
m	meters
mg	milligrams
min	minute
mol	moles
nmol	nanomoles
N_2	Nitrogen
NH_4^+	Nitrate
NO	Nitrogen Monoxide
NO_x	Nitrous oxides
NO_3^-	Ammonium
$\text{O}^1(\text{D})$	Electronically excited oxygen atoms
O_2	Oxygen
O_3	Ozone
OH	Hydroxyl Radical
P	Pressure, Phosphorus
Q	Flow rate
R	Universal Gas Constant
T	Absolute temperature

UV	Ultraviolet
v	velocity
V	CH ₄ consumption rate or Volume
V _{max}	Maximum CH ₄ consumption rate

Abbreviation	Definition
CAPP	Canadian Association of Petroleum Products
CO ₂ (eq.)	Carbon dioxide equivalents
EBRT	Empty bed residence time
EPS	Exopolymeric substances
GHG	Greenhouse gas
GWP	Global warming potential
IPCC	Intergovernmental Panel on Climate Change
MBF	Methane Biofilters
MC	Moisture content
MMO	Methane monooxygenase
NAP	Non-aqueous phase
pMMO	Particulate methane monooxygenase
RuMP	Ribulose monophosphate
sMMO	Soluble methane monooxygenase
SOUR	Specific Oxygen Uptake Rate
TCA	Tricarboxylic acid
TPPB	Two-phase partition bioreactor

Chapter One: Introduction

1.1 General

Global climate change refers to persisting variations in the Earth's climate excluding natural changes observed over comparable periods of time. Natural factors such as massive volcanic eruptions to slight variations in Earth's orbit have affected the Earth's climate historically, giving rise to periods of glaciation followed by warmer periods (Venhaus, 2012). Solar radiation is the main power source for the Earth's climate system. Since average temperature of the planet has remained constant over centuries, the solar energy received by the Earth's surface must be in balance with the energy reflected back from the surface. Of the incoming energy (i.e. short wave radiations), half is absorbed by the surface, 30% is emitted back to the space by aerosols, clouds, and the surface (i.e. albedo), and 20% is absorbed by the atmosphere. Certain atmospheric constituents including carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), water vapour (H₂O), and other greenhouse gases (GHGs) absorb most of the long-wave radiations of the Earth's surface. This phenomenon, known as the greenhouse effect, increases the temperature of the first two layers of the atmosphere as well as the Earth's surface (Cubasch et al., 2013). Anthropogenic, industrial, and biogenic sources enhance the greenhouse gas effect by emitting more GHGs that are significant drivers for the climate change.

The strength of GHGs is commonly compared to CO₂, mainly since recent increases in GHG effects have been linked to increased CO₂ concentrations. Also, CO₂ is relatively inert in the atmosphere. With an atmospheric lifespan much shorter than that of CO₂, to be discussed later, the relative GHG impact of CH₄ with respect to CO₂, also known as Global Warming Potential (GWP), is defined depending on the time span considered. With a GWP of 28-34 over a time period of

100 years, and 85 over a window of 20 years, CH₄ contribution to heat trapping and global climate change is significant (Caulton et al., 2014; Myhre et al., 2013).

Anthropogenic CH₄ has natural gas and petroleum systems as its largest contributors above landfills, wastewater treatment plants, rice paddy agriculture, and livestock farms. In addition to anthropogenic emissions, CH₄ is produced naturally under low O₂ conditions through fermentation processes by methanogenic bacteria (Conrad, 1996). Biogenic sources include emissions predominantly from wetlands, termites, wildfires, and small quantities from the oceans (EPA, 2010).

CH₄ accounts for approximately 13% of Canada's total emissions, and 14.3% of the global anthropogenic GHG emissions (Chai et al., 2016; Environment Canada, 2014). In 2013, Government of Canada estimated annual CH₄ emissions in Canada to be 24 Mt of the carbon dioxide equivalents (CO₂ eq.) from landfills, and also 24 Mt of CO₂ eq. from the petroleum industry (Government of Canada, 2013). Since pre-2014 reports have used a global warming potential of 25 for CH₄, modified emissions based on 2013 Intergovernmental Panel on Climate Change (IPCC) report value of 28-34 can be recalculated as 65 Mt of CO₂ eq. Also, the report solely considers CH₄ releases from the petroleum industry that are intentional, i.e. CH₄ that can be captured or flared if desired. Abandoned oil and gas wells and leakages from hydraulic fracturing provide pathways for subsurface migration and venting to the atmosphere (Howarth, 2014; Kang et al., 2014). Such emissions are either not included or underestimated in emission inventories. Therefore, the actual preventable CH₄ emissions is even higher than 65 Mt.

Canadian Association of Petroleum Products (CAPP) claims that total CO₂ eq. of CH₄ from the oil and gas sector in year 2000 were estimated to be 46% of the total emissions (Clearstone

Engineering Ltd., 2005). Increased anthropogenic contribution to GHG production has caused the globally averaged surface concentrations of CH₄ to rise from 722 ± 25 ppb in year 1750 to 1803 ± 22 ppb in 2011 (Myhre et al., 2013). This value has been increasing by 0.5% annually in the past 30 years, as shown in Figure 1-1. Over the past 250 years, CH₄ concentrations have increased by 250%, surpassing CO₂ concentrations which doubled over the same period (Chai et al., 2016).

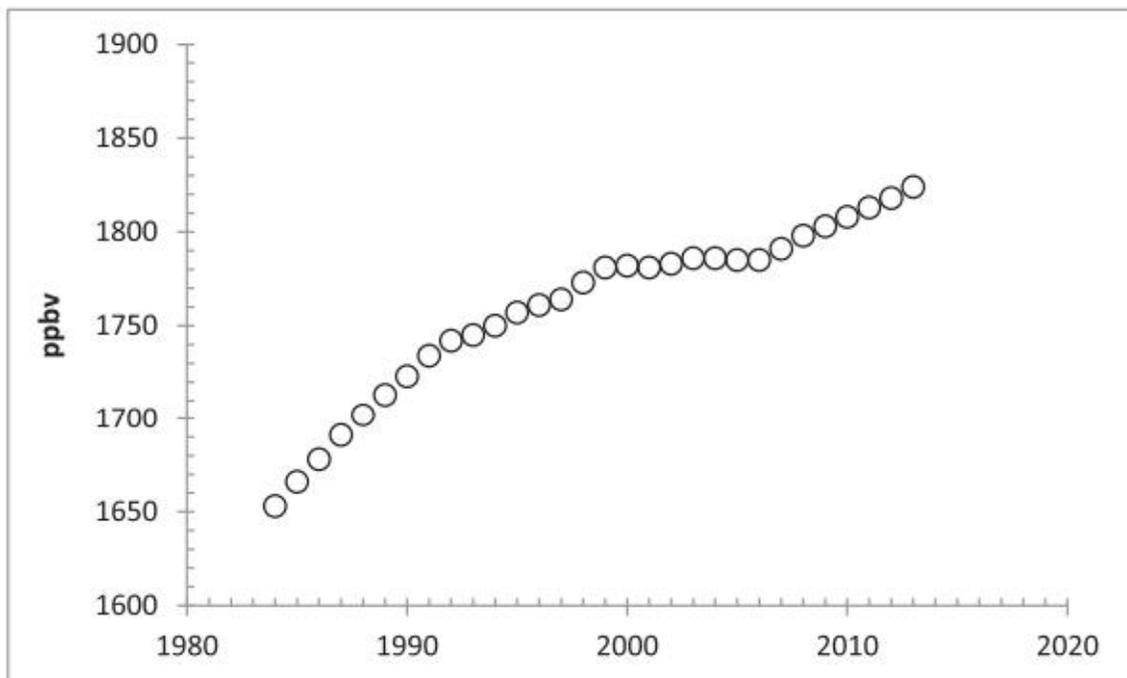


Figure 1-1 Recent trends in global atmospheric methane concentrations (adapted with permission from (Chai et al., 2016).

Therefore, research in CH₄ economy is essential in meeting the current society's energy needs in a sustainable manner, as well as fulfilling our G7 commitment to decarbonising the global economy over the course of the century (G7, 2015). Developing effective managerial techniques

for addressing CH₄ emissions from anthropogenic sources is a critical step towards mitigating its impact on the global warming phenomenon.

In fact, CH₄ capture and treatment programs have proven to be efficient in reducing its GHG effects in short time, as the element's atmospheric lifetime of 12.4 years is very much lower than CO₂'s. One exercised option regarding CH₄ emissions is to flare the gas, combust without energy recovery. Flaring is a simple and rather maintenance-free alternative when the logistics of the considered site do not support energy recovery infrastructures or other CH₄ emission control technologies (Chai et al., 2016; Goldsmith et al., 2012). If the flow rates of the surplus gas are too low or too intermittent, or if the heating value of the gas is too low to sustain combustion, gases are instead simply released to the atmosphere (Johnson and Coderre, 2012a). Because of its high energy content, CH₄ is also commonly used to produce electricity, combined heat and power (CHP), compressed to be vehicle fuel (CNG), or other oxygenated fuels. However, such applications rely mostly on the economics of the site as well as availability of utility infrastructures, regulations, and sufficient availability of the gas (Chai et al., 2016). This option is rarely practiced in most oil and gas producing regions as flaring and venting sites are mostly distributed among smaller areas (Johnson and Coderre, 2012b). Moreover, flaring produces other pollutants in addition to CO₂ such as particulates, sulphur dioxide¹, carbon monoxide (CO) and other by-products of incomplete combustion (Johnson and Coderre, 2012a).

A simple, cost-effective and environmentally-friendly alternative to flaring is biological oxidation, a technology based on CH₄-oxidising bacteria, known as methanotrophs. Various

¹ In cases where the flare gas contains sulphur-containing compounds such as hydrogen sulphide (H₂S)

biological methods used for CH₄ elimination include biofiltration, bioscrubbing, biotrickling filters, and airlifts that will be discussed in great detail in Chapter 2. Biofiltration offers the simplest and most cost-effective implementation and operation. Biofilters have been widely used during the past two decades (Lebrero et al., 2016). Biofilters have the potential to replace flaring when flow rates are low (lower than 15–30 m³/h) or intermittent, concentrations are low (below 20–30% v/v), or the treating gas contains sour gas (H₂S) without producing any of the secondary pollutants. Methane biofilters (MBFs) entail a layer of granular material on which the methanotrophs reside. CH₄ acts as the energy and carbon source for the microbial population and is oxidised to CO₂ (Mancebo et al., 2016). The mechanism is affected by a variety of parameters including CH₄ concentrations and flow rates, O₂ availability, temperature, moisture content, and the granular media used.

Despite the significant role of CH₄ biofilter design on its performance, insufficient effort has been made to understand and model operational procedures, and modify classical practices in order to exploit the method's potential versatility. Although high CH₄ oxidation capabilities of actively-vented biofilters have already been established, a comprehensive study is needed for to optimize their design configuration.

1.2 Scope and Objectives

The scope of this work is to develop an efficient configuration for active CH₄ biofilters. With the aim of finding the most suitable design configuration for actively-aerated CH₄ biofilters, a multiple level air injection system is proposed in this work to ensure that O₂ availability is no longer a limitation to CH₄ degradation. Having O₂ enter the system at several inlets along the biofilter bed is a promising approach towards CH₄ oxidation all through the thickness. In order to

address the general research question regarding the multiple level air injection system, the following questions are investigated further:

- What are the optimum numbers of air injection points in a multi-injection biofiltration system?
- How is the biofilter performance affected by variations in inlet gas flow rates and residence time?
- What is the minimum air flow rate required to maintain a satisfactory oxidation rate?

To answer the above-mentioned research questions, the following objectives need to be met;

- Determine the effects of injection points on MBF performance;
- Determine the effects of gas residence time through changing inlet flow rates on MBF performance;
- Determine the minimum O₂ requirements (minimum inlet air flowrate) to keep the system efficiency at a certain level.

To be able to apply the conceptual design into practice achieving a reliable performance in terms of CH₄ mitigation, ease of installation, operation, maintenance, and reasonable associated costs is guaranteed. Therefore, the last objective is particularly important when it comes to operating mentioned systems in field as delivering high air flow rates is challenging. If one can achieve the same level of oxidation efficiency at lower O₂ levels, large-scale models of the discussed design can be operated at lower financial and mechanical costs.

As this study is mainly focused on actively aerated MBF configurations, maintaining the media used to achieve favorable conditions in terms of porosity, moisture holding capacity, nutrient supply, temperature and pH adjustments is outside the scope of this work, and can be the subject of future work.

1.3 Overall Approach

In order to further understand and improve the system for full-scale implementation, its behavior under different operating parameters and inlet flow rates needs to be investigated. Biofilter column experiments have been carried out for the present study, as they provide a better representation of full-scale replica compared to batch experiments. A first set of columns are run with air injected at stoichiometric levels to answer the first two research questions regarding the effects of number of injection points and inlet flow rates. A second set of columns are then run with varying inlet air to CH₄ flow rate ratios to determine the minimum air requirements of the proposed aerated systems.

In order to identify present gaps in this area and how the current research fits in, Chapter 2 presents a background on the fate of atmospheric CH₄, the role of methanotrophs in CH₄ oxidation, and various methods employed to address CH₄ emissions. The related literature and studies on the influencing factors on biofiltration will be discussed later in Chapter 2, followed by further discussions on how the current work integrates with past endeavours in the field. Chapter 3 expands on the experimental methods and materials used in running the column experiments. Doehlert experimental design (also known as uniform shell design) was the experimental protocol of choice in the current study; this method is described in detail in Chapter 3, along with the involving factors and their levels. Leaf compost from the City of Calgary landfill has been used as the packing material; characteristics, method and level of compaction of the material are also described in Chapter 3. Chapter 4 discusses the performance of lab-scale CH₄ biofilter columns and the results obtained. An Analysis of Variances (ANOVA) is performed on the results obtained from the columns run at stoichiometric air flow rates to evaluate the extent of effects of variables

of study and their interactions. Chapter 5 incorporates the conclusions of the present work and future directions of related work.

Chapter Two: Literature Review

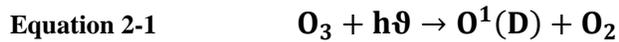
2.1 Overview

To establish a desirable design and operation of CH₄ biofilters, an understanding of how various physical mechanisms can affect biofilter performance is necessary. In this chapter, some of the most important parameters in operation of biofilters are discussed by referring to some of the work previously done on this subject.

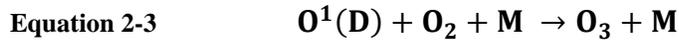
This review starts with a dialog on the chemical changes the CH₄ molecule undergoes in the atmosphere in section 2.2. Section 2.3 is focused on CH₄ mitigation strategies employed to date. Although each of these methods is descriptive on their own, the focus on this section is on biofilters. Section 2.4 deals with methanotrophs and their role in CH₄ oxidation. Section 2.5 is devoted to factors that affect the CH₄ biofiltration, such as temperature, moisture content, nutrient availability, pH, and inlet concentrations. In section 2.6 CH₄ oxidation reaction kinetics and the underlying mathematical equations are discussed. Finally, section 2.7 describes various biofilter configurations used to treat CH₄ in previous studies.

2.2 Atmospheric Methane Chemistry

Oxidation of CH₄ in the atmosphere affects the chemical state of the atmosphere through the products of the reaction and the destruction of their reactants. Atmospheric CH₄ decays to CO₂ and H₂O. The most important sink in atmospheric CH₄ is its reaction with the gas phase hydroxyl radical, OH. The pathway is not simple, and involves several reactions. Also, some intermediates are removed through precipitation. Through this chain of reactions, as further expanded in the equations to below, CO₂, CO, H₂O, and CH₂O are produced. The reaction sequence starts with the ultraviolet (UV) light breaking ozone (O₃) atoms.



Most of the electronically excited oxygen atoms, $O(^1D)$, produced in the above reaction are reduced in their collisions with N_2 and O_2 :



Where, M corresponds to O_2 , N_2 or any other third body with which collisions stabilise the O_3 atom. Some of the $O(^1D)$ atoms produced react with water vapor (H_2O) and generate OH :



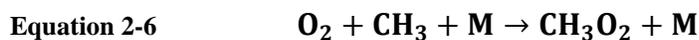
Gas phase OH radicals will then destroy CH_4 following reaction Equation 2-5:

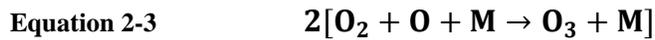
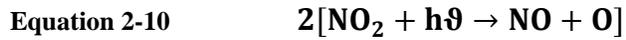


Equation 2-5 is accountable for destroying most of CH_4 emitted in the troposphere (levy, 1971). 7-11% of the remaining is destroyed in the stratosphere by OH , Chlorine atoms (Cl), or $O(^1D)$. 1-10% is also consumed by soil bacteria (Born et al., 1990; Lelieveld et al., 1998). Minor quantities of CH_4 are further passed onto the mesosphere where they are broken photolytically by very short wavelength UV light (Cicerone and Oremland, 1988).

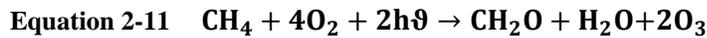
The mechanism of CH_4 reactions in the atmosphere vary substantially in cases of high and low nitrous oxides (NO_x) concentrations. At high NO_x concentrations, such as polluted tropospheric air and all stratosphere, CH_4 oxidation begins with reaction Equation 2-5 and produces hydrogen oxides (HO_x), formaldehyde (CH_2O), and ozone.

The first series of reactions produced CH_2O via reactions Equation 2-6 to Equation 2-10:

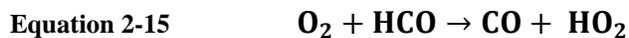
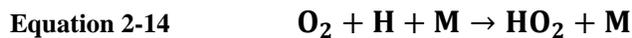




Therefore, the net reaction of CH₄ oxidation in cases of high NO_x concentration leading to production of CH₂O is written as in Equation 2-11. In fact, since NO_x species are produced and later destroyed along the process, they act solely as the catalyst for CH₄ oxidation reactions.



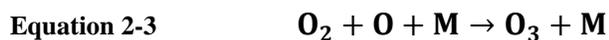
The produced CH₂O is converted to Carbon Monoxide (CO) through three reaction pathways as elaborated below:



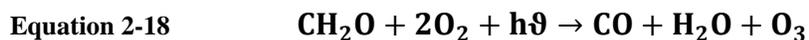
Resulting in the net reaction of CH₂O oxidation leading to production of O₃ and OH radicals can be summarized as in Equation 2-16 .



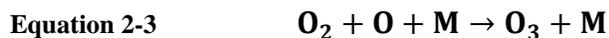
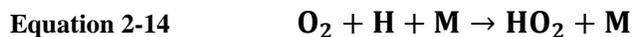
The other pathway from CH_2O to CO is;



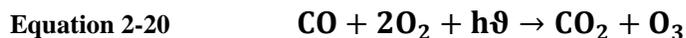
And thus the net reaction will be:



The CO produced in the above equation is further oxidized to produce O_3 and CO_2 . This final step, still occurring at the presence of high NO_x species, is broken down as follows:



Resulting in the net reaction of



Therefore, when high concentration of NO_x species are available, oxidation of CH_4 produces O_3 as well as OH radicals, depending on the relative fraction of CH_2O (Cicerone and Oremland, 1988). The principal reaction and species in CH_4 oxidation in the case of abundant NO_x are also presented in Figure 2-1. As indicated, CH_4 reacts with OH to produce CO_2 , H_2O , CO, and H_2 and various intermediate products as discussed above.

Crutzen et al. (Cicerone and Oremland, 1988; Lelieveld et al., 1998) calculated that average relative fractions of Equation 2-12, Equation 2-16, and Equation 2-20 in the troposphere are 50-60%, 20-25%, and 20-30% respectively. Under such conditions, oxidation of one mole of CH_4 generates 3.7 moles of O_3 and 0.5 mole of OH radical. It should be noted that for these reaction pathways to occur, there should be sufficient NO available for HO_2 to preferentially react with NO rather than O_3 and for CH_3O_2 to react with NO rather than HO_2 . Model results from the same study have indicated that NO mole fractions higher than 5 to 10 ppt are sufficient for the reactions to occur.

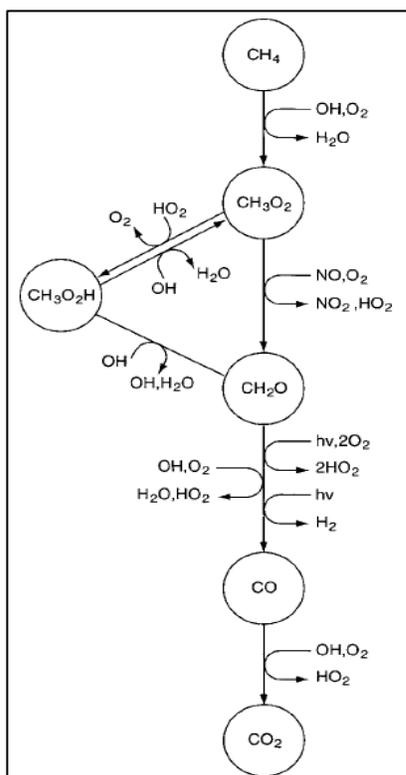


Figure 2-1 Major reactions in CH₄ oxidation chain in NO_x rich air direct path to CH₂O dominates, whereas in NO_x-poor air CH₂O is mostly formed through the CH₃O₂H pathway (Reprinted with permission, Lelieveld, Crutzen, and Dentener 1998)

Figure 2-1 highlights the production of CO as a result of atmospheric CH₄ oxidation, an indirect effect of CH₄ on the climate that is often overlooked. Atmospheric lifetime of CO is approximately two or three months, after which it is converted to CO₂. Logan (Logan et al., 1981) suggested that CH₄ oxidation produces about 8×10^{14} g of CO annually, which yields 0.34×10^{15} g of CO₂. Another indirect effect of CH₄ concentration increases, is the additional production of O₃ in the presence of NO_x, especially in the upper troposphere where O₃ is a potentially a greenhouse gas (Cicerone and Oremland, 1988).

2.3 Methane Elimination Methods

CH₄ lifetime is calculated as the atmospheric content divided by the removal rate. However, various atmospheric reactions can slow CH₄ removal. Therefore, CH₄ lifetime is reported as perturbation life times, with values from 12.4 ± 1.4 years as used by IPCC to 14.4 years (Kirtman et al., 2013; Lashof and Ahuja, 1990). IPCC estimated CH₄ life time at 11.2 years with respect to OH radicals, 120 ± 24 years with respect to additional sinks (e.g. bacterial uptake in soils), 150 ± 50 years to stratospheric loss, 200 ± 100 years to chlorine loss. Therefore, the total atmospheric lifetime of CH₄ is estimated at 9.25 ± 0.6 years, and a perturbation lifetime of 12.4 ± 1.4 years (Myhre et al., 2013).

Compared to the 100-year lifetime of CO₂ in the atmosphere, the 12 year residence time of CH₄ suggests that mitigation strategies can in fact be efficient in short-time (Myhre et al., 2013). Oxidation of one tonne of CH₄ produces 2.75 tonnes of CO₂ in ideal conditions, reducing the net greenhouse gas emissions by 87% (Hayes, 2004). Thus CH₄ removal by its conversion into CO₂ can contribute significantly to the reduction of greenhouse gas effects. CH₄ pollution control technologies already employed include a variety of thermal and biological methods explained as follows.

2.3.1 Thermal Treatment

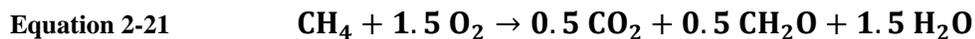
When conditions are right, capturing CH₄ and further processing it in thermal systems for energy recovery is a promising strategy for mitigating gaseous CH₄ emissions (Hettiarachchi et al., 2011). However, incineration facilities are costly to operate as it is often expensive to maintain high temperatures. Therefore, their application may not be economically justifiable when dealing with low concentrations, and low or intermittent flow rates (Huber-Humer et al., 2008). Besides,

the process generates substantial quantities of other more potent greenhouse gases including nitrous oxide (N₂O) as well as highly toxic contaminants such as dioxin on the side (Jaffrin et al., 2003).

2.3.2 Biological Treatment

Soil bacteria are considered a global sink for CH₄ as they oxidize the gas and reduce CH₄ flux to the atmosphere. Simulating and enhancing these natural oxidation processes by CH₄-oxidizing bacteria in the presence of O₂, biological methods have found increasing application among mitigation strategies (Park et al., 2002). These methods are competitive alternatives to traditional technologies, especially when flow rate and concentration requirements are not met for incineration. They offer advantages such as high removal efficiencies, reduced operational costs, and environmental friendliness (Lee et al., 2013; Wani et al., 1997). The process serves CH₄ as the carbon and energy source for methanotrophs, and subsequently oxidizes the element to produce CO₂, with a lower global warming potential, alongside other by-products including water and cellular biomass, all of which are environmentally benign.

CH₄ oxidation in general is a multifaceted process involving a variety of contributing factors and their interactions. The process can be explained using the chemical reaction below, as suggested by Equation 2-21 (Chanton et al., 1995).



Where, CH₂O denotes biomass produced. Generally, in biological treatment methods, the contaminant gas as well as O₂, must be transferred from the gas phase to aqueous phase, and then to the biofilm, where they are consumed by microorganisms (Lebrero et al., 2012). Major air-phase biological reactors are biofilters, biotrickling filters, bioscrubbers, stirred tanks and airlifts. The

principal removal mechanisms are the same for all mentioned systems, however they vary in the phases of microbes (fixed or suspended) and the state of the liquid (flowing or stationary).

Biological CH₄ treatment is limited by mass transfer from gas to aqueous phase due to its poor water solubility. One solution to this problem is the addition of an organic liquid or solid medium with more affinity for CH₄ than water. Such two phase treatment systems are called two-phase partition bioreactors (TPPBs) and the organic medium is known as transfer vector. Turbulent systems (i.e. stirred tanks and airlift reactors) can be operated as TPPBs. Application of transfer vectors increases the gas/water interfacial contact area by disruption of gas bubbles, mass transfer, and general CH₄ uptake by microbial population as a result of increased solubility (Galindo et al., 2000; Quijano et al., 2010). Silicone oil, long-chain hydrocarbons, and fluorocarbons (i.e. C₁₀F₈) are some of the most common transfer vectors used in TPPBs (Rocha-Rios et al., 2011). However, high operational costs associated with implementation of TPPBs, as well as costs of transfer vectors, dispersing them in water, and problems regarding their recovery from the culture medium have posed limitations to their commercial scale application (Rocha-Rios et al., 2013). Therefore, application of laminar contactors (biotrickling filters, bioscrubbers, and biofilters) are commercially more attractive as they have lower power requirements and often have adequate performances (Rocha-Rios et al., 2013). A description of each of the systems is provided in the following section.

2.3.2.1 Airlifts

Air lifts are composed of two chambers; an upper chamber of relatively larger dimensions which functions as a growth chamber, and a lower smaller diameter mixing chamber as illustrated schematically in Figure 2-2. When in operation, CH₄ is bubbled gently by means of an inlet at the

bottom of the lower chamber. As gas travels up the cell culture medium, it displaces the liquid in the lower chamber and carries cells upwards. In a cyclic manner, media from the upper portion of the chamber travel downward and sweep over its tilted floor to replace the displaced cells at the bottom portion. This continuous movement keeps the cells in suspension helping to keep O_2 concentrations high enough for maximum cell growth (Familletti, 1987) .

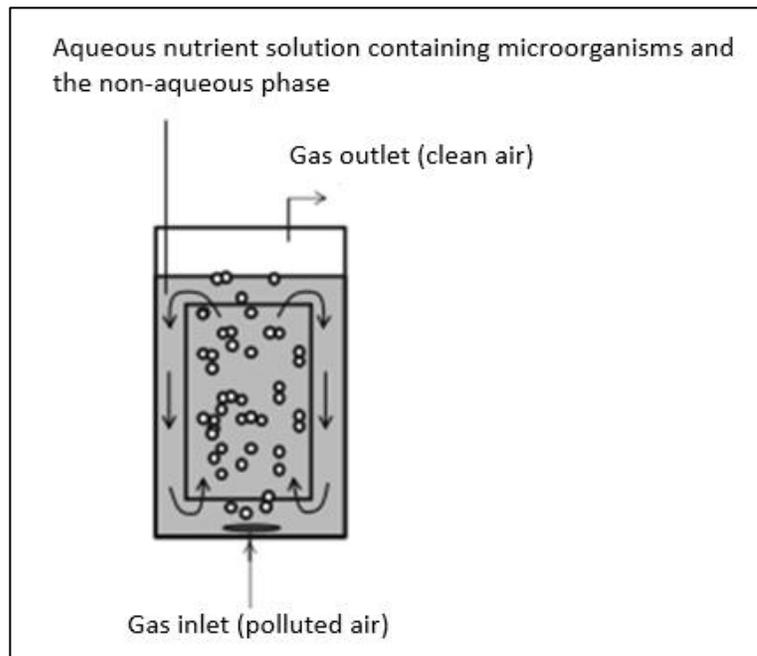


Figure 2-2 Schematic diagram of a concentric tube airlift (Adapted with permission from (Lopez et al., 2013))

Rocha-Rios et al. (Rocha-Rios et al., 2011) investigated the potential of organic liquid and solid vectors to enhance CH_4 mass transfer in an internal loop airlift reactor operated with gas circulation. They used Silicon oil 200 cSt (S200) and Desmopan DP9370A (DP9370) as the vectors due to their high CH_4 affinity, biocompatibility, and non-biodegradability. The results suggested a 100% and 136% increase in O_2 mass transfer for S200 and DP9370, respectively. However, no significant enhancement in CH_4 degradation was observed in the presence of vectors.

Therefore, solely on the results of this study, it can be concluded that microbial activity rather than mass transport is the limiting factor in CH₄ biodegradation.

2.3.2.2 Stirred Tanks

Stirred tank bioreactors increase effective contact rate or contact efficiency between the immobilized microorganism population and the liquid containing the gas to be treated. To that end, such tanks include a vessel for receiving the liquid to be treated, a carrier member of porous material accommodating the microbial community, and a drive unit for moving the carrier unit. The drive unit or agitator is required to provide the adequate momentum, heat and mass transfer, and homogenization of suspensions through mixing (Kuriyama, 1998). A schematic diagram of a stirred tank bioreactor is presented in Figure 2-3.

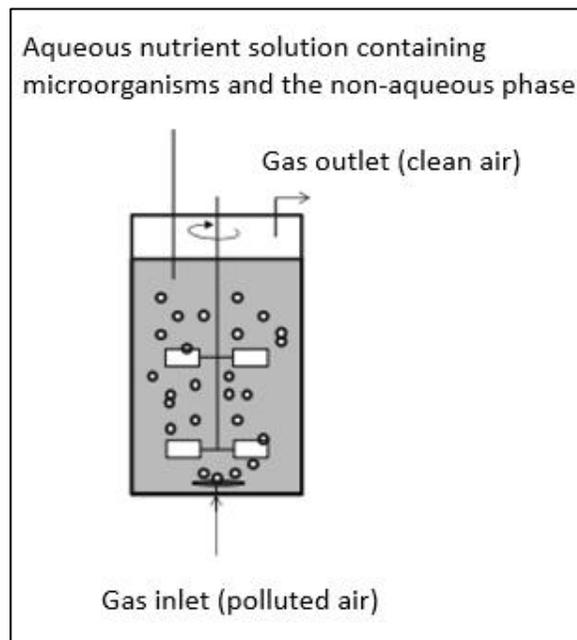


Figure 2-3 Schematic diagram of a stirred tank (Adapted with permission from (Lopez et al., 2013))

2.3.2.3 Bioscrubbers

Bioscrubbing involves absorption of contaminants into a liquid, mainly water, sprayed counter-currently in a treatment vessel. The water containing the target contaminant species is pumped to an activated sludge tank where they are degraded by freely suspended microorganisms (Devanny et al., 1999). Therefore, a bioscrubber consists of a scrubber tower and an activated sludge tank as shown in Figure 2-5. Unlike biofilters, the liquid phase in bioscrubbers is constantly in motion, recirculating over the two reactors and the microbial consortium is suspended in the liquid.

Bioscrubbers are more energy intensive compared to traditional biofilters as operations occur in separate tanks, and the activated sludge tank often needs stirring, aerating and periodic removal of sludge (Wang et al., 2009). Furthermore, application of bioscrubbers is limited for gases with dimensionless Henry constant lower than 5-10 e.g. CH₄ (Henry's law constant of 27 (Hemond and Fechner, 2015)) . This is due to the decreased specific surface area for gas-water exchange (Van Groenestijn and Hesselink, 1993).

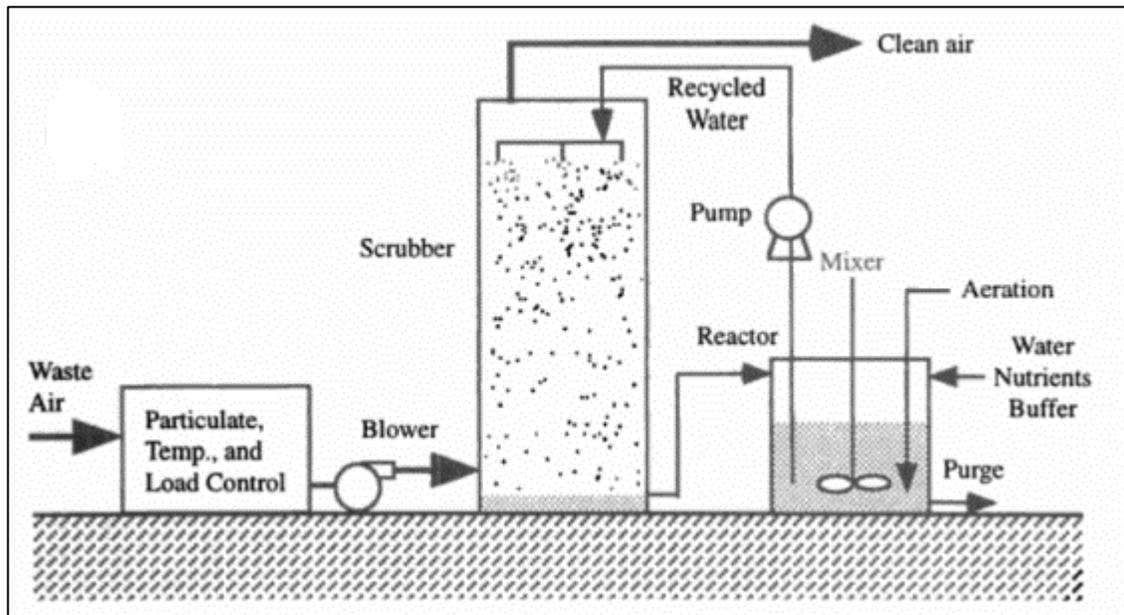


Figure 2-5 Schematic diagram of a bio-washer or bio-scrubber. The adsorption and biodegradation steps are separated (Copied with permission from (Deviny et al., 1999)).

2.3.2.4 Biotrickling filters

Biotrickling filters are an intermediate between bioscrubbers and biofilters in that the liquid phase is constantly recirculated through the packed bed as in bioscrubbers, but both adsorption and degradation occur in the same reactor as in biofilters (Van Groenestijn and Hesselink, 1993). A schematic representation is shown in Figure 2-6 . In a biotrickling system, gas contaminants are adsorbed in a liquid phase prior to passing through a column filled with inert porous material, on which microorganisms are immobilized. Among examples for inert packing material are plastics, resins, ceramics, rocks, and granular activated carbon. Because of the inert nature of the column material, biotrickling filters need to be inoculated. Required nutrients are introduced through irrigation of a nutrient-rich solutions (Lebrero et al., 2012). Organic material, such as compost, cannot be used as the support media as they will absorb water, therefore limiting available space for free air movement.

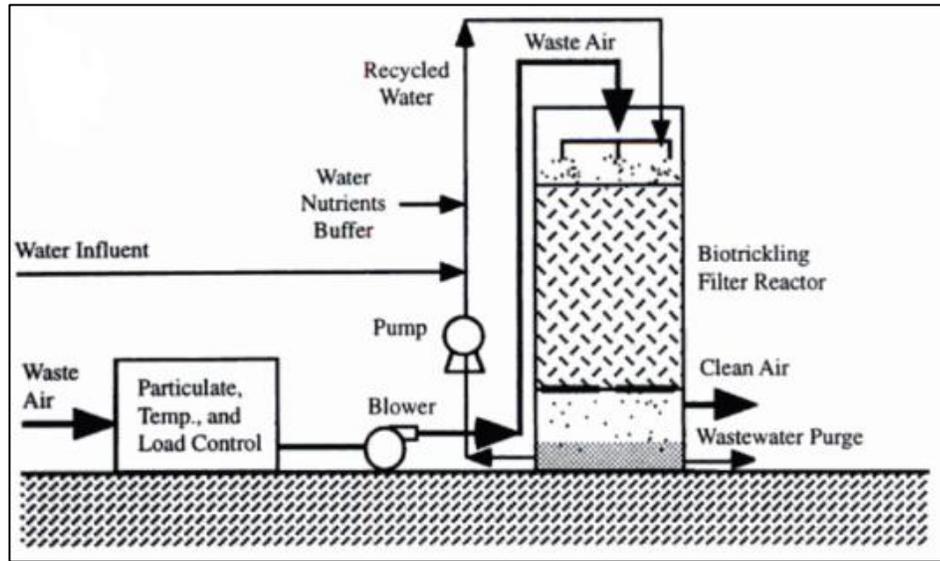


Figure 2-6 Schematic diagram of biological trickling filter. Because the packing material is covered by a biofilm, adsorption and biodegradation are achieved in the same reactor (Copied with permission from (Deviny et al., 1999)).

Operating parameters such as moisture content, temperature, pH, nutrient availability, etc. can be accounted for through the nutrients solution (Deviny et al., 1999). However, the system comes with disadvantages such as high pressure drop, decreased removal efficiency, and high operational costs. Such problems are aggravated with high liquid flow rates, or excessive biomass growth bringing about plugging issues, channelling, formation of anaerobic zones, and failing to yield homogeneous temperature and concentration profiles. These lead to limited application for biotrickling filters in CH₄ abatement (Lebrero et al., 2012).

2.3.2.5 Biofilters

Biofiltration is the application of microorganisms supported on a biologically active surface to treat contaminants in an air stream. Biofilters operate under the idea that contaminants are transferred from gas-phase into an aqueous biofilm layer on the media, where they are then biodegraded by the bacterial population present (Lee et al., 2013). As this process takes place in

ambient atmospheric pressure and temperatures, it offers the advantage of lower operational costs compared to other alternatives. As opposed to the trickling filters where the interparticle space is mainly water-filled, in these systems the space is largely occupied with air to reduce pressure drop, excess leachate, and production of anaerobic areas (Devinny et al., 1999).

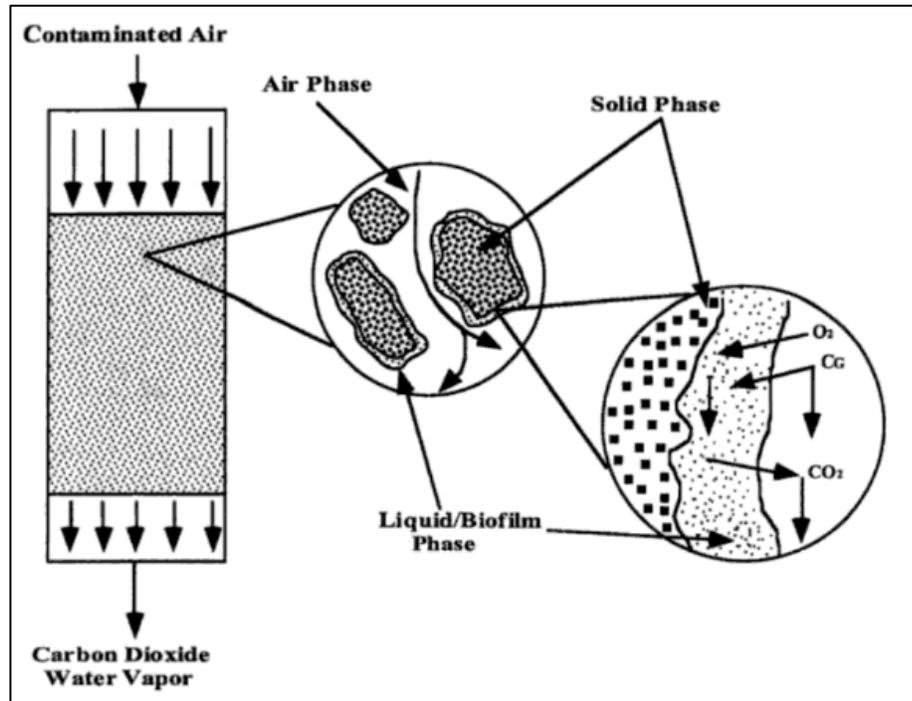


Figure 2-7 Internal mechanism of biofiltration. Contaminated air (CG) passes through the filter bed medium with O₂ and sorbs into a microbial biofilm/liquid phase attached to the filter medium. Microbes convert the contaminant to CO₂ and water (Copied with permission from (Devinny et al., 1999).

In classic biofilters, the structure contains a 1 to 1.5 m thick layer of a porous material, usually compost, soil or peat. The contaminated gas, usually pre-humidified to avoid drying out of the bed, passes through the packed layer through a network of perforated pipes at the base of the soil.

Due to its low water solubility, CH₄ removal using biofiltration is relatively challenging (Lee et al., 2013). Therefore, the optimal biofilter configuration compatible with CH₄ characteristics in

terms of degradability and solubility is an important factor. In order to make the species more bioavailable, some researchers have recommended utilization of surfactants to increase solubility and increase partitioning into the aqueous phase. One other approach is to reduce flow rates such that microorganisms have more contact time with the contaminant species and yield higher degradation rates. Some researchers have attempted modifying the flow rates through application of an adsorption/desorption agent, such as activated carbon, on which contaminants are adsorbed during high flow periods and slowly desorbed at a lower flow rate (Wang et al., 2009). As this study is mainly focused on the MBFs, the rest of this chapter is focused on reviewing previous studies on this technology.

2.4 Role of methanotrophs in CH₄ Oxidation

Methanotrophic bacteria, or methanotrophs, are the engine of the biofilter technology. They are a subset of a group of bacteria known as methylotrophs. Methanotrophs are able to use CH₄ as their sole carbon and energy source, and are the specific bacteria responsible for CH₄ oxidation. Methanotrophs are generally identified by their morphology, intracytoplasmic membrane structures, and DNAs (Hanson and Hanson, 1996; Nikiema et al., 2007).

Decomposition of CH₄ via methanotrophic activities consists of three main steps (as schematically illustrated in Figure 2-8);

(i) The first reaction includes conversion of CH₄ to methanol using the enzymes known as methane monooxygenase (MMO). The use of MMOs to catalyze the degradation of CH₄ to methanol is the key characteristic of methanotrophs. MMOs use two reducing equivalents to break the O–O bond of di-Oxygen and pass one of the oxygen atoms to a hydrocarbon substrate. The

other oxygen atom is then assimilated into the C–H bond of CH₄ to form methanol (Chan et al., 2004).

(ii) The second step involves conversion of methanol to formaldehyde (CH₂O).

(iii) The final step is further decomposition of CH₂O into CO₂ or cell components which are necessary to growth of methanotrophs (Auman and Lidstrom, 2002; Hanson and Hanson, 1996; Nikiema et al., 2007).

A branch point in oxidation of CH₂O to CO₂ is the assimilation through either the ribulose monophosphate (RuMP) or the serine pathway (Nielsen et al., 1997). Figure 2-8 demonstrates CH₄ metabolism, the role of CH₂O as an intermediate, and further synthesis of intermediates.

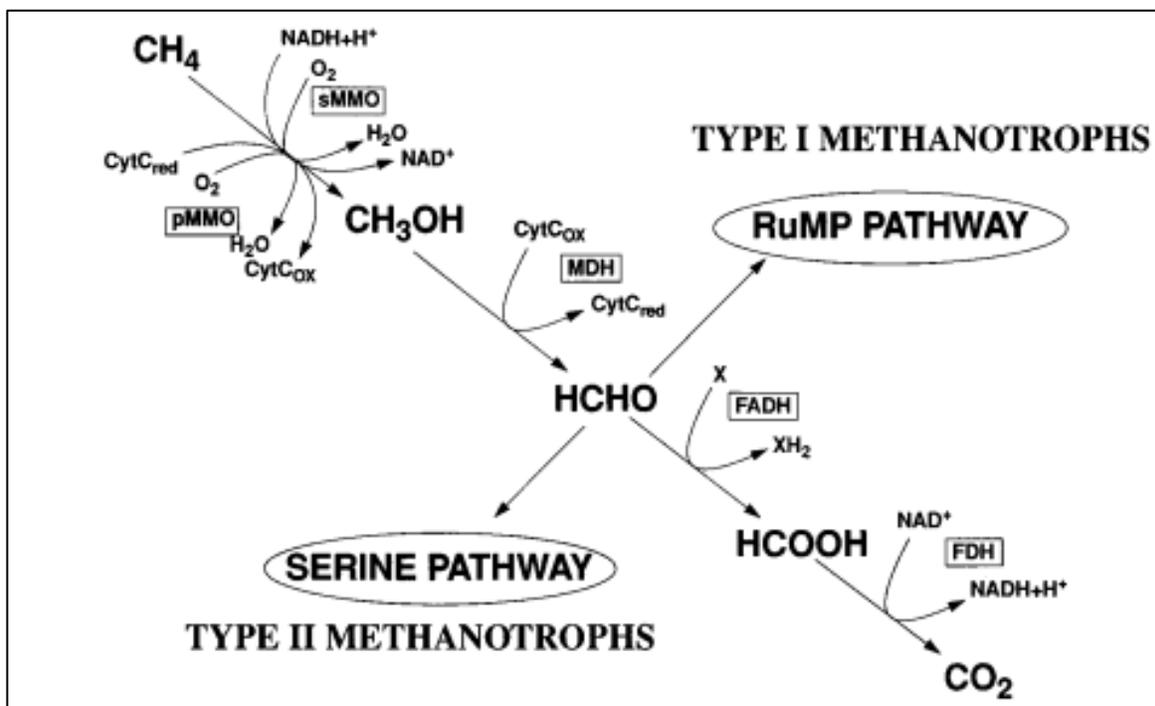


Figure 2-8 Pathways for the oxidation of CH₄ and assimilation of formaldehyde. Abbreviations: CytC, cytochrome c; FADH, formaldehyde dehydrogenase; FDH, formate dehydrogenase (Copied with permission from (Hanson and Hanson, 1996)).

Based on their structure of internal membranes, methanotrophs are generally classified as type I and type II, both of which are identified with a particulate or membrane-associated MMO (Graham et al., 1993). The two classes differ in several other aspects including their ability to fix molecular nitrogen, as well as their dependency on copper for growth. Type I methanotrophs are incapable of fixating molecular nitrogen, require copper for growth, assimilate CH_2O via the ribulose monophosphate (RuMP), and lack a tricarboxylic acid (TCA) cycle. Type II organisms are able to fixate molecular nitrogen, are able to function in the absence of copper, assimilate formaldehyde through the serine pathway, and possess a complete TCA cycle (Graham et al., 1993). A smaller group of methanotrophs, type X, are found to have a mixture of both type I and type II characteristics.

The enzyme responsible for the initial breakdown of CH_4 , MMO, occurs in two forms (Auman and Lidstrom, 2002);

(i) Particulate MMO (pMMO), also known as membrane-bound MMO

(ii) Soluble MMO (sMMO), also known as Cytoplasmic MMO

While all known methanotrophs contain pMMO, only a subgroup possesses sMMO. Additionally, in methanotrophs containing both expressions of MMO, the cellular location of the MMO depends on the concentration of copper during growth (Auman and Lidstrom, 2002; Nielsen et al., 1997). At low copper to biomass ratios, CH_4 oxidation is mostly observed in the soluble fraction and referred to as the soluble MMO (sMMO). At high copper to biomass ratios, the enzyme activity is observed in the cell membrane and is referred to as the membrane associated or particulate MMO (pMMO) (Dispirito J. Gullledge, J. C. Murrell, A. K. Shiemke, M. E. Lidstrom, and C. L. Krema., 1992). In most natural CH_4 oxidation environments, copper is not a limiting

factor. Therefore, the role of sMMO is not known precisely. However, in simulated CH₄ bioremediation practices, copper could become limiting, indicating sMMO presence (Auman and Lidstrom, 2002; Bowman et al., 1993). Therefore, pMMO is found in all methanotrophic bacteria while sMMO has only been isolated from certain subspecies of methanotrophs (Chan et al., 2004).

The biochemical and genetic composition of sMMO has been studied to great depth by Bowman et al. (Bowman et al., 1993). It has been isolated from four different methanotrophs and is known to be a three component enzyme. This iron-containing enzyme consists of three proteins A, B, and C. However, the molecular structure of pMMO is less known due to the inconsistency of the enzyme after cell disruption and difficulties in extraction of the pure active enzyme (Bowman et al., 1993; Dispirito J. Gullledge, J. C. Murrell, A. K. Shiemke, M. E. Lidstrom, and C. L. Krema., 1992).

Besides their differences in molecular structure, the two enzymes differ in their substrate. sMMO is capable of oxidizing straight-chain, branched-chain alkanes, alkenes up to 8 carbons long, CH₄, and cyclic and aromatic compounds. sMMO is also capable of oxidizing trichloroethylene (TCE) several times faster than pMMO (Bowman et al., 1993). pMMO, however, is more specific and is able to oxidize alkanes and alkenes up to 5 carbons long. Because the oxidation of these substrates, except CH₄, does not support methanotrophic population growth, it is known as co-oxidation (Dispirito J. Gullledge, J. C. Murrell, A. K. Shiemke, M. E. Lidstrom, and C. L. Krema., 1992).

2.5 Factors Affecting CH₄ Oxidation in Biofilters

In order to ensure a system with high CH₄ oxidizing productivity, low operational and investment costs, ease of maintenance and operation, an optimal combination of various factors

should be considered. Generally, the assimilation capacity of the biofiltering system is governed by how well the inlet gas stream and O_2 are transported through the porous media. This is affected by a variety of factors including average operating temperature, moisture of the packing bed, formation of Exopolymers (EPS), as well as media characteristics. All of which change over time, as microbial activities generate heat, moisture. And biomass along the process. The operation temperature increases during summer months enhancing microbial activities. This leads to higher oxidation rates and higher temperatures inside the system. Conversely, temperature rises induce moisture loss (Hettiarachchi et al., 2011). Other factors affecting the system performance are availability of nutrients and CH_4 loading rate as determined by the inlet concentration and gas velocity (Hettiarachchi et al., 2007; Nikiema et al., 2009). These factors and their effects on MBF operation are elaborated further in the following section.

2.5.1 Filter Bed

The packing media should enhance microbial population growth, thus should possess the following features;

- High specific surface area for microorganisms to grow on,
- Favorable porosity,
- Near-uniform particle size distribution to improve gas distribution throughout the bed,
- Good water retention capacity to prevent material desiccation and cracking,
- High nutrient content, and
- pH-buffering capacity (Bohn, 1996; Lebrero et al., 2012).

Among the most commonly employed materials are compost, peat soils, woodchips, and flax straws. Stability of the media is another important parameter, especially when it comes to

material durability. Self-compaction of the bed leads to reduced porosity, limited gas transport, and increased pressure drop. Compost, although very frequently used in packing biofilters, is a less stable material. More recent studies have attempted to use bulking material (e.g. ceramic, lava rock, activated carbon, flax straw, woodchips and wood shavings, perlite, and plastics) alongside compost to increase media life. However, as organic material degrade over time and self-compaction is unavoidable in loose material structures, amendments such as adding bulking agents are only a temporary answer to the problem. Eventually, the packing material eventually needs to be changed after several years of operation (Auria et al., 2000; Gaudin et al., n.d.; Hirai et al., 2001; Woertz et al., 2002).

2.5.2 Methane Concentration

According to the Michaelis-Menten reaction kinetics, CH₄ oxidation is a function of the gas concentration as described as;

Equation 2-22
$$V = \frac{V_{max} \times C_{CH_4}}{K_m + C_{CH_4}}$$

V is the CH₄ consumption rate in $\frac{g/day}{g \text{ dry weight}}$

C is the concentration of CH₄

V_{max} is the maximum CH₄ consumption rate in $\frac{g/day}{g \text{ dry weight}}$

K_m is the half-saturation constant

At low CH₄ concentrations, the rate of CH₄ oxidation is proportional to the concentration; whereas at high CH₄ concentrations, the oxidation rate is independent of the concentration and has the maximum value of V_{max} (Stein et al., 2001). Czepiel et al. (Czepiel et al., 1996) demonstrated

a linear relationship between V_{\max} values and CH_4 concentrations with a regression coefficient of 0.68.

2.5.3 Oxygen Concentrations

Due to the aerobic nature of methanotrophs, CH_4 oxidation naturally occurs where CH_4 and O_2 are both present (King and Adamsen, 1992). Therefore, the presence of O_2 and its concentration are determining factors to CH_4 oxidation. CH_4 oxidation has been reported to decline substantially at O_2 concentrations below 3%, but varies only marginally at O_2 concentrations above 3% (Bender and Conrad, 1995; Czepiel et al., 1996; Stein and Hettiaratchi, 2001). O_2 concentrations ranging from 0.45 to atmospheric levels of 20% are sufficient to support type I and II methanotrophic cultures (J.H. Wilshusen et al., 2004).

The maximum depth at which microbial populations can function depends on the depth to which O_2 can penetrate. This is key when designing biofilters as it dictates the thickness of the packing media layers. The maximum O_2 penetration depth is a function of various factors including permeability of the bed material, porosity, moisture content, and compaction level. The displacement of air in the packing media matrix due to the movement of CH_4 and O_2 consumption as a result of oxidation reactions also affect the penetration depth (Scheutz et al., 2009; Stein and Hettiaratchi, 2001).

2.5.4 Temperature

As with all biological reactions, temperature is an essential factor in CH_4 oxidation. Methanotrophic activity is usually well established over mesophilic temperatures of 10 – 45°C, with the optimum being in the range of 25 – 37°C (Czepiel et al., 1996; De Visscher et al., 1999; Hanson and Hanson, 1996; Hettiarachchi et al., 2011; Swanson and Loehr, 1997).

Although microbial population can adapt to both high and low temperatures, dramatic decreases have been observed at temperatures below 10°C and above 65°C. Omelchenko et al. (Omelchenko et al., 1993) isolated a group of psychrophilic obligate methanotrophs from the tundra soil in polar Ural to verify O₂ activities at low temperatures. Optimal activities were observed at a temperature range of 3.5 – 10°C and minimal growth was recorded at 20°C.

Temperature is a controlling factor in determining the prevalent type of bacteria in a certain environment (type I or type II). Type I methanotrophs are reported to have lower optimum temperatures compared to type II methanotrophs, making them more dominant at temperatures close to 10°C rather than 20°C (Börjesson et al., 2004, 1998; Gebert et al., 2003; Scheutz et al., 2009).

The correlation of oxidation reactions with temperature is described by the Arrhenius equation. As such, the oxidation rate increases exponentially with temperature to reach a certain maximum (V_{max}), after which it continuously decreases with further increase in the temperature (Hettiaratchi et al., 2000). The temperature coefficient (Q_{10}), is used as a measure of the rate of change in a biological system as a result of increasing the temperature by 10°C. It has been reported that at temperature range of 10 and 30°C, V_{max} increases exponentially with Q_{10} values. The values are calculated from the average oxidation rates ranging between 1.7- 4.1 (Czepiel et al., 1996; De Visscher et al., 2001; Park et al., 2005; Scheutz et al., 2009). Scheutz and Kjeldsen (Scheutz and Kjeldsen, 1997) observed a steep decline in oxidation rates upon increasing the temperature further to 40°C. As such, at 50°C microbial activity was completely inhibited. A Similar behaviour was reported by Czepiel et al. (Czepiel et al., 1996) who reported no activity at 45°C.

De Visscher et al. (De Visscher et al., 2001) observed a stronger influence of temperature on oxidation at CH₄ concentrations of 10,000-20,000 ppm compared with 0-3000 ppm. They attributed their observations to the effect of temperature on CH₄ solubility in water. Therefore, at low initial CH₄ concentrations mass transfer between the gas and liquid phase is likely a more limiting factor in oxidation compared to temperature effects. However, at high initial CH₄ concentrations, the oxidation reaction is no longer phase-transfer limited and more enzymatically influenced, which is why temperature effects are more highlighted (Scheutz et al., 2009). Thus although low temperatures promote higher gas solubility in water, biofilter temperature is commonly maintained in the moderate range for better microbial growth and operation (Lebrero et al., 2012).

The heat required to maintain the system at the optimum temperature range can be provided through the inlet gas and metabolic activity of microorganisms. CH₄ biodegradation produces heat on the side, maintaining temperatures at a favorable range within the biofilter bed. This can by itself support biofiltration even in freezing temperatures (Wani et al., 1997).

2.5.5 Moisture content

Moisture content is a critical parameter in biofilter application as microbial biological activity depends highly on moisture availability. In order to be biologically available to methanotrophs, CH₄ has to be solved in water. Biofilter moisture content is also the transport means for nutrient supply (Scheutz et al., 2009).

Excess water content takes up pore spaces slowing down gas transport in the porous medium. When the degree of saturation reaches values close to 85%, air-filled pore spaces are not interconnected and gases have to diffuse in water. The molecular diffusion is 10⁴ times lower in

water compared to that in air. This will limit CH₄ and O₂ availability to methanotrophs thereby reducing system's oxidation efficiency (Cabral et al., 2004; Scheutz et al., 2009). Blockage of O₂ transport also promotes the formation of anaerobic zones in the biofilter and decreases oxidation rates (Czepiel et al. 1996; Hettiaratchi et al. 2000; King and Adamsen 1992; Shareefdeen and Singh 2005).

Alternatively, low humidity levels decrease the rate of biological reactions of CH₄ oxidation, due to microbial physiological response to water deficiency. In addition, low moisture contents bring about the development of fractures causing short-circuit channeling. Bergamaschi et al. (Bergamaschi et al., 1998) reported that nearly 70% of direct CH₄ emissions in European landfill covers are via cracks and fissures.

The optimum moisture content depends on a variety of factors including the type of porous media, pore structure, and porosity (Shareefdeen and Singh, 2005). For a more uniform CH₄ removal within a biofilter system, the material used should be capable of sustaining sufficient moisture content while maintaining a high pore space volume for gas exchange. In 2002, Park et al. (Park et al., 2002) studied the effects of moisture content, soil fertility, CH₄ application rate, and incubation period on CH₄ oxidation in loamy sand biofilters. They suggested that optimum conditions consisted of a water content of 13% by weight, bed thickness of 30 cm, incubation time of 30 days, and application rate of 524.8 g/m²/day and achieved an oxidation rate of 435.2 g/m²/day under the optimum conditions. Effects of soil fertility, characterized by NO₃-N were found to be insignificant. However, their study lacked results of long-term operation of such biofilters, which may affect conclusions on the effects of soil fertility and bed medium stability on CH₄ oxidation. Optimum moisture contents ranging between 15 and 60% (by weight) have been

suggested for soil and compost filter beds (Boeckx and Van Cleemput, 1996; Czepiel et al., 1996; Wani et al., 1997; Whalen et al., 1990).

Water is produced to some extent alongside CH₄ degradation, and is lost through evaporation at the surface (Hettiarachchi et al., 2011). The exothermic nature of the process also accounts for some moisture loss across the biofilter volume. Therefore, water requirements depend both on the heat generated and the humidity of the inlet stream. In case of desiccation, water is supplied through surface irrigation (spray nozzles and sprinklers), or pre-humidifying the gas stream (Shareefdeen and Singh, 2005).

2.5.6 Gas contact time

Gas contact time, also referred to as *true residence time*, is defined as the actual time a parcel of gas remains inside the biofilter in order to be treated. It can be expressed in terms of gas flow rate and bed porosity as follows (Shareefdeen and Singh, 2005);

Equation 2-23
$$\tau = \frac{V_f \times \theta}{Q}$$

V_f is the bed volume in m^3

Q is the volumetric air flow rate in m^3/s

θ is the bed porosity defined by the volume of void space by total volume of medium

The term “*Empty Bed Residence Time*” (EBRT) is more commonly used to refer to the time for a parcel of air to travel through an empty bed biofilter surface as the porosity of filter bed changes over time. EBRT is generally expressed as:

Equation 2-24 **EBRT = $\frac{V_f}{Q}$**

All terms previously defined. As evident from Equation 2-23 and Equation 2-24 , EBRT is larger than the true residence time, because of the space occupied by the support media. Therefore, the true bed residence time is more indicative of the treatment time. In general, the higher EBRT or true residence time, the better the contaminant removal (Wang et al., 2009). Typical residence times for removal of trace gases vary from approximately 25 seconds for low concentration VOCs to several minutes for higher concentrations (Leson and Winer, 1991).

CH₄ oxidation, however, calls for higher residence times due its low water solubility; therefore, residence times of several hours to days have been reported in literature (Du Plessis et al., 2003; Nikiema et al., 2005). As dictated by Equation 2-24, very high flow rates result in low contact times leaving the degradation reaction incomplete. In addition, high flow rates tend to wash away the moisture trapped around the particles causing faster desiccation of biofilter bed (Delhom, 2005).

2.5.7 pH levels

CH₄-oxidizing activities generally require an optimal pH of 5.5 to 8.5, a range preferred by most methanotrophic bacteria (Bender and Conrad, 1995; Scheutz and Kjeldsen, 2004; Shareefdeen and Singh, 2005). Biofilter pH-value depends mainly on the properties of the material used in filter bed. Loamy material usually have higher buffer capacities, therefore are less prone to acidification, whereas sandy substrates can undergo pH values as low as 4.5 (Scheutz et al., 2009).

Hilger et al. (H. Hilger et al., 2000) experienced a drop in pH near the top of soil columns subjected to landfill gas. They linked the pH decrease to the buildup of oxidation intermediates such as methanol and formic acid. The researchers also observed a rise in pH levels at the bottom layers of column, owing to reduction reactions caused by reduced pore spaces and low O₂ concentrations. pH rise at low-O₂ regions was also attributed to nitrogen (N) fixation by type II methanotrophs leading to production of ammonium NH₄. Four H⁺ ions are used per mole of NH₄ produced (White, 1996). Addition of lime to columns increased CH₄ uptake, proposing that pH is a controlling factor in CH₄ oxidation.

In general, methanotrophic communities are capable of adapting to changes in background pH values and withstand acidic pHs of as low as 4. However, pH adjustments are preferred when the intermediate by-products depress the system's pH (Quigley et al., 2004).

2.5.8 Nutrients

CH₄ can be used as the carbon source for microbial reactions once introduced to the microorganism culture in the biofilter media (Huber-Humer et al., 2008). Other elements such as nitrogen (N), phosphorus (P), and minerals such as copper (Cu) should be further supplied to the system to stimulate biological reactions (Wani et al., 1997). P and N are more essential to the synthesis of cellular tissue, thus are more rate-limiting in CH₄ oxidation (Stein and Hettiaratchi, 2001). Anthony (Anthony, 1975) reported a relatively high N demand of 0.25 mole per mole of assimilated CH₄. This means that in circumstances where CH₄ to N ratio is 10 or higher, unavailability of N can lead to decreased or ceased CH₄ oxidation rates. Long-term N limitations can also cause decreased bacterial growth and synthesis which terminates microbial oxidation of CH₄ (Scheutz et al., 2009). This reduction can be amended to some extent by fixation of

atmospheric N by type II methanotroph, although the reaction is energetically more demanding compared to inorganic N consumption. In fact, De Visscher and Van Cleemput (De Visscher and Van Cleemput, 2003) suggested three phases for CH₄ biodegradation with respect to exposure-time to inorganic N.

- The initial stage yielded a high growth rate for methanotrophs, especially type I bacteria, with more dependence on inorganic N availability.
- The second stage marked by a decrease in biodegradation of CH₄, due to N limitations, lasted for a few weeks until a steady-state oxidation was reached.
- The last stage began an observed rise in microbial population growth, most probably dominated by N-fixating Type II bacteria. This final phase led to a broader peak of methanotrophic activities, independent of inorganic N.

Inorganic N (ammonium [NH₄⁺] and nitrate [NO₃⁻]) can either enhance or inhibit CH₄ degradation reactions depending on CH₄ and N concentrations, background pH, and types of methanotrophs present (Scheutz et al., 2009). At high N concentrations, NH₄⁺ is known to have inhibitory effects on methanotrophic activities as a result of substrate competition between N and CH₄ at enzymatic levels. The inhibition has also been attributed to competitions between methanotrophs and nitrifiers (Moiser et al., 1991). Once NH₄⁺ is added, nitrifiers can dominate methanotrophs and result in reduced CH₄ oxidation due to the lower consumption of CH₄ by nitrifiers (Dunfield and Knowles, 1995). Boeckx and Cleemput (Boeckx et al., 1996; Goldman et al., 1995; Sitaula et al., 1995; Steudler et al., 1996) tested the effects of N on CH₄ oxidation in soil batch experiments at NH₄⁺ concentrations of 25, 50, and 75 mg/kg. They observed an inverse relationship between CH₄ oxidation and NH₄⁺ addition. They also found that this reduction

decreased at higher moisture contents. Because NH_4^+ turnover (oxidation rate) is also lower at increased moisture contents, they argued that N transformation processes such as nitrification have a more significant effect on CH_4 oxidation inhibition than the actual NH_4^+ concentrations.

Natural packing media of compost contains the essential required nutrients, minimizing the need for additional amendments (Shareefdeen and Singh, 2005). In cases of biologically available nutrient shortages, design modifications such as direct nutrient injection or leachate circulation should be accounted for.

2.5.9 Exopolymeric substances

The formation of heavy weight polysaccharides, known as Exopolymeric substances (EPS) is commonly related to biological CH_4 degradation. Accumulation of EPS is known to cause a decline in oxidation rates after long-term operation of laboratory simulations of landfill covers or passive biofilters. This may be due to clogging of pore spaces, restricting gas transport, or short-circuiting and channeling of gas through medium (Haubrichs and Widmann, 2006; Streese and Stegmann, 2003; J. H. Wilshusen et al., 2004). EPS formation consequently increases the pressure drop across the biofilter, which in turn affects the operation costs (Delhom, 2005; Streese and Stegmann, 2003).

EPS are heavy weight polymer formations which mostly serve as an anchorage for bacteria to soil surfaces. These macromolecular polymers are not generally considered as sources of carbon or energy for methanotrophic bacteria as they cannot be re-metabolized (H. A. Hilger et al., 2000). They often form a barrier layer of biofilm because of their adsorptive surface and cation exchange properties. EPS structures promote nutrient accumulation and metal immobilization. This leads to formation of a barrier biofilm layer between microorganisms and substrate gas (Fletcher, 1992).

Wilshusen et al. (J.H. Wilshusen et al., 2004) reported that the areas with EPS formation had the highest methanotrophic population counts, the most dominantly type I bacteria, and highest batch maximum oxidation rates (V_{max}). Gebert and Grongroft (Gebert and Groengroeft, 2006; Gebert and Gröngröft, 2006) observed no significant effects of EPS formulation in a passively-aerated biofilter packed with loosely packed clay pellets. Scheutz et al. (Scheutz et al., 2009) linked this observation to the intermittent supply of landfill gas to the biofilter as well as regular aeration which they believed prevented EPS formation.

Although both type I and type II methanotrophs produce EPS, Malashenko et al. (Malashenko et al., 2001) suggested that mesophilic methanotrophs with the RuMP pathway of C1-compound assimilation (i.e. type I) are actually more active in producing EPS than bacteria operating the serine cycle (i.e. type II). Interestingly, it has also been reported that accumulation of EPS can affect bacterial population; EPS build-up limits O_2 transport and promotes favourable conditions for Type-II bacteria growth (Whittenbury and Dalton, 1981). Wilshusen et al. (J.H. Wilshusen et al., 2004) studied the effect of O_2 concentration on the formation of EPS. They observed two methanotrophic activity peaks in the course of 6 months for both high and low O_2 environments. The dominant bacteria population shifted from type I to type II between the peaks likely due to the production of EPS and promotion of microaerophilic conditions. They also found that EPS activity was 250% higher in high O_2 environments compared to low O_2 concentration, due to the higher microbial activity.

2.6 Gas Transport and Biological Reactions

Biological treatment in MBFs begins with dissolution of gas in water. The dissolved gas is moved by diffusion and advection. Ultimately, biotransformation oxidizes CH_4 into CO_2 , water,

by-products, and biomass. The slowest step of the mentioned processes defines the required contact time for CH₄ degradation, this controls the biofilter efficiency. This section expands on the underlying mechanisms of each of the steps mentioned (Devanny et al., 1999).

2.6.1 Gas Transport Mechanisms

Gas transport in porous media is governed by a combination of diffusion, dispersion, and advection. Diffusive mass transport is mostly driven by concentration gradients across the biofilter in a microscopic scale, whereas dispersive transport is commonly related to random eddies and flow tortuosity. Advection is associated with pressure gradients across the biofilter, mainly pressure differences between the injected gas and atmospheric pressure. The mentioned mechanisms are independent and additive.

At equilibrium, the partition between gas and liquid is dependent on the chemical's vapour pressure and solubility, and is described by Henry's law. Henry's law states that concentrations in water are proportional to chemical's partial pressure in the air, and the constant of proportionality is called the Henry's law constant (k_H or H) (Hemond and Fechner, 2015). According to the ideal gas law, the partial pressure of a chemical in the gas phase is directly proportional to the number of moles per unit volume of the chemical. Alternatively, Henry's law constant can be expressed as the ratio of the concentration of the chemical in the gas phase to its concentration in the aqueous phase;

Equation 2-25 $C(g) = HC(aq)$

$C(g)$ is the concentration or pressure in gas phase in g/L of air or atm

$C(aq)$ is the equilibrium concentration in the aqueous phase in mol/L or g/L of water

H is Henry's law constant in $\frac{\text{atm.L}}{\text{mol}}$ or $\frac{\text{g/L}}{\text{g/L of water}}$ (dimensionless) depending on the units used for gas phase and liquid phase concentrations.

As the air moves through the biofilter, concentrations vary significantly from region to region, with values most likely higher near the gas inlet and lower near the outlet. Even along the biofilm thickness, higher concentrations are expected on the surface of the biofilm layer and lower values deeper in the biofilm. Nonetheless, it is possible for concentrations to be at equilibrium in each region of the biofilter volume (Deviny et al., 1999).

Advective gas transport in a porous medium is expressed by Darcy's law, highlighting its dependency on pressure gradient (Zheng and Bennett, 1995).

Equation 2-26 $\mathbf{J}_i = \mathbf{v}C_i$

C is molar concentration,

v is the velocity of each species (m/s) determined by;

Equation 2-27 $\mathbf{v} = -\frac{k_z}{\mu} \frac{\partial P}{\partial z}$

k_z is the intrinsic permeability of the porous medium (m^2)

μ is the viscosity of gas mixture (Pa.s)

$\partial P/\partial z$ is the pressure gradient in the z direction (Pa/m). Pressure is determined using the ideal gas law as;

Equation 2-28 $\mathbf{P} = \mathbf{RT} \sum_{k=1}^i C_k$

P is the pressure (Pa)

R is the universal gas constant ($\text{Pa.m}^3/\text{Kmol}$)

T is absolute temperature (K)

i is the number of gas species present in the mixture

Viscosity of gas mixture (μ) is determined using values for individual gases as expressed in Equation 2-29 (Stein et al., 2001);

$$\text{Equation 2-29} \quad \mu = \sum_{k=1}^i \frac{\mu_k}{1 + \sum_{l=1}^i \theta_{kl} \frac{y_k}{y_l}}$$

y_l and y_k are molar fractions of mixture components k and l (mol/mol)

μ_k and μ_l are individual viscosities of gas components k and l (Pa.s)

θ_{kl} is defined as follows;

$$\text{Equation 2-30} \quad \theta_{kl} = \frac{[1 + (\frac{\mu_k}{\mu_l})^{\frac{1}{2}} (\frac{M_l}{M_k})^{\frac{1}{4}}]^2}{\sqrt{8[1 + (\frac{M_l}{M_k})^{\frac{1}{2}}]}}$$

M_k and M_l are molecular weights of components k and l (g/mol) respectively.

Fick's 1st law of diffusion provides a description of the molar flux of species relative to their mean molar velocity and concentration gradient. Fick's 1st law can be written as;

$$\text{Equation 2-31} \quad \mathbf{J}_i = \mathbf{D}_i \nabla C_i$$

C_i is the molar density (mol/m³) of gas i

D_i is the dispersion coefficient of gas i (m²/s)

In biotrickling filters and bioscrubbers, the turbulent movement of air is a result of both advection and eddy diffusion.

The combination of both mechanisms is called convection. The dispersion coefficient is defined as a combination of molecular diffusion and mechanical dispersion for each individual species. Dispersion is a result of velocity variations in pore spaces. Therefore;

Equation 2-32 $\mathbf{D}_i = \mathbf{D}^m + \alpha_D |\mathbf{v}|$

D^m is the molecular diffusion of each individual species in soil (m^2/s)

α_D is dispersivity (m)

Near the water-gas interface, molecular diffusion becomes dominant as the flow is laminar. Therefore, water-gas partitioning is the main rate limiting process in biofilters because the molecular diffusion is slower than convection (Devanny et al., 1999).

The continuity equation is used to describe the additive effects of the afore-mentioned mechanisms;

Equation 2-33 $\frac{\varepsilon \partial C}{\partial t} = -\nabla \mathbf{J}_i + \mathbf{R}_i$

ε is the air-filled porosity

R_i is the rate of reaction (production or consumption) of gas species i

$\nabla \mathbf{J}_i$ is changes of mass flux with location

As previously noted, the general flux is a combination of both dispersion and advection;

Equation 2-34 $\mathbf{J}_i = \mathbf{v} \mathbf{C}_i - \mathbf{D}_i \nabla \mathbf{C}_i$

Gas diffusion coefficients are to a high extent controlled by pore volume, tortuosity paths, and water content (Stein et al., 2001). Therefore, gas diffusion coefficients in porous media are independent of the nature of the diffusing gas and depend solely on material characteristics (Abichou et al., 2015; Jin and Jury, 1996; Stein et al., 2001). Several researchers have

experimentally indicated that gas diffusion coefficients in unsaturated porous media as a function of porosity since diffusion occurs solely through the interconnected void space (Allaire et al., 2008). There is abundant literature on empirical, semi-physical, and physical models for gas diffusion in porous media. A list of such models is presented in Table 2-1.

Table 2-1 Gas diffusion models in porous media

Reference	Model	Annotation
Welty, Wicks and Wilson (Welty et al., 1984)	$D_s/D_a = \varepsilon / \tau$	τ is tortuosity.
Millington and Quirk (Millington and Quirk, 1961)	$D_s/D_a = \varepsilon^{10/3} / \varphi^2$ or $D_s/D_a = \varepsilon^2 / \varphi^{2/3}$	Φ is the total porosity of the porous media
Penman (Penman, 1940), Van Bavel (vanBavel, 1952), Flegg (Flegg, 1953), Dye and Dallavalle (Dye and Dallavalle 1958)	$D_s/D_a = a\varepsilon$ or $a\varepsilon + b$	a and b are constant, they vary with different porous media.
Buckingham (Buckingham, 1904) Marshall (Marshall, 1959) Millington (Millington, 1959)	$D_s/D_a = \varepsilon^\tau$	τ is tortuosity (dimensionless). $\tau = 2$ (Buckingham, 1904) $\tau = 1.5$ (Marshall, 1959) $\tau = 1.33$ (Millington, 1959)
Moldrup et al. (Moldrup et al., 1999)	$D_s/D_a = \varphi^2 (\varepsilon/\varphi)^{2+3/c}$	2+3/c is an correspondent to the tortuosity model for describing unsaturated hydraulic conductivity.

D_s and D_a are gas diffusion coefficient in soil and air, respectively in cm/s

ε is the air-filled porosity

φ is the total porosity

2.6.2 Microbiological reactions

In biological environments, single or double substrate limitation may occur where the growth of an organism requires the presence of two substrates. In cases where the growth rate is limited by one substrate at a time, a non-interactive model is applied (Bader, 1978). Mathematically put, the reaction rate is equal to the lowest growth rate and can be predicted using

a single-substrate model. The Michaelis-Menten kinetics is most commonly used for enzymatic reactions, given by Equation 2-35:

Equation 2-35
$$R = \frac{d[P]}{dt} = \frac{V_{\max}[S]}{K_m + [S]}$$

R is the reaction rate

$[P]$ is the concentration of reaction product

$[S]$ is the substrate concentration

V_{\max} is the maximum rate achieved by the system

K_m is the Michaelis constant, defined as the substrate concentration at which the reaction rate is half of the maximum value (V_{\max})

The Monod kinetics is known as a chain of enzymatically mediated reactions with a limiting step described by Michaelis- Menten kinetics. It is assumed that enzymes catalyze reactions by forming an enzyme-substrate complex initially which will later decay to either the original substrate or the product (Bungay, 1994; Monod, 1949). Therefore, Monod equation is identical to Equation 2-35.

An interactive model is used when both substrates are present in concentrations less than their saturated levels (Bader, 1978). Since the overall growth is then affected by both substrates, the double-Monod equation is derived by simply multiplying two single-substrate limited models as formulated in Equation 2-36:

Equation 2-36
$$R = \frac{V_{\max_1}[S_1]}{K_{m_1} + [S_1]} \times \frac{[S_2]}{K_{m_2} + [S_2]}$$

Double Monod reaction kinetics will be used to model CH₄ and O₂ consumption through biofiltration. The reaction kinetics can thus be presented as;

Equation 2-37
$$R = \frac{V_{\max CH_4} [S_{CH_4}]}{K_{mCH_4} + [S_{CH_4}]} \times \frac{[S_{O_2}]}{K_{mO_2} + [S_{O_2}]}$$

S_{CH_4} and S_{O_2} are concentrations of CH₄ and O₂, respectively.

Gebert et al. (Gebert et al., 2003) studied oxidation reaction kinetics in a landfill biofilter with an average CH₄ loading of 11g CH₄ m³/h and a maximum CH₄ loading of 60 g CH₄ m³/h. Their findings followed a double Michaelis-Menten saturation kinetics with a V_{\max} of $1.78 \frac{\mu mol CH_4}{h g ww}$ and a K_{mCH_4} of 15.1 μmol for CH₄. The values reported by Gebert et al. were higher than those presented in previous studies for soil. Bender and Conrad (Bender and Ralf Conrad, 1992) studied kinetic parameters of CH₄ oxidation in four different oxic soils both in a fresh state and under 20% CH₄ incubation. They found a Michaelis-Menten kinetics with K_m values ranging between 30-51 nmol CH₄ and V_{\max} of $0.7-3.6 \frac{nmol CH_4}{h g dw}$ soil for fresh soils. Interestingly, in pre-incubated soils they observed a biphasic behaviour with two different sets of kinetic parameters;

(i) A high-affinity activity with low K_m values of 13-470 nmol CH₄, and low V_{\max} values of $2.1-150 \frac{nmol CH_4}{h g dw}$ soil (similar to the behaviour in fresh soils)

(ii) A low-affinity activity with high K_m values of 1740-27,900 nmol CH₄ , and high V_{\max} values of $270-3,690 \frac{nmol CH_4}{h g dw}$ soil

The authors believed that the low-affinity activity is related to methanotrophic bacteria activity as also observed in soil covers subjected to natural gas with high CH₄ mixing ratios (>5%). Similar values were observed by Benstead and King (Benstead and King, 1997) in their study on

forest soils exposed to atmospheric CH₄. They experienced an increasing capacity with increasing CH₄ concentration up to a V_{max} of 1.0 $\frac{\text{nmol CH}_4}{\text{h g dw}}$ soil, and K_m of 10 nmol.

Ordaz et al. (Ordaz et al., 2014) investigated the effect of introducing a non-aqueous phase (NAP) using a technique derived from the in-situ pulse respirometry on CH₄ biodegradation kinetics. The in-situ pulse respirometry technique used in this work involved injection of different volumes of CH₄ in the headspace of a gas-tight stirred tank reactor filled with 1 L of O₂-saturated water. Their results suggested 30%, 120%, and 150% enhancement in O₂ uptake rate, K_m, and V_{max}, respectively, compared with the control system with no NAP addition.

2.7 Methane Biofilter Configuration

Due to their versatile design options, biofilters can be set up depending on their application purposes and desired removal efficiency. In general, biofilters can be classified based on their mode of aeration, flow orientation, and setting (Leson and Winer, 1991).

2.7.1 Mode of aeration: Active vs. passive

Classical approaches in biofiltration have typically used passive aeration, in which case atmospheric air interaction on the surface is the only O₂ source, and gas flow is controlled by the pressure difference between the biofilter and ambient air (Gebert and Groengroeft, 2006). Having O₂ enter in the opposite direction as the influent gas, leads to the reduction of system efficiency via formation of restricted CH₄ oxidation zone and not taking advantage of the full potential of filter bed thickness. A schematic diagram of passive landfill MBF is illustrated in Figure 2-9 .

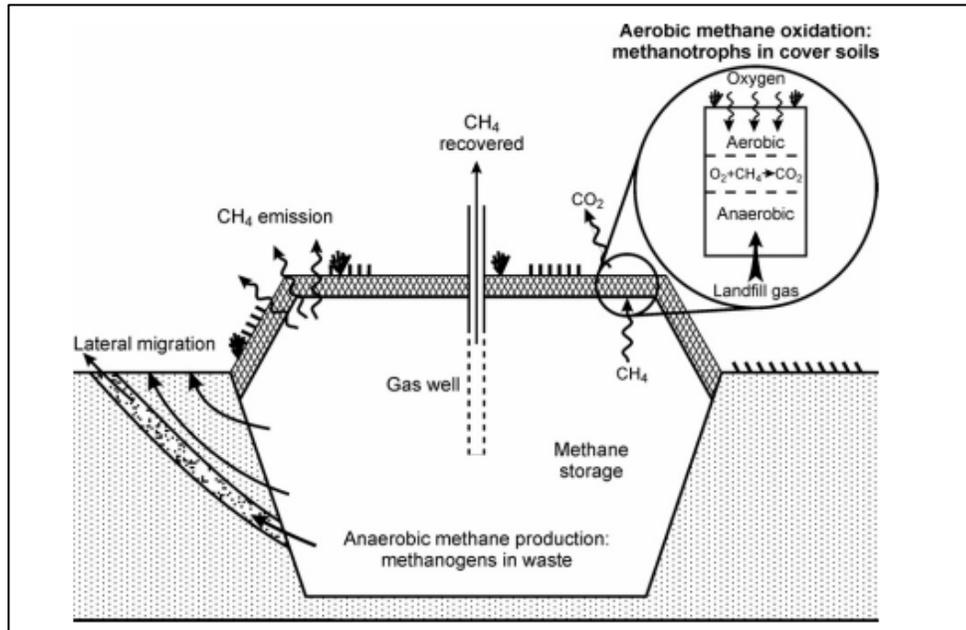


Figure 2-9 Schematic of a passive MBF for landfill gas emission control (Reprinted with permission from (Scheutz et al., 2009)).

Gebert et al. (Gebert et al., 2003) studied CH₄ oxidation capacity, and methanotrophic population of a passively-vented landfill biofilter with an average CH₄ loading of $11 \frac{g CH_4}{m^3 h}$. The biofilter consisted of a two-chamber up-flow system, for which the ventilation was passive, depending only on pressure build-up inside the landfill. It was found that the number of methanotrophic bacteria increased over the first year of operation, from an initial value of $4.5 \times 10^6 - 2.6 \times 10^7 \frac{cells}{g dw}$, and stabilizing at $1.3 \times 10^8 - 7.1 \times 10^9 \frac{cells}{g dw}$. As expected, the variations in ambient temperature in winter time adversely affected the top layers of the biofiltration system and decreased the microbial population. Oxidation rate trends in the topsoil were directly correlated with microbial count and adversely affected by the moisture content in this study. Similar observations were reported by Jones and Nedwell (Jones and Nedwell, 1993) for landfill cover soil and Henckel et al. (Henckel et al., 2000) for forest soil. Maximum oxidation rates (V_{max}) ranging

between 5.3 and $10.7 \frac{\mu g CH_4}{h g dw}$ were measured in the month of November. Similar ranges of oxidation rates were reported by Whalen et al. (Whalen et al., 1990) and Kightley et al. (Kightley et al., 1995). Conversely, Boeckx et al. (Boeckx et al., 1996) studied landfill cover soil subjected to fluxes ranging from 5.9 to $914.3 \frac{ml CH_4}{m^2 d}$ and reported considerably lower values of V_{max} (0.88 - $10.86 \frac{ng CH_4}{h g dw}$). The investigations by Borjesson et al. (Börjesson et al., 1998) on a landfill cover soil made of sewage sludge were an order of magnitude higher, with values ranging from 7.68 to $152 \frac{\mu g CH_4}{h g dw}$.

Nevertheless, as previously discussed, CH_4 oxidizing bacteria are obligate aerophiles, preferring O_2 concentrations below atmospheric levels (Mancinelli, 1995). Therefore, biofilter performance is better supported through active introduction of O_2 to the biofilter profile (Gebert and Gröngröft, 2006; Haubrichs and Widmann, 2006).

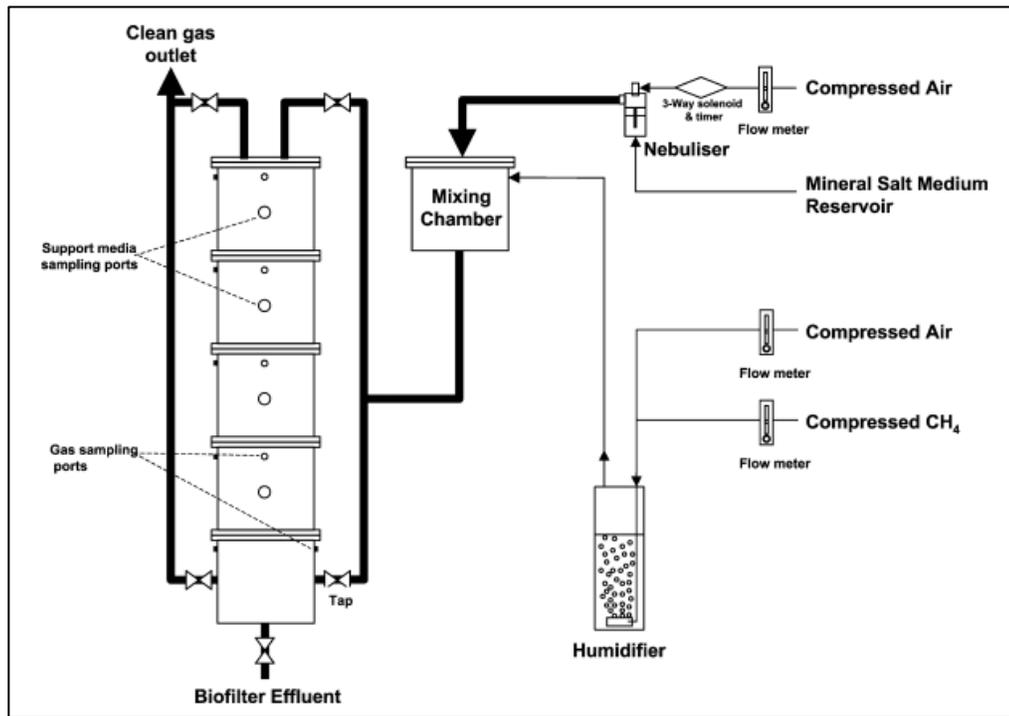


Figure 2-10 schematic representation of a lab-scale active MBF set-up (Reprinted with permission from (Du Plessis et al., 2003)).

Streese and Stegmann (Streese and Stegmann, 2003) investigated the potential of actively aerated MBFs in treating landfill gases by introducing a mixture of air and CH₄ to four bench-scale and one field-scale biofilters. The gas mixture was moisturized using a scrubber before being injected to the columns in a down-flow mode. CH₄ with concentrations varying between 0.58-3.5% v/v was examined on three different filter media; (i) compost, (ii) a mixture of compost, peat and wood fibres, and (iii) intermittent layers of compost and wood fibres. Their results indicated that biofilter performance declined for compost after five months of operation due to O₂ holdup by EPS accumulation, whereas a mixture of compost, peat and wood fibre presented a more stable performance due to a “fluffy” texture. Although the decrease in system performance after long-

term operation for higher concentrations can also be attributed to insufficient contact time, such effects were not accounted for in this study.

Haththotuwa (Haththotuwa, 2005) studied the performance of actively aerated MBF columns subjected to a mixture of CH₄ and air at the bottom. Columns were packed with soil and fed at loading rates ranging from 407 to 1212 g/m³/day in 7 stages. The study suggests a maximum oxidation rate of 705 g/m³/day for actively vented MBF columns.

A recent study was conducted by Haubrichs and Widmann on actively aerated MBFs (Haubrichs and Widmann, 2006). They studied actively vented columns injecting air in 1/2, 1/3, and 1/6 proportions along the biofilter height. Biofilter columns were packed with compost, a mixture of compost and woodchip, and a mixture of compost and paper pellets. Their results confirmed better distribution of CH₄ oxidation through the biofilter height and suggested enhancement of oxidation rate by a factor of 5.5 compared to passive systems. However, EPS formation and O₂ transport clogging were observed after long-term operation of the columns, causing the lower part of the biofilter to go anaerobic. Mean efficiency values of 96% and 93% were obtained for the compost and compost/woodchips biofilters, respectively. These efficiencies were decreased to 90% as a result of a 20% decrease in inlet air flow rates. As very high O₂ flow rates cause low residence times and low flow rates may be rate limiting for CH₄ oxidation, an optimum ratio between O₂ input flow rate and CH₄ loading is yet to be determined. Also, the results obtained here have little applicability in the field-scale, as the concentrations were representative of those normally encountered in poor landfill gases where application of active aeration with variable air flow rates may not be economical.

In 2011, Lee et al. (Lee et al., 2013) studied CH₄ removal using aerated biofilters and biotrickling filter, where CH₄ was introduced from the bottom in a mixture along with air, ethylene, acetone, n-butanol, and ammonia to the PVC columns. They used a mixture of 40% woodchips, 40% Straw and 20% compost as their packing media. Their results suggested no CH₄ removal in the biofilters. This was attributed to either very low residence time of 30 seconds employed (which is not favourable regarding the low solubility of CH₄), or the presence of other contaminants alongside CH₄ (which inhibit methanotrophic culture growth). Also, the presence of ammonia in the contaminant stream likely released ammonium ion, and lowered the pH below neutral levels optimum for methanotrophic activities.

2.7.2 Flow direction: up-flow vs. down-flow

Air flow direction is of critical importance to guarantee proper system loading (Quigley et al., 2004). Down-flow operation may come with advantages such as better moisture control and drainage handling. When operated down-flow moisture is more easily provided through further additional of water at the top portion. This area is more likely to be desiccated due to the reaction heat generated and inlet air supply. In contrast, up-flow operation can be beneficial in terms of easier leachate removal and prevention of CH₄ accumulation in the headspace, taken that CH₄ is lighter than air with a density of approximately 0.66 Kg/m³ at 20°C (Haubrichs and Widmann, 2006). Gebert and Grongroft (Gebert and Groengroeft, 2006) also investigated performance of an up-ward flow passively-aerated biofilter for treatment of residual landfill gas. They found that for long-term operations of such systems CH₄ oxidation is strongly limited to O₂ supply which is controlled by the dynamics of the site, atmospheric pressure, and wind flows. In such circumstances, CH₄ loading rates and operating temperatures can highly affect biofilter

performance. This calls for more research on the application of active aeration and the influence of corresponding controlling parameters.

2.7.3 Setting: closed vs. open

Open biofilters are more commonly operated in landfills as robust systems with no need for irrigation and heating. CH_4 is usually supplied and distributed at the bottom through an upward flow, and O_2 is made available through surface diffusion. Passive ventilation of these systems, and their susceptibility to ambient climatic conditions brings about reductions in system efficiency. Fully contained systems provide better control over operating conditions, such as temperature and moisture content, and inlet gas fluxes (Huber-Humer et al., 2008). In such systems, O_2 is supplied through the gas supply network which adds to higher capital and operational costs.

Hettiaratchi et al. (Hettiarachchi et al., 2011) investigated the operation of a pilot-scale MBF located at Wildcat-Hills gas metering station, in Alberta, Canada, and compared the results to that of a one-dimensional model. Their model, serving as a screening design tool, included advective and dispersive gas transport, as well as heat and moisture fluxes and biological reactions. The MBF monitored had a wooden structure, packed with leaf compost of 37 cm thickness. As for the CH_4 oxidation rate, no oxidation was observed in cold months and an oxidation efficiency of 90% was experienced any other time. This suggests that the application of a closed biofiltration scheme in colder climates, especially regions with high diurnal temperature changes.

Chapter Three: Theoretical and methodological Considerations

3.1 Overview

This chapter is intended to expand on the theoretical and methodological procedures adopted to choose the value of background parameters, such as bed material characteristics (e.g. moisture content, organic carbon fraction, porosity, etc.), flow rates, and compaction effort.

Section 3.2 includes information on compost characterization methods.

3.2 Compost characteristics

3.2.1 Organic Carbon Fraction

Organic matter content is used as an estimate of the total organic carbon content. The loss-on-ignition method (LOI) is used for determination of the organic matter (Schumacher, 2002). The method involves placing 10 grams of dry samples which have been passed through at most a 10 mm screen in ceramic crucibles and heating them in a furnace at a temperature of 440°C overnight. Samples are then cooled and weighed, and the organic matter fraction is determined as the difference between the initial and final mass divided by the initial mass (ASTM International, 2014; Schumacher, 2002). The organic fraction of the compost used in this study was measured at 34.2% on a dry weight basis.

Equation 3-1
$$\% \text{ OC} = \frac{m_{\text{dry}} - m_{\text{burned}}}{m_{\text{burned}}} \times 100$$

m_{dry} is the mass of samples after drying in the oven at 110°C, in grams (g),

m_{burned} is the mass of samples after burning in the oven at 440°C, in grams (g), and

OC is the organic content in percentage of dry compost, in percentage (%).

3.2.2 Moisture Content

Moisture content is determined gravimetrically by measuring the loss of mass after samples were heated overnight at a temperature of 110°C in accordance with ASTM standard (ASTM International, 2014). Firstly, the wet mass of samples and crucibles are measured and recorded. Samples are then heated in the oven at a temperature of 110°C for 12 hours. Subsequently the samples are weighed again and the moisture content is determined using Equation 3-13. The moisture content values are expressed as percentage of dried compost.

Equation 3-2 $\% \text{ MC} = \frac{m_{\text{wet}} - m_{\text{dry}}}{m_{\text{dry}}} \times 100$

m_{wet} is the mass of samples before drying in grams (g),

m_{dry} is the mass of samples after drying in grams (g), and

MC is the moisture content in percentage of dry weight (% dw).

Since moisture content is held constant in all columns, its value is set at an optimum value as studied by Pokhrel (Pokhrel, 2006). He developed CH₄ oxidation curves based on moisture content, temperature, and organic matter for different soil and compost mixtures. In his study on soil and compost biocaps, he examined the maximum CH₄ oxidation rate (V_{max}) for different moisture content and temperature conditions. This value is always less than the optimum CH₄ oxidation rate ($\text{opt-}V_{\text{max}}$) obtained at the optimum moisture content and temperature. A graphical presentation of his work is presented in Figure 3-1. In order to determine the minimum and maximum required moisture content (to achieve an oxidation rate of at least 80% of $V_{\text{max-opt}}$) as well as the optimum value for the present research, CH₄ oxidation curves are used for a 100% compost with an organic matter content measured according to the procedure explained in 3.2.1.

1. A vertical line is drawn from the point representing the percentage organic content on the horizontal axis until it intersects the optimum V_{\max} curve. Continuing the intersection point horizontally to the right and towards the $V_{\max\text{-opt}}$ axis, we find the optimum V_{\max} value to be approximately 35 $\mu\text{mol/g}$ of dry compost.
2. To have the CH_4 oxidation rate within 80% of its optimum value, we aim to find the minimum and maximum moisture contents corresponding to a V_{\max} value of 28 $\mu\text{mol/g}$ of dry compost (0.8 $V_{\max\text{-opt}}$). Therefore, the curves representing 0.8 $V_{\max\text{-opt}}$ on the sides of the $V_{\max\text{-opt}}$ curve are followed until they meet the vertical line of the 34.2% organic matter in step 1.
3. Two horizontal lines are drawn to the left from the intersection points in step 2 to cross the vertical axis representing moisture content. The minimum and maximum moisture

contents on a dry weight basis are found to be 40% and 90%. The optimum moisture content is obtained by intersecting the vertical line of step 1 with the 1.0 $V_{\max\text{-opt}}$ curve.

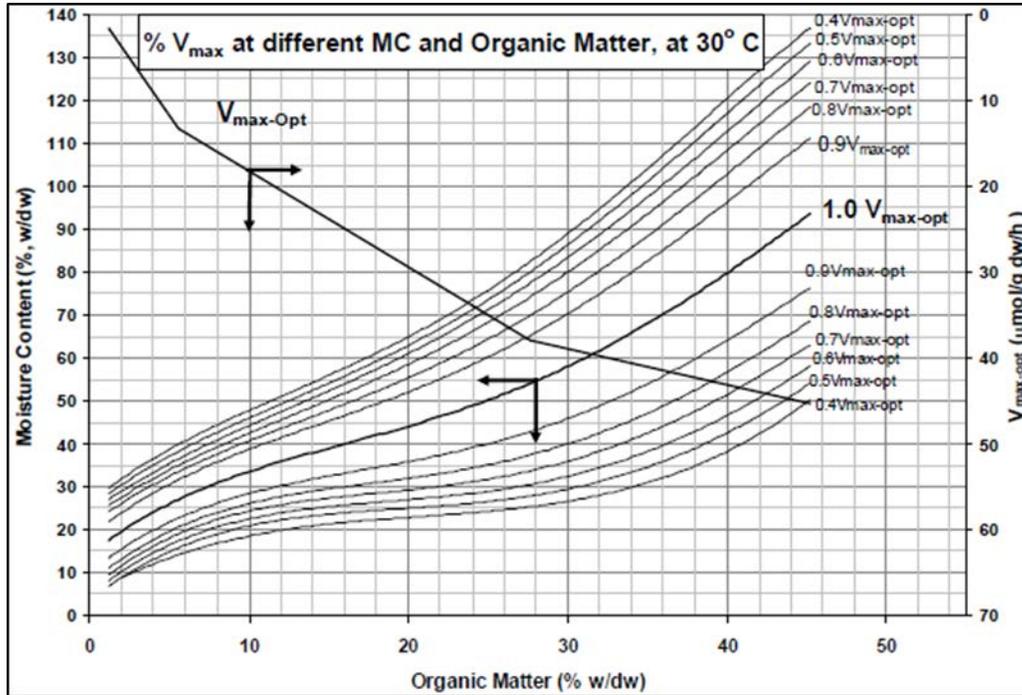


Figure 3-1- Methane Oxidation Curves (MOCs) for V_{\max} at different Moisture Contents (Adapted with permission from (Pokhrel, 2006)).

3.2.3 Water Holding Capacity

Water holding capacity refers to the water content remaining after the soil has been saturated and allowed to drain freely, and is determined using simple funnel experiments (Klute, 1986). A filter paper was wetted, drained, folded and fitted into a funnel. The total weight of the funnel and wet filter paper was recorded. Funnels were then filled up to the top with oven dried compost samples. The volume of the funnel was 500 cm³. The funnel was clamped to a stand. The bottom tip of the funnel was connected to a tube connected at the other end to a burette filled with water. The water-filled burette was clamped to the stand at the same level. Water slowly released from the burette removing air from the compost. Samples were then saturated in this way. After

saturation, the tube was disconnected and the total weight of saturated samples and funnels were measured. A beaker was then placed at the bottom of each funnel to collect the water drained from saturated compost samples. Allowing the water to drain for 4 to 5 hours, the weight of the funnels and wet samples were determined and water holding capacity of the compost was calculated accordingly. During the experiment, funnels were covered to prevent moisture loss from the top surface.

3.2.4 Compost Maturity Index

The term “stability” is used to refer to the highest degree of decomposition with resistance to further decomposition (Wichuk and McCartney, 2010). Immature and unstable compost introduces a number of errors when packed in the columns. Further aerobic decomposition of organic materials depletes O₂ and develops anaerobic “pockets” (*Compost maturity index*, 2001; Wichuk and McCartney, 2010). Moreover, the high degree of microbial activities in unstable compost have the potential to cause self-heating, which is particularly dangerous in cases of large volumes of material, trapping of flammable gases, and expansion of bagged compost (Brinton, 2000; Marthur et al., 1993; Wichuk and McCartney, 2010). Such adverse effects highlight the need to evaluate compost maturity and stability before packing the columns.

Compost maturity and stability tests are categorized into physical (e.g. pile temperature, colour and odour), chemical (e.g. organic matter, pH, and Carbon: nitrogen ratio) and biological (e.g. respiration and enzyme activity) parameters (Wichuk and McCartney, 2010). The most commonly used methods for estimating compost stability are based on respirometry (*Compost maturity index*, 2001). Respirometry is referred to measuring the CO₂ produced or alternatively the O₂ consumed by microorganisms. These indices are used as an indicator of the degree

biological activity remaining in the compost. Higher values of CO₂ evolution (or O₂ consumption) suggest high microbial activity, high respiration rates and less compost maturity. The most frequently cited respirometry methods in the literature are Oxygen Uptake Rate (OUR), Carbon Dioxide Evolution Rate, Solvita® respiration test, and compost self-heating (Wichuk and McCartney, 2010). Although the results from these test are not exactly similar, Brewer and Sullivan (Brewer and Sullivan, 2001) concluded that all these methods can equally differentiate between stable and unstable compost. In the remaining of this section, each of these methods will be briefly discussed. This discussion ends in selecting the method of preference and is followed by the results obtained.

3.2.4.1 Oxygen uptake rate

Specific Oxygen Uptake Rate (SOUR) is defined as the rate of O₂ consumption by microorganisms in a biological system per unit time per unit mass of total solids (U.S. Environmental Protection Agency, 2001). As compost reaches a stable state, the amount of present biodegradable organics decreases, and so do the microbial activities and O₂ consumption rate. Therefore, this parameter can theoretically be related to the extent of compost stability (Tiquia, 2005).

Lasaridi et al. (Lasaridi et al., 2000) suggested a SOUR less than 2.5 mg O₂.g⁻¹ of Volatile Solids (VS).h⁻¹ for stable compost, with values less than 1.5 mg O₂.g⁻¹ VS.h⁻¹ indicating very stable compost. Biologic suggests a threshold of 0.4 O₂.g⁻¹ VS.h⁻¹ (Bio-Logic Environmental Systems, 2008).

SOUR is more advantageous when examining aerobic respiration. Since CO₂ can be produced either aerobically or anaerobically, SOUR is not always the most precise method in evaluation of compost maturity (Bio-Logic Environmental Systems, 2008).

3.2.4.2 Carbon dioxide evolution rate

CO₂ evolution rate is used as a measure for compost maturity and stability as it indicates the decrease in microbial activity as compost matures through time. This test is the preferred method in the majority of composting facilities as it offers the following advantages over similar respiration methods (Switzenbaum et al., 1997):

1. The insensitivity of CO₂ production to anaerobic microbial activities.
2. Low cost, simple procedure, and more precise results

In order to be considered mature and stable, a compost must meet a CO₂ evolution rate of less than 4 mg of C (as CO₂) per gram of organic matter per day as defined by Canadian Council of Ministers of the Environment (CCME) (Canadian Council of Ministers of the Environment, 2005).

3.2.4.3 Dewar self-heating test

This method uses a special vessel or vacuum flask (called a Dewar vessel) with a known volume and monitors compost temperature inside the vessel over the course of a few days under accurately controlled conditions. The increase in temperature can be related to microbial activities and therefore compost stability.

This method is advantageous to its counterparts as it is easily applicable to compost from different feedstock and allows for better control of ambient conditions (Bio-Logic Environmental

Systems, 2008; Brinton, 2000; Wichuk and McCartney, 2010). Nonetheless, some of the disadvantages of this method include;

1. Lack of sufficient sensitivity, it can only distinguish between stable and unstable compost but not the levels in between (Bio-Logic Environmental Systems, 2008; Brinton, 2000; Wichuk and McCartney, 2010).
2. Over-sensitivity to moisture content; the higher the water content, the more energy is required to cause a certain temperature rise (Sullivan and Miller, 2001; Wichuk and McCartney, 2010).
3. The analysis is often time-consuming; several days are needed to obtain the results (Switzenbaum et al., 1997).
4. Non-optimal operating conditions; Operating conditions are normally not at their optimum values including salinity, pH, over-heating and reheating of the compost sample caused by uneven access of microorganisms to substrate affect the results (Brinton et al., 1995; Wichuk and McCartney, 2010).

3.2.4.4 Solvita ® test

Wood Ends Research Laboratory developed a method to evaluate compost maturity on a scale of 1 to 8 using the Solvita Digital Colour reader. Solvita chemistry gels react to the concentrations of CO₂ and NH₃. This method allows for the distinction between “raw”, “active”, and “finished” compost, with 1 indicating a completely raw compost, and 8 indicating the final mature product with resilience to further decomposition (Brinton, 2000; Sullivan and Miller, 2001; Wallace, 2005; Wichuk and McCartney, 2010).

Although this method is accurate for practical compost characterization purposes, it needs some level of standardization with regards to temperature and moisture content. The moisture contents should not be too high or too low, and the temperature should be maintained between 20 and 25°C (Brinton, 2000; Wichuk and McCartney, 2010).

3.2.5 Material properties

Sufficient air-filled porosity is a key factor for optimum CH₄ consumption, and is essential to guarantee air diffusion, adequate retention time, as well as minimized pressure drop (Huber-Humer et al., 2008). We specifically draw our focus to air-filled porosity and water content of the filter bed material, as these are the two most important characteristics of the biofilter media controlling gas transport.

The two defining parameters in packing columns are porosity and moisture content. In material characterization, porosity is expressed as the ratio of void volume to total volume according to Equation 3-3;

Equation 3-3
$$\phi = \frac{V_V}{V_T} = 1 - \frac{\rho_d}{\rho_s}$$

Where, V_V is the volume of voids, V_T is the total volume of the packing bed, ρ_d is the dry bulk density, and ρ_s the particle density.

The porosity values of 30-80% are recommended to ensure both gas plug flow and low pressure drop, according to the literature (Devinny et al., 1999). By definition the particle density is mass of particles divided by the volume occupied by particles. Mass of compost particles is then determined by:

Equation 3-4
$$M_s = \rho_s \times V_s$$

Where, M_S is the mass of solid particles, and V_S is the volume occupied with compost particles.

The laboratory columns used in this study are 14 cm in diameter packed to a height of 70 cm. Thus the volume occupied by soil can be determined as follows; With all terms previously defined.

$$\text{Equation 3-5} \quad V_T = \frac{(0.14)^{0.2} \times 0.7 \times \pi}{4} = 0.107 \text{ m}^3$$

$$\text{Equation 3-6} \quad V_s = V_T - V_v = (1 - \phi) \times V_T$$

Subsequently the gravimetric moisture content on a wet weight basis is defined as the quantity of water in the bulk mass.

$$\text{Equation 3-7} \quad MC = \frac{M_w}{M_T} \times 100 = \frac{M_w}{M_s + M_w} \times 100$$

Where, MC is the moisture content in %, M_w is mass of water, M_T is the total mass of the packing bed and M_s is the mass of solid phase (compost particles).

Mass of water in kg is calculated as:

$$\text{Equation 3-8} \quad M_w = \frac{M_s \times MC}{1 - MC}$$

Alternatively, gravimetric moisture content can also be defined on a dry weight basis:

$$\text{Equation 3-9} \quad MC = \frac{M_w}{M_s} \times 100$$

As discussed in Chapter Two, on the one hand sufficient moisture content is essential for survival and metabolism of the microorganism population. On the other hand, excessive moisture results in the formation of anaerobic zone, induces self-compaction of the bed material and leads to incomplete gas treatment. Moisture content values of 40 to 70% (depending on the material

used) on a dry weight basis are considered favourable based on previous studies (Leson and Winer, 1991; Streese and Stegmann, 2003).

Given that the density of water is 1 g/cm^3 , volume of the water is calculated using:

$$\text{Equation 3-10} \quad V_W = \frac{M_w}{1 \text{ g/cm}^3} C$$

The volume occupied by air is then calculated as the total void volume subtracted by the volume taken up by water:

$$\text{Equation 3-11} \quad V_a = V_T - V_W - V_S$$

Air-filled porosity (ϵ) is defined as the proportion of bulk volume filled with air;

$$\text{Equation 3-12} \quad \epsilon = \frac{V_a}{V_T}$$

Where V_a is the volume occupied with air.

With wet bulk mass (M_T) defined as the sum of masses of water and solid particles, wet bulk density (ρ_b) of the mixture can be calculated as:

$$\text{Equation 3-13} \quad \rho_b = \frac{M_T}{V_T}$$

Then the dry bulk density (ρ_d) is determined using:

$$\text{Equation 3-14} \quad \rho_d = \frac{M_S}{V_T}$$

The liquid film formed on the surface soil particles reduces air-filled porosity, effective gas passages and varies pore geometry. Since the material used in packing the columns is 100% compost with a high moisture holding capacity, the thickness of this liquid layer is expected to be higher than that of soil. Therefore, ensuring a minimum air-filled porosity depending on the moisture content and porosity level is important to guarantee gas diffusion. The values reported

by other researchers for the minimum air-filled porosity at which gas diffusion ceases range between 5% (Xu and Gupta, 1992) to 29% (Hagan, 1941; Marshall, 1959). Pokhrel et al. (Pokhrel et al., 2011) suggested the use of Equation 3-15 to determine the minimum air-filled porosity.

Equation 3-15 $\epsilon_{\min} = (0.0793 \times \phi^{0.32})^{1/1.92}$

3.2.6 Flow rates

As for lab-scale CH₄ flow rates to be used, a down-scale of the flow rates for which a pilot-scale of the proposed CH₄ biofilter to be operated will be used. Oxidation rates in passive biofilters are usually 200-300 g/m³/day. One similar study on actively aerated MBFs suggests that oxidation efficiencies in active MBFs are 200% higher than passively-aerated columns (Haththotuwa, 2005). CH₄ oxidation rate for active biofilters is assumed to be 500 g/m²/day. For a biofilter thickness of 1 m:

Equation 3-16 **Oxidation rate** = $\frac{500 \text{ g/m}^2/\text{day}}{1 \text{ m}} = 500 \text{ g/m}^3/\text{day}$

Defining oxidation rate as;

Equation 3-17 **OR** $\left(\frac{\text{g}}{\text{m}^3/\text{day}}\right) = \frac{Q (C_{in} - C_{out})}{V}$

Where, Q is the volumetric flow rate in m³/day, C_{in} and C_{out} are the inlet and outlet concentrations in g/m³, and V is the biofilter volume in m³. For the lab columns with inner diameters of 14 cm filled up to 0.7 m, we can substitute values as;

Equation 3-18 $500 \text{ g/m}^3/\text{day} = \frac{Q (C_{in} - C_{out})}{\pi \times (0.14)^2 / 4 \times 0.7}$

The inlet CH₄ concentration is 99%, converted to g/m³ (using ideal gas law in NTP conditions):

$$\text{Equation 3-19} \quad \frac{0.99 \text{ m}^3 \text{ of methane}}{1 \text{ m}^3 \text{ of air}} \times \frac{1 \text{ mole}}{24.46 \text{ L CH}_4} \times \frac{1000 \text{ L}}{1 \text{ m}^3} \times \frac{16 \text{ grams}}{1 \text{ mole}} = 647.6 \frac{\text{g}}{\text{m}^3}$$

For an efficiency of 95%, the degraded concentration will be:

$$\text{Equation 3-20} \quad (C_{\text{in}} - C_{\text{out}}) = 0.95 C_{\text{in}} = 0.95 \times 647.6 = 615.22 \frac{\text{g}}{\text{m}^3}$$

Then the lab-scale flow rate can be calculated as follows:

$$\text{Equation 3-21} \quad Q = \frac{\pi \times (0.14)^2 / 4 \times 0.7 \times 500 \text{ g/m}^3 / \text{day}}{615.22 \frac{\text{g}}{\text{m}^3}} = 0.00875 \text{ m}^3 / \text{day} = 6.07 \text{ ml/min}$$

Taking the density of CH₄ as 0.66 kg/m³ in NTP conditions (at a temperature of 20°C and a pressure of 1 atm.):

$$\text{Equation 3-22} \quad \text{Mass flow rate of methane} = \left(0.00875 \frac{\text{m}^3}{\text{day}}\right) \times 0.66 \frac{\text{kg}}{\text{m}^3} =$$

$$0.00585 \frac{\text{kg}}{\text{day}}$$

$$\text{Equation 3-23} \quad \text{Hourly mass flow rate of methane} = 0.243 \frac{\text{g}}{\text{hr}}$$

The value obtained here is set as the minimum flow rate for which lab-scale MBF columns are run. Flow rates are changed according to the experimental design, as discussed in Section 4.4.1. According to the stoichiometric equation for CH₄ degradation, 22.4 L of CH₄ requires 44.8 L of O₂. So, the volumetric flow rate of O₂ should be twice as that of methane:

Equation 3-24 **Average $(Q_{lab})_{O_2} = 0.045 \frac{m^3}{day}$**

Taking the density of O₂ as 1.33 kg/m³ in NTP conditions:

Equation 3-25 **Mass flow rate of O₂ = 0.0598 $\frac{kg}{day}$**

Air is 23% O₂ by mass:

Equation 3-26 **Mass flow rate of air = 0.26 $\frac{kg}{day}$**

3.2.7 Gas residence time

Empty bed gas residence time (EBRT) is calculated by dividing the reactor volume by gas flow rate. Generally, higher residence times guarantee higher removal efficiencies (Limbri et al., 2013). Sly (Sly et al., 1993) suggested a 70% improve in CH₄ oxidation efficiency by increasing the residence time from 5 to 20 minutes. Although EBRT values in the order of seconds to minutes are typically applied for VOC removals, higher values are suggested for CH₄ degradation (Limbri et al., 2013; Nikiema et al., 2007). Some of the values used in literature for CH₄ EBRT are presented in Table 3-3. With a Henry's law constant of more than 30 at 20°C (Rettich et al., 1981), CH₄ low water solubility could be one of the reasons behind higher residence times suggested for the gas.

Table 3-1- EBRT comparisons of EBRT values for lab-scale CH₄ biofilters (Limbri et al., 2013).

Packing material	Inlet Loading (g/m ³ /h)	EBRT (min)	Elimination Capacity (g/m ³ /h)	Removal Efficiency (%)	Operating Conditions	Reference
Glass tubes	24.7	15	17.3	70	1% CH ₄ ; nutrient recycled rate :0.5-3 L/day	(Sly et al., 1993)
Pine bark compost	400.6	50	280.4	70	0.5%CH ₄ ; nutrient mixed with inlet gas	(Du Plessis et al., 2003)
Soil	36-72	114-228	30-52	71-83%	CH ₄ from natural gas npipeline at 2,3,4 ml/min	(Park et al., 2002)
Gravel	71.2	4.3	29.2	41	0.7-0.75% CH ₄ ; nutrient addition: 1.5 L/day	(Nikiema et al., 2005)
Compost	65.8	4.3	12.5	19	0.7-0.75% CH ₄ ; nutrient addition: 1.5 L/day	(Nikiema et al., 2005)
Compost/Perlite (40:60)	15	42	8.25	55	0.8% CH ₄ ; no nutrient added	(Melse and Van Der Werf, 2005)
Gravel	28	4.2	12.5	43	0.025-0.43%CH ₄ ; nutrients :1.6 L/day	(Girard et al., 2011)
Gravel	30	6	16.2	54	0.3% CH ₄ ; nutrient addition 1.5 L/day	(Veillette et al., 2012)

For the purposes of this study and to obtain an estimate for the time required for CH₄ biodegradation without having to overestimate the biofilter volume, we consider the following three mechanisms governing CH₄ elimination:

1. Gas transport through the biofilter packing media
2. Aqueous CH₄ diffusion through the biolayer
3. Degradation kinetics (Wani et al., 1997)

In this section, diffusion time scales for gas-phase transport and gas-water exchange are compared to determine which process is rate-limiting. Conservatively, it is assumed that CH₄ transport through the packing media is diffusion dominated for now, as it will lead to a larger residence time. Advection-dominated residence time depends on the flow rates in operation and will be calculated and discussed in detail in Chapter Four.

Diffusion time-scale can be defined as (Socolofsky and Jirka, 2005);

Equation 3-27
$$t = \frac{x^2}{2D}$$

Where, t is the diffusion time-scale in seconds, x is the thickness of layer in cm: bed thickness for gas-phase transport, and biofilm thickness for liquid-gas exchange, and D is CH₄ diffusion coefficient (in water or air) in cm²/s.

For gas phase transport of CH₄, a bed thickness of 30 cm, and a diffusion coefficient of $6.903 \times 10^{-6} \text{ m}^2 \cdot \text{s}^{-1}$ at 20°C (Pokhrel et al., 2011) have been assumed. CH₄ diffusion in the bed thickness is calculated as 1.8 hours based on Equation 3-27. As for the gas-water exchange, a diffusion coefficient of $1.49 \times 10^{-9} \text{ m}^2 \cdot \text{s}^{-1}$ for CH₄ in water at a temperature of 20°C (King and Adamsen, 1992; Nikiema et al., 2009) has been assumed. The diffusion time scale for aqueous film

thicknesses within the range of 0.1 to 100 μm has been calculated using Equation 3-27. Diffusion time varies from values lower than a second to a few seconds. Therefore, it can be concluded that of the two mechanisms (CH_4 diffusion-dominated transport and aqueous CH_4 transport through the biolayer), oxidation is mostly limited by transport through the porous media thickness (King and Adamsen, 1992).

As for the biodegradation reactions time-scale, the double Monod model has been used to describe the kinetics of biodegradation within the biofilm layer as introduced previously in Equation 2-37.

$$\text{Equation 2-37} \quad \mathbf{R} = \frac{V_{\max\text{CH}_4}[\text{S}_{\text{CH}_4}]}{K_{\text{mCH}_4} + [\text{S}_{\text{CH}_4}]} \times \frac{[\text{SO}_2]}{K_{\text{mO}_2} + [\text{SO}_2]}$$

The focus of this research is on actively-aerated MBFs, therefore O_2 concentrations are not rate-limiting and for simplicity the Monod model for one substrate is used in carrying the calculations:

$$\text{Equation 3-28} \quad \dot{\mathbf{R}} = \frac{V_{\max\text{CH}_4}[\text{S}_{\text{CH}_4}]}{K_{\text{mCH}_4} + [\text{S}_{\text{CH}_4}]}$$

CH_4 degradation rate can be written as

$$\text{Equation 3-29} \quad \mathbf{r} = \frac{X_b}{Y} \mu$$

r is the consumption rate,

X_b is the density of biomass in the biofilm in g/m^3 ,

Y is the biomass yield coefficient in $\text{g biomass}/\text{g CH}_4$,

μ is the specific growth rate of the microorganisms within the biofilm in $1/\text{s}$ (Nikiema et al., 2009)

Subsequently, the term $k(T)$ is defined as the maximum substrate utilisation rate which is temperature dependant. This dependence can be explained using the van't Hoff-Arrhenius equation (Tchobanoglous et al., 2003).

Equation 3-30 $\frac{\mu_m}{Y} = k(T) = k_{20^\circ\text{C}} \times \theta^{T-20}$

μ_m is the maximum specific growth rate of the microorganisms within the biofilm in 1/s,

$k(T)$ is the maximum substrate utilisation rate at the temperature T in 1/s,

$k_{20^\circ\text{C}}$ is the maximum substrate utilisation rate at 20°C in 1/s with an experimental value of 1.464×10^{-5} 1/s (Delhomenie et al., 2008)

The degradation rate is doubled with a temperature increase of 7°C (from 20°C to 27°C).

Therefore, θ is calculated at 1.104 (Nikiema et al., 2009).

Substituting values in Equation 3-30, one will get

Equation 3-31 $k(T) = 1.464 \times 10^{-5} \times 1.104^{T-20}$

Therefore, with the elimination rate defined as a function of CH₄ concentration in the biofilm layer and temperature, Equation 3-29 can now be rewritten as:

Equation 3-32 $r = f(S_{\text{CH}_4}, T) = \frac{X_b k(T) [S_{\text{CH}_4}]}{K_{m\text{CH}_4} + [S_{\text{CH}_4}]}$

CH₄ concentration in the biofilm layer is dependent on the gaseous CH₄ concentrations as described by Henry's law in Equation 2-25:

Equation 2-25 $C_{\text{CH}_4} = H \times S_{\text{CH}_4}$

Equation 3-32 is then rewritten as:

Equation 3-33 $\int_{C_{\text{in}}}^{C_{\text{out}}} \frac{k_m + C/H}{X_b K(T) C/H} dc = \int_0^t dt$

Integration will lead to :

Equation 3-34 $\int_{C_{\text{in}}}^{C_{\text{out}}} \frac{k_m + C/H}{X_b K(T) C/H} dc = \left[\frac{H \cdot (k_m \cdot \ln(C) + \frac{C}{H})}{X_b K(T)} \right] = t$

Substituting values, for an elimination efficiency of 95% (i.e., $C_{out} = 0.05 C_{in}$), t is estimated at 6 minutes, supporting the previous assumption of CH₄ diffusion time-scale being the rate-limiting mechanism.

As previously described, lab columns are filled up to a height of 70 cm and air is injected at either one, two, or three levels. Therefore, the shortest time of travel occurs in the column with three air injection points. The effective bed thickness used in the estimation of time of travel here is one-third of the total column thickness with the assumption that CH₄ degradation occurs evenly through the column height. Gas residence time in lab columns are calculated and compared to the values suggested here in section 5.3.

3.2.8 Compaction Procedure

Compaction is defined as the reduction of porous material volume by expulsion of air from the voids without changing the moisture content.

The compaction test procedure is that suggested by ASTM International for laboratory compaction characteristic of soil using standard effort (ASTM International, 2007). The test method entails placing selected soil at desired moisture content in three layers into a mold. The mold shall be cylindrical in shape, made of rigid metal and have a standard capacity. The mold used here had an average inner diameter of 4 in. (101 mm) and an average height of 4.584 in (116 mm). The mass of mold and mold base plate was determined and recorded. Sieved compost was placed in three layers in a mold in volume. Each layer was compacted by blowing a 5.50 lbf (24.47 N or 2.5 kg) rammer a certain number of times. Layers should be of equal thickness after compaction. The rammer should fall freely through a distance of 12 in. (304 mm) from the surface of the sample. Blows were applied in such a way to ensure coverage of the whole surface.

Following compaction of the last layer, the base plate and collar were removed and the surface of the specimen was trimmed with a knife. The mass of specimen was determined and recorded. The resulting wet bulk unit weight was determined using Equation 3-35.

Equation 3-35 $\rho_b = \frac{(M_t - M_{md})}{V}$

ρ_b is moist density of the compacted specimen in g/cm³ or kg/m³,

M_t is mass of moist soil in mold and mold in g or kg,

M_{md} is mass of compaction of mold in g or kg,

V is volume of compaction mold in cm³ or m³.

The procedure is repeated for different number of blows to establish a relationship between the unit weight and compaction level for the soil.

In order to find the required compaction energy, three samples with at the same moisture level were prepared for the standard proctor test. Samples were compacted at different levels with 10, 5, and 0 drops of the standard rammer at three layers. Then, the resulting wet bulk density was plotted against the number of rammer blows. This graph is presented in Figure 3-2. Interpolating based on the trend line equation shown on the graph, the total number of blows required to achieve the desired bulk density can be determined.

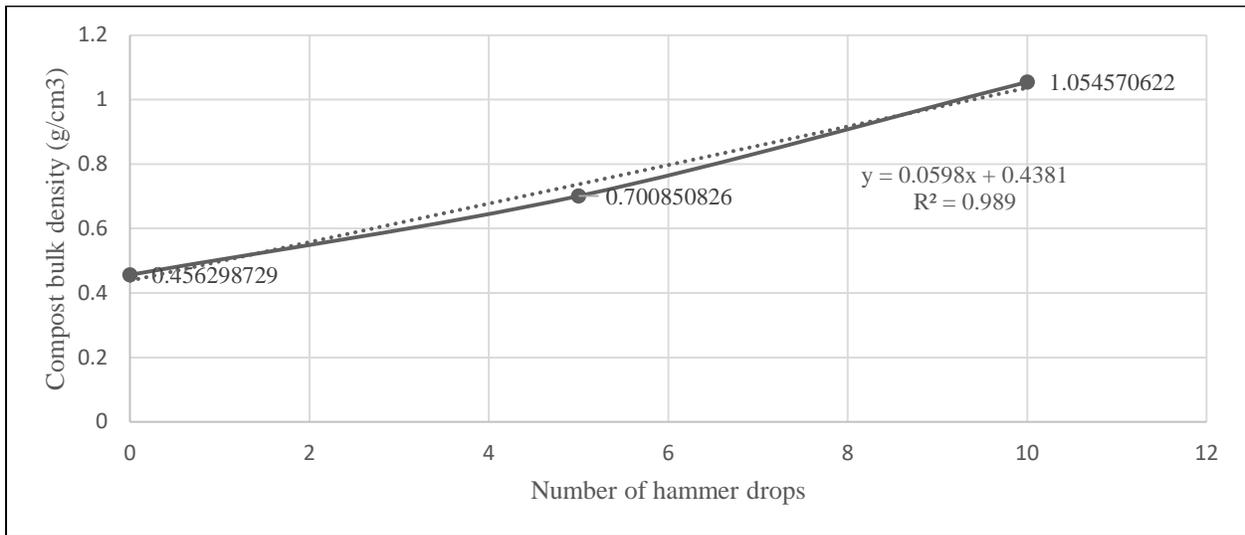


Figure 3-2 Number of hammer drops vs. compost bulk density (g/cm³) in the standard proctor test

Compaction energy can be determined using Equation 3-36.

Equation 3-36
$$E = \frac{N \times L \times W \times H}{V}$$

E is the compaction energy per unit volume of the sample in J/m³,

N is the number of rammer drops,

L is the number of layers,

W is the weight of the rammer in N ,

H is the height of drop in m,

V is the volume of the mold in m³.

For the standard proctor test graphed in Figure 3-2 with the calculated number of hammer drops, the compaction energy is calculated using Equation 3-36.

Chapter Four: Materials and Methods

4.1 Overview

The overall goal of this investigation is to develop optimum design criteria for active CH₄ biofilters in order to minimize point source CH₄ emissions. To that end, laboratory column experiments with different CH₄, air flow rates, and configurations are conducted. The collected data are compiled and analyzed to investigate the effects of air injection levels. Subsequently, the effects of O₂ content are investigated by changing air to CH₄ flow rate ratio and the minimum O₂ requirements to maintain CH₄ oxidation are determined. Column experiments have been chosen for the purposes of this study as they simulate the extent of O₂ penetration due to the advective flows and microbial consumption in a down-scaled biofilter volume.

4.2 Variable definition

Before progressing with development of the most efficient configuration for the proposed multiple level air injection CH₄ biofilters, experiments have to be run to examine the effects of various air injection levels at different CH₄ and O₂ loading rates. Such effects are mainly influenced by O₂ transport (advective and dispersive gas transport) across the porous medium, as well as the extent of O₂ consumption by microorganisms. Before carrying on with set-up of the experiments and the experimental design protocol applied, a clear definition of all the variables involved in this study is given in this section. The parameters involved can be classified as follows:

- **Nuisance variables:** Nuisance variables can be identified, have significant effects and can be measured, but are not controlled in the context of the present study such as;

1. Temperature
2. Moisture content

3. pH levels
4. Nutrients levels available
5. Pressure drop

These parameters can further be categorized as controllable and uncontrollable nuisance variables, as described below:

(I) Controllable nuisance variables, whose levels can be controlled and set along the experiments, such as moisture content, temperature, pH levels, and nutrients available.

(II) Uncontrollable nuisance variables, which cannot be controlled but are measured, such as pressure drop.

- **Primary variables:** Primary variables can be manipulated and studied, such as:

1. Levels of air injection (the three options being tested are air injected at one, two, or three levels in this study)
2. Flow rates (which can also be studied through effects of residence time)
3. Air to CH₄ flow rate ratio

The values and different levels used for each of these primary variables are determined according to the experimental design used, discussed in detail in section 3.4.

- **Uncontrollable variables:** Uncontrollable variables are difficult to be manipulated or measured, such as the extent of EPS formation, its variability with time, its effect on gas transport clogging and overall biofilter performance.

4.3 Operating conditions

4.3.1 Compost characteristics

Three samples of the compost used in this study were prepared and burned as explained above and the average organic content was measured at 34.2%.

4.3.1.1 Moisture Content

Moisture content is determined gravimetrically in accordance with ASTM standard (ASTM International, 2014) as explained in 3.2.2. Moisture content is held constant in all columns and is set to an optimum value as determined by Pokhrel (Pokhrel, 2006). The procedure adopted to determine the optimum moisture content is discussed in 3.2.2. For an organic fraction of 34.2% optimum value chosen for moisture content in this study is set as 60%.

4.3.1.2 Water Holding Capacity

Following this procedure described in 3.2.3, the water holding capacity was calculated at 71.4% on a dry weight basis.

4.3.1.3 Compost Maturity Index

In our evaluation of the compost stability, the costs associated with Solvita test were unreasonable. Since the CO₂ Evolution Rate method offers more simplicity and precision for estimation of CO₂ production, the CO₂ Evolution Rate method has been adopted in this study. The test involves measuring the amount of CO₂ released in the headspace of a closed container containing wet compost of known mass and volume at a known temperature over time. The moisture content of the samples was adjusted to approximately 50%, then the samples were pre-incubated in a chamber with a 100% humidity (to avoid moisture loss) at a temperature of 37°C for 3 days. After the pre-incubation stage, the samples were placed in glass jars with sealed caps,

from which 2 mL samples were taken daily and the evolution of CO₂ was monitored over a four-day period. The results were reported in grams of CO₂ evolved per unit mass of organic matter per day and average over the five samples ran in this study. The average CO₂ produced after a four-day period was measured at 3.089 mg per gram of organic matter per day. This value is less than the 4 mg of CO₂ per gram of organic content per day as defined by CCME. Therefore, the compost used for packing the columns used in this study is assumed to be stable.

4.3.1.4 Material properties

The material used in the present study is solely leaf compost obtained from the City of Calgary East landfill. As previously noted, since the main objective of the column experiments is to compare the performance of different O₂ injection levels under varying flow rates, the type of material used is not a major concern in this study, as long as it remains the same within all the experiments. Physical parameters of the compost, such as average particle sizes, particle density, organic content, and water holding capacity are determined according to commonly-used standard methods. These values are tabulated in Table 4-1.

Table 4-1 Compost characteristics used for CH₄ biofilter columns

Parameter	unit	value
Biofilter material	-	Compost
Organic Content	%	35
Particle size	mm	<2.36
particle density	kg/m ³	2.28
Water Holding Capacity	%	71.4

For the purposes of this study, moisture content was maintained at 60% dw and porosity at 0.73 throughout all columns. These values are not manipulated among the columns. Therefore, once sieved, the initial moisture content of the compost was measured as described in Section 3.2.2 and water was added accordingly. Columns were packed following a Standard Proctor compaction method to ensure porosity is kept at 0.73 in all columns as discussed in Section 3.2.8. With the values adopted for moisture content and porosity, the rest of the parameters were calculated using Equations 3-3 to 3-14. The values obtained are presented in Table 4-2.

Table 4-2 Laboratory columns material characterization

parameter	Unit	Value
Moisture content (MC)	% (dw)	60
Porosity (ϕ)	-	0.73
Air-filled porosity (ϵ)	-	0.5
Dry bulk density (ρ_d)	g/cm ³	0.60
Wet bulk density (ρ_b)	g/cm ³	0.98

The equation suggested by Pokhrel (Pokhrel et al., 2011) is used to determine the minimum air-filled porosity.

Equation 3-15 $\epsilon_{\min} = (0.0793 \times \phi^{0.32})^{1/1.92}$

For a total porosity of 0.73, a minimum air-filled porosity of 0.25 is required. This value is on the higher end of values found in the literature likely since the above equation is derived based on the results of compost and compost-soil mixture samples. The higher capacity of compost and other organic soils in withholding moisture around the particles leads to reduced air-filled porosity available for gas transport.

4.3.2 Flow rates

The minimum flow rate are determined based on the down-scale of field operating conditions as described in 3.2.6. According to the experimental design, detailed in Section 4.4.1, columns are exposed to increasing flow rates of CH₄ ranging from 5.85 g.day⁻¹ (5.68 ml/min) to 18.51 g.day⁻¹ (18 ml/min) in a step-wise manner, for which the CH₄ conversion rate is measured based on the outlet CH₄ concentration.

4.3.3 Compaction Procedure

As discussed previously in Section 4.3.1.4, the desired wet bulk density in biofiltration columns was 0.98 g/cm³ with a moisture content of 60% dw. According to the Standard Proctor compaction test on samples with the same moisture content and using resulting graph of Figure 3-2, a total number of 9 blows are required to achieve a 0.98 g/cm³ density.

Compaction energy is then using Equation 3-36 to be 212776.62 J/m³ for 9 hammer blows.

Equation 3-36
$$E = \frac{N \times L \times W \times H}{v}$$

The hammer used for compaction of columns was 1910.2 g in weight. The columns were 0.14 m in diameter, packed in seven layers until a height of 0.7 m. Therefore, the number of drops for each layer were calculated to be 8 using Equation 3-36.

4.4 Experimental protocol

To evaluate and compare design configurations, as well as select optimum parameters under which the final product can robustly operate, column experiments are run to optimize air injection levels for CH₄ oxidation. With CH₄ oxidation rate as the response variable, a response surface, on which the optimum range of values for each controlling variable are defined, should be developed. The experimental process is summarized in the steps below and shown in Figure 4-1;

Step 1: The method and size of the experimental design was identified. A two-factor Doehlert matrix was chosen for the first stage. CH₄ flow rates and number of levels at which air is injected are the variables in the first set. In the second stage, air flow rate to CH₄ flow rate ratios are changed along with the previous two factors based on a three-factor Doehlert design. Doehlert design was chosen because of its advantages over conventional designs as elaborated in Section 4.4.1.

Step 2: The three input variables and their range of values were defined based on the design matrix. The highest number of levels was chosen for the most significant factor.

Step 3: The experiments established were run and the results were recorded and plotted.

Step 4: The results were analyzed for significance of effects of each input variable, their influence on the response variable and optimum levels.

These steps will be discussed further in sections 4.4.1 through 4.4.3.

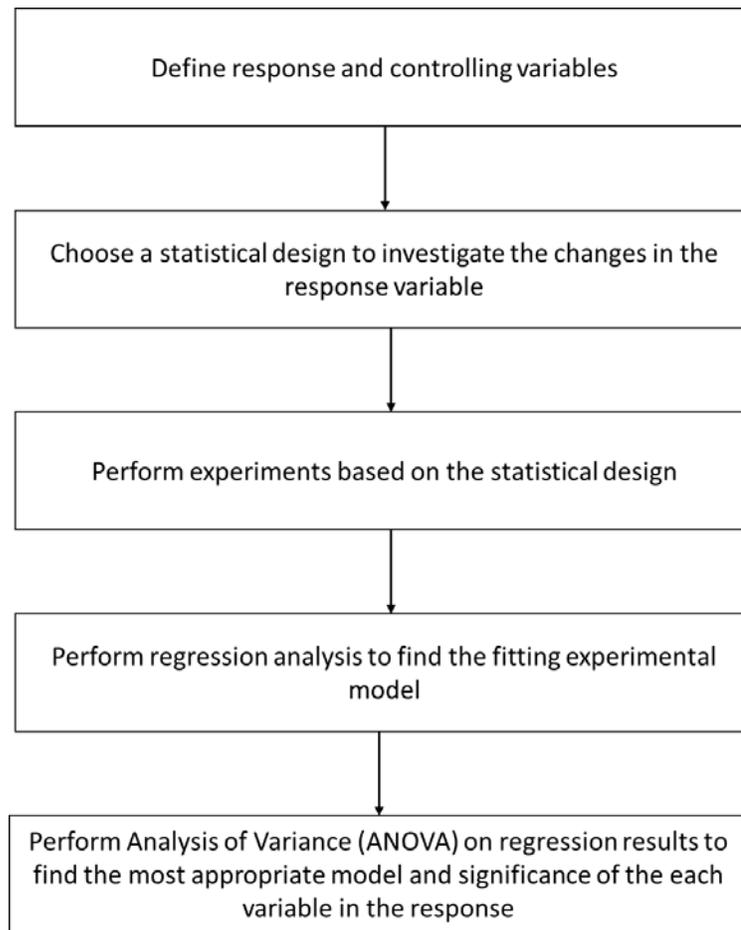


Figure 4-1 Experimental procedure in optimizing O₂ levels for the multiple level air injection MBF.

4.4.1 Experimental design

In this context, the second-order uniform shell design proposed by Doehlert (Doehlert, 1970) is applicable. Before expanding on this design, its experimental domain, and how the factors are distributed, we will illustrate the reasons why some of the most common design methods are not applicable in this study. A one-factor-at-a-time approach is not only time-consuming but also fails to account for the possible interactions between different components . An interaction is defined as the failure of one parameter to produce the same response with changes in another factor

(Montgomery, 2001; Nechar et al., 1995). Variation of all studied parameters simultaneously through multivariate experimental designs allows for studying interaction effects.

A full factorial design is useful in estimation of first-order main effects and interactions. It is therefore applied in preliminary studies where a large number of factors are involved among which the most predominant variables need to be highlighted. A full factorial design with N factors requires 2^N experiments. For 3 factors, this design generates 8 experimental points. This method fails to determine second-order effects, therefore it is not applied in the present work (Araujo and Brereton, 1996; Ferreira et al., 2007). Second-order designs are more powerful in expressing the curvature of the response surface and approximating a response function (Rossi and Haupt, 2007).

Star designs are formed by $2N+1$ points, N being the number of factors to be studied. These points are formed by moving an equal step size from the centre point (0,0,0) along each axis in the positive and negative direction. For 3 factors, a star design required 7 experiments as shown in Table 4-3.

Table 4-3 Star design for three factors

No.	X₁	X₂	X₃
1	0	0	0
2	-1	0	0
3	+1	0	0
4	0	-1	0
5	0	+1	0
6	0	0	-1
7	0	0	+1

Although star designs need fewer number of experiments compared to full factorial designs, they fail to account for interactions. This is evident from Table 4-3 where multiplication of any two columns will produce a result of zero (Araujo and Brereton, 1996).

A Central Composite Design (CCD) is the result of overlaying a factorial design on a star design producing 2^N+2N+1 points to define linear, quadratic and interaction terms in the polynomial model. In a rotatable CCD, the points generated by the factorial design are located at ± 1 distance from the centre point, and those generated by the star design are positioned at $\pm[2^N]^{1/4}$ (Araujo and Brereton, 1996). In this format, the results provide equal information in all directions. However, the number of experiments required to generate a quadratic models with interaction model is higher in comparison with the Doehlert design. To study the effects of two and three factors, a CCD requires nine and 15 experiments whereas the Doehlert design requires 7 and 12, respectively.

The drawbacks of the mentioned experimental design methods can be eliminated by incorporating all the affecting parameters by the Doehlert design (Doehlert, 1970). The uniform shell design developed by Doehlert is used in this study; as it comes with the following advantages:

1. It allows analysis of each of the factors at different number of levels with fewer experiments compared to the central composite design (Doehlert, 1970; Ferreira et al., 2007; Nechar et al., 1995). Generally, it is preferred to assign a large number of levels to the factor with the more significant effect to get the maximum information on its effects.
2. Interestingly, Doehlert designs allow for possible introduction of new variables into the design during the course of the experimental study. As long as all variables of interest are already incorporated to the experiment at their average value (at the centre point) from the

start, they can be manipulated later without loss of the already run experiments (Ferreira et al., 2007). We took advantage of this feature in order to develop a base line for the optimization problem with minimum number of experiments. The first set of columns were run for the stoichiometric air to CH₄ flow rates, changing only the injection points and CH₄ flow rate. Once the main effects and interactions of these parameters were studied, different air to CH₄ flow rate ratios were also investigated through additional experiments.

3. The efficiency of any experimental design is expressed as the number of the coefficients of the regression model divided by the number of experiments. Doehlert design uses fewer number of experiments compared to the central composite design or Box-Behnken design and therefore is more efficient (Gonzalez and Gonzalez-arjona, 1999).
4. Modifications of the levels of parameters in the study can also be easily made with minimum loss of results through displacing the hexagon towards regions with the desired parameter values (Araujo and Brereton, 1996; Doehlert, 1970; Ferreira et al., 2007). This feature is of great value for our application, especially in studying effects of flow rate levels for which a wide range of promising values should be tested.

The complete design contains $d^2 + (d+1)$ points, with d being the number of parameters. Applied to our optimization study, a two variable Doehlert experimental design involves a total number of 8 experimental runs including one replicate at the centre points. With three factors, 12 runs are required to obtain a quadratic optimization function with the interaction effects involved. The form of the experimental design includes a centre point (0,0) and all the remaining points on a sphere of radius 1.

A second-order model for a process involving two factors, x_1 and x_2 , and a response variable, Y , can be described as;

$$\text{Equation 4-1} \quad Y = a_0 + a_1x_1 + a_2x_2 + a_{11}x_1^2 + a_{22}x_2^2 + a_{12}x_1x_2$$

Y is the response variable, CH₄ oxidation rate (g/m²/day),

x_1 and x_2 , are the independent variables corresponding to CH₄ unit mass flow rates (g/m²/day) or volumetric flow rates (ml/min), and numbers of levels of air injection,

$a_0, a_1, a_2, a_{11}, a_{22},$ and a_{12} are model coefficients estimated from the regression model.

With flow rate and the level of air injection as our controlling factors, the designs can be generated for two factors following a regular simplex in two-space. The levels of injection are changed in three levels, and flow rates in five levels. With the centre point set as (0,0), an equilateral triangle is developed for the first three runs of experiments, with points (0,0) , (0.5,0.866) and (1,0). Other points of the design are determined by subtracting the initial set of points from each other (Doehlert, 1970). As mentioned before, an advantage of this approach is that a neighboring region can easily be examined for different flow rate values by adding a few runs as presented in Figure 4-2 (Lundstedt et al., 1998).

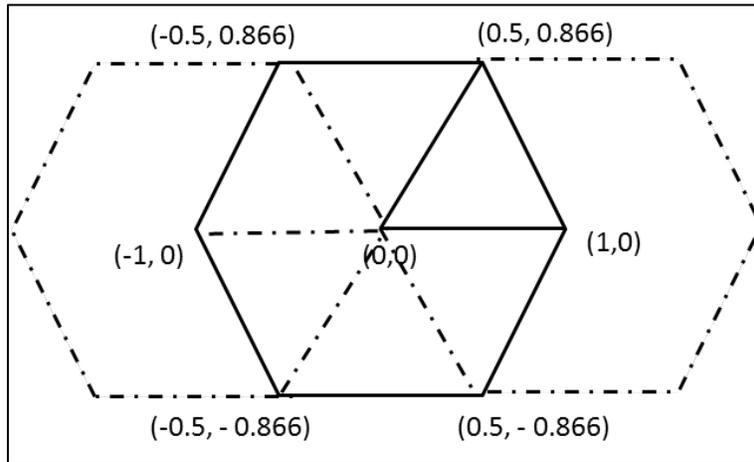


Figure 4-2-Two factor design based on the uniform shell design (starting points of the simplex are marked with an equilateral triangle).

With the air flow rate changing from 60 to 180 ml/min, we have obtained the values tabulated in Table 4-4 for the air flow rate levels in each experiment of the first stage.

Table 4-4 Experimental design matrix based on Doehlert approach (two-factor design)

No.	Experimental design coordinate	No. of air injection points	CH ₄ Flow rate (ml/min)	CH ₄ Loading rate (g/m ² /day)
1	(0,0)	Two	12	741.53
2	(1,0)	Two	18	1112.30
3	(0.5,0.866)	Three	15	926.92
4	(-0.5,0.866)	Three	9	556.15
5	(-1,0)	Two	6	370.77
6	(-0.5,-0.866)	One	9	556.15
7	(0.5, -0.866)	One	15	926.92

Once an optimum combination of flow rates and injection levels and possible interactions of the two factors were obtained, we proceeded by introducing a third controlling variable, inlet air

flow rate to CH₄ ratio. An experimental design based on a three-factor Doehlert network was applied. The design uses air to CH₄ flowrate ratios between 2.5 to 12. With the new parameter introduced, 13 experimental runs are required to fit a second-order regression model to the data with the centre defined as before (Araujo and Brereton, 1996; Doehlert, 1970; Lundstedt et al., 1998). Using the regular simplex model in three-space, the resulting points on a cuboctahedron. The experimental domain is presented in Figure 4-3. The corresponding experimental values were determined as in Table 4-5 (Doehlert, 1970).

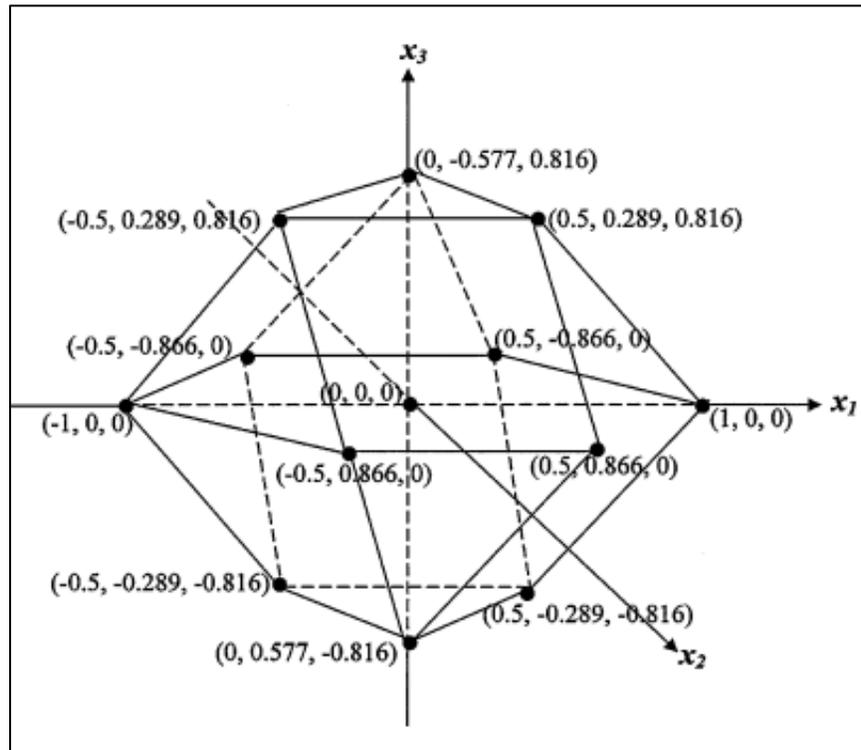


Figure 4-3 3D view of the Doehlert experimental domain. The dots represent the experiments of the Table 4-5 (Adapted with permission from (Imandi et al., 2007)).

Table 4-5 Experimental design matrix based on Doehlert approach (Three-factor design)

No.	Experimental design coordinate	Air flow rate values	CH ₄ Flow rate (ml/min)	Loading rate (g/m ² /day)	No. of air injection points
1	(0, 0, 0)	10:1	12	741.53	Two
2	(1, 0, 0)	15:1	12	741.53	Two
3	(0.5,0.866,0)	12.5:1	18	1112.30	Two
4	(0.5,0.289,0.816)	12.5:1	14	864.78	Three
5	(0.5,-0.866,0)	12.5:1	6	370.77	Two
6	(-1, 0 ,0)	2.5:1	12	741.53	Two
7	(-0.5,-0.866,0)	5:1	6	370.77	Two
8	(-0.5,-0.289,-0.816)	5:1	10	617.70	One
9	(-0.5,0.866,0)	5:1	18	1112.30	Two
10	(-0.5,0.289,0.816)	5:1	14	864.78	Three
11	(0.5,-0.289,-0.816)	12.5:1	10	617.70	One
12	(0,-0.577,0.816)	10:1	8	494.16	Three
13	(0,0.577, -0.816)	10:1	16	988.33	One

The results of the experiments carried out will lead to development of a second-order regression model with three factors as follows;

$$\text{Equation 4-2 } Y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_1^2 + a_{22}x_2^2 + a_{33}x_3^2 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3$$

Y is the response variable, CH₄ oxidation rate (g/m²/day),

x_1 , x_2 , and x_3 are the independent variables corresponding to CH₄ unit mass flow rates (g/m²/day) or volumetric flow rates (ml/min), numbers of levels of air injection and air to CH₄ flow rate ratios,

a_0 , a_1 , a_2 , a_3 , a_{11} , a_{22} , a_{33} , a_{12} , a_{13} , and a_{23} are model coefficients estimated from the regression model.

A response surface is then developed to optimize the O₂ levels with respect to air and CH₄ flow rates as well as number of injection levels.

4.4.2 Response Surface Methodology

Response Surface Methodology (RSM) is intended to lead the researcher rapidly and efficiently to the vicinity of the optimum region (Imandi et al., 2007). Prior to performing the experiments, the region is of course unknown, this is why choice of the experimental design method is of great importance. A design that produces an estimate with equal accuracy in all directions provides a more unbiased response surface. Experiments were carried out as per the design discussed in 4.4.1. Second-order polynomial equations were estimated for the response as elaborated in Equations 4-1 and 4-2. This regression model helps obtain a more precise understanding of the involved factors paired with the response surface.

4.4.3 Analysis of Variances

Using regression analysis and a multiple factor ANOVA, the developed model is validated, and the significance of each of the factors for which tests were carried out along with their interactions is investigated. All calculation are performed using SPSS statistics software (IBM Corporation) and 3D graphs are plotted using SigmaPlot (Systat Software Inc.). This section details on the logic of ANOVA. ANOVA parameters for the list squares fit of a regression model can be defined as those found in Table 4-6.

Table 4-6 ANOVA table for the least squares fit of a linear model (Ferreira et al., 2007)

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares
Regression	$SS_R = \sum_i^m \sum_j^{n_i} (\hat{y}_i - \bar{y})^2$	$p-1$	$MS_R = SS_R/(p - 1)$
Residual	$SS_r = \sum_i^m \sum_j^{n_i} (y_{ij} - \hat{y}_i)^2$	$n-p$	$MS_r = SS_r/(n - p)$
Lack of fit	$SS_{lof} = \sum_i^m \sum_j^{n_i} (\hat{y}_i - \bar{y}_i)^2$	$m-p$	$MS_{lof} = SS_{lof}/(m - p)$
Pure Error	$SS_{pe} = \sum_i^m \sum_j^{n_i} (y_{ij} - \bar{y}_i)^2$	$n-m$	$MS_{pe} = SS_{pe}/(n - m)$
Total	$SS_T = \sum_i^m \sum_j^{n_i} (y_{ij} - \bar{y})^2$	$n-1$	

The parameters in the formulations above are described as follows,

n_i is the number of replicates at the i^{th} level,

m is the number of distinct levels of the independent variables,

p is the number of parameters in the model (also the number of coefficients in the model),

n is the total number of observations,

SS_R is the sum of squares of differences between all the response values predicted by the regression model and their grand mean, with $p-1$ degrees of freedom.

SS_r is the sum of squares of differences between the predicted and the experimental values, with $n-p$ degrees of freedom. Therefore, it can be inferred that large sum of squares values for variations in regression and small sum of squares for the variations in the residual will result in a model that most accurately explains the experimental data,

SS_T is the summation of the two variations above,

SS_{lof} is defined as the sum of squares of differences between each individual predicted value and the average of experimental values with $m-p$ degrees of freedom, and

SS_{pe} is the sum of squares for the difference between the experimental values at each level and their average at the same level and has $n-m$ degrees of freedom.

The ratio SS_R/SS_T will determine how much of the variations in the data is explained by the model and is known as the R^2 . R^2 can only roughly give an understanding of how descriptive the model is of the experimental data. More rigorous judgments have to be made based on the F-test for lack-of-fit and the significance of the regression.

To perform the F-test on the regression lack-of-fit, one has to compare the ratio of SS_{lof}/SS_{pe} to the F-value found on the table for $m-p$ and $n-p$ degrees of freedom at the desired level of significance. Once the model is approved at the desired level of significance as being sufficiently descriptive of the data, a significance test can be performed on the regression. Regression significance can be test by comparison of the ratio of SS_R/SS_T to the F-value found from the table with $p-1$ and $n-p$ degrees of freedom. Having checked the regression significance, ANOVA has to be performed on the values obtained for each coefficient.

4.5 Laboratory-scale Biofilters

Figure 4-5 is a schematic diagram of the compost columns used for this research. Columns are made of rigid plexiglass with an inner diameter of 14 cm. The top and bottom of each column are bolted using two plexiglass endcaps fitted with rubber O-rings. Walls of the column are checked for leakage prior to running the columns. A perforated plate is located near the end of the column to support packing media. The perforated plate covered with fine mesh is disposed on top

of a 10 cm layer of gravel to ensure even gas distribution across the column surface area. Sampling ports are evenly distributed along the height of the columns at 10 cm intervals allowing for periodic gas sampling. Ports are threaded for 1/8" NPT fittings and are fitted with 1/4" Swagelok adaptors. CH₄ (99% purity) flows upward in the column to prevent its accumulation in the headspace. Air is injected through a number of brass perforated probes at desired levels. Probes were punched in a staggered way as shown in Figure 4-4.



Figure 4-4 Aeration probes

Treated gas stream flows through the top, where it is exhausted to the fume hood. The columns are located in the laboratory where the temperature is held at 20°C and supported in a steel structure for safety purposes as shown in Figure 4-6.

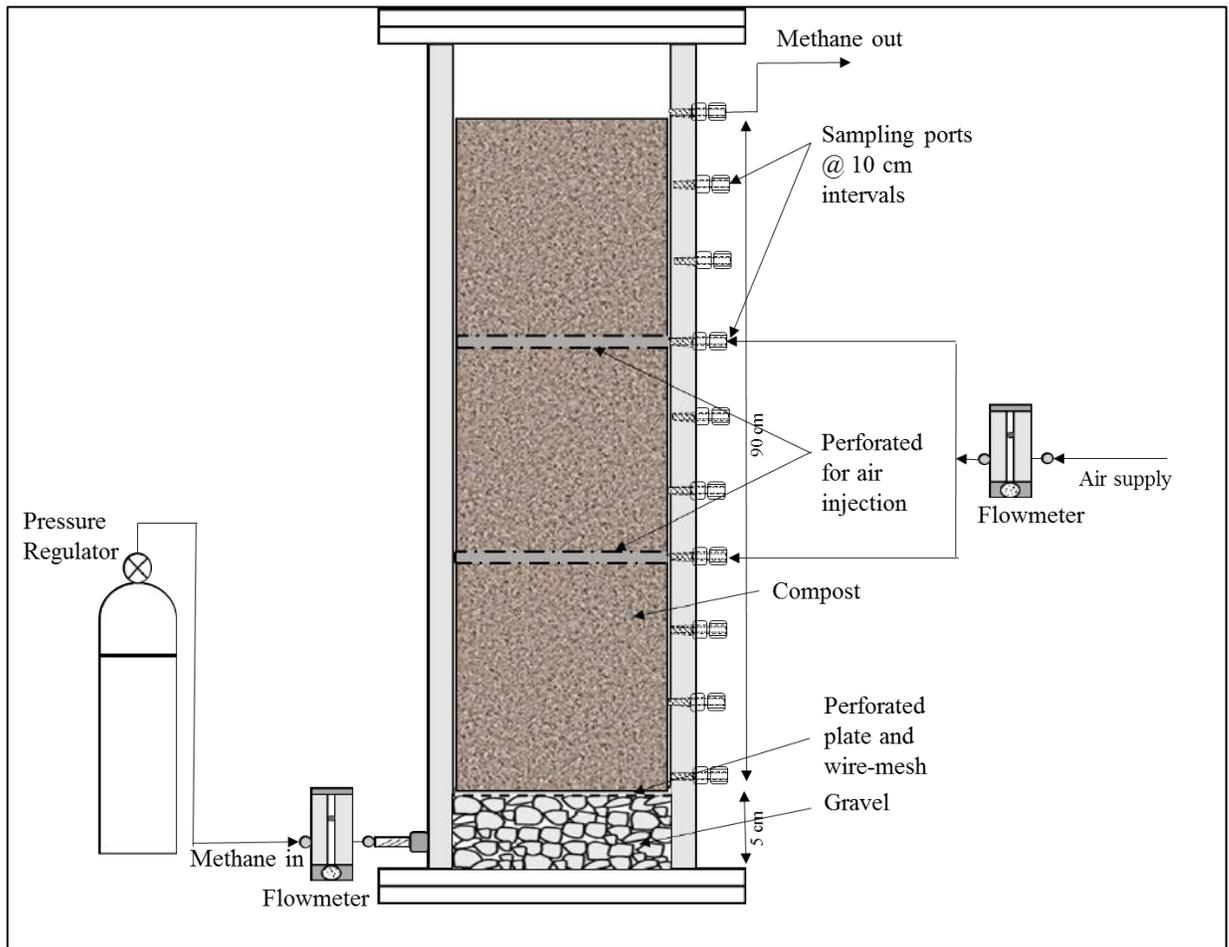


Figure 4-5- Schematic of laboratory biofilter column

Following the start-up of the experiments, all columns are operated under the operating scheme presented in Table 4-4, receiving identical concentrations of CH_4 at a mass flow rate of 6 ml/min (or 370 g/m²/day). For the first set of experiments, flow rates are varied at 5 stages as presented in Table 4-4. Running the columns long enough to reach an oxidation efficiency of 100%, the next step is run increasing the inlet flow rate to next flow rate, 9 ml/min, corresponding to a CH_4 loading rate of 555.9 g/m²/day. CH_4 loading rate is increased in this step-wise manner in the following stages.



Figure 4-6 Photograph of biofilter columns

4.6 Biofilter Packing Media

In classical CH₄ biofilters filter bed thickness was limited by the extent of O₂ transfer, therefore thicknesses higher than 50 cm have been found to be essentially impractical. Because of the active introduction of air in the current study, it is expected that the bed thickness can be

stretched to values as high as 1 m. With the 10 cm gravel layer on the bottom, and a 5 cm headspace at the top for surface gas collection, the compost height in the columns was set to 70 cm.

Granular material used is leaf compost from City of Calgary, sieved with a 2.5 mm screen to retain particles less than 2.5 mm in diameter. The granular medium is lightly packed in the column in 10 cm increments up to a height of 70 cm to ensure uniform density.

4.7 Gas Sampling System

An array of nine four way plastic-lined valves consist the gas sampling system, located every 10 cm along the height, allowing for periodic gas sampling from the biofilter inlet, outlet, and different levels along the height. This will allow for regular monitoring of gas concentration profiles. Gas samples are analyzed for CH₄, N₂, O₂, and CO₂ concentration using gas chromatography as discussed in further detail in section 4.8.

4.8 Gas Chromatography (GC)

Gas samples collected during the column and batch experiments were analyzed for CH₄, O₂, CO₂, and N₂ using a Hewlett Packard Micro-Gas Chromatograph with Thermal Conductivity Detector (TCD)s.

Chromatography involves the separation of a the entities of a mixture as a result of their partitioning between two different phases. The first phase is stationary, has a large surface area and is in contact with the second phase in motion. The sample entities must be stable, with a vapour pressure of approximately 0.1 Torr (13.33 Pa) at the operating temperature, and interact with the column material (a liquid or a solid stationary phase) and the mobile phase (carrier gas). The mobile phase is a chemically inert carrier gas, commonly nitrogen, helium, and argon (Grob and Barry, 2004). The carrier gas of choice in this study was helium. The moving phase system

contains a molecular sieve to remove water and other impurities. The stationary phase is a micro-layer of liquid adsorbent on an inert solid inside a glass or metal column. Hence the term gas-liquid chromatograph refers to the mobile and stationary phases, respectively.

The sample is injected to the column through a microsyringe. The gaseous compounds interact with the walls of the column, covered with the stationary phase as they are swept by the carrier gas stream. The rate at which gas molecules progress along the column depends on the type of the molecule and the adsorption strength. Since each molecule has a different rate of progression, gas constituents are removed at a different time (known as the compound's retention time). The column is located in an oven with controlled temperature (Dojcinovski and Anakievski, 2013). A diagram of the GC system is shown in Figure 4-7.

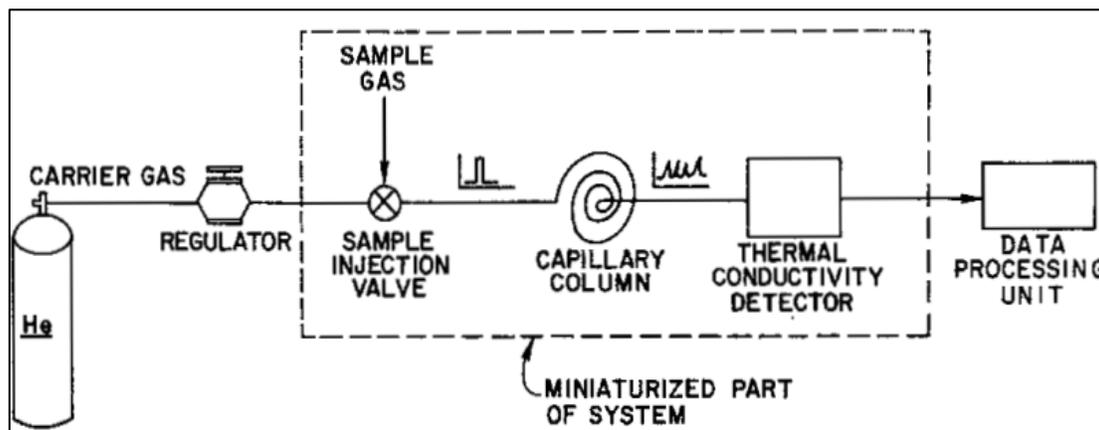


Figure 4-7 Block diagram of a gas chromatography system Reprinted, with permission, from (Terry et al., 1979))

As the components are separated, they exit the column at different times and are detected and identified electronically at the exit. The least-sorbed constituent advances first and is the first to be detected. The column's effluent is therefore a series of peaks separated by regions of pure reference gas. To detect these peaks, the output is sent over a detector which measures a specific property of the mixture. The detector for the GC used in the current study uses thermal conductivity

to distinguish peaks. The TCD senses variations in the thermal conductivity of the column effluent and compares it to that of the carrier gas (or the reference gas). Since the thermal conductivity of most carrier gases are much higher than the column output compounds, once the gas stream exits the column, its thermal conductivity is reduced, and a detectable signal is produced and recorded as a function of time. Therefore, the identity of the gas component is determined from its retention time in the column and its concentration is determined from the area under its output peak (Terry et al., 1979). The analytical conditions for the GC used in the present study are presented in Table 4-7.

Table 4-7 Gas chromatography system specifications

Carrier gas	He
Column	Poraplot-Q (4m × 0.32mm I.D., 10mm df) Packed with MS-5A molecular sieve (10m × 0.32mm I.D., 30 mm df)
Sample size	2-5 ml
Sample time	10 sec
Injection time	100 msec
Column Oven temperature	100°C
TCD sensitivity	Low
Peak integration software	Hewlett-Packard EZ-Chrom

4.9 Biofilter Performance Assessment

Two parameters, biofiltration elimination capacity and oxidation efficiency, are used to evaluate and compare performance of different columns. Biofilter elimination capacity is determined in grams of CH₄ per m³ of bed per hour using;

Equation 4-3
$$EC = \frac{Q(C_i - C_0)}{V}$$

Q is the volumetric CH₄ flow rate in m³/h,

C_i and C_{out} are CH_4 concentration in the inlet and outlet flows in g/m^3 ,

V is the volume of column packed bed in m^3 (Nikiema et al., 2007).

Biofiltration oxidation efficiency can also be calculated in % using;

Equation 4-4
$$RE = \frac{Q_{in}C_{in} - Q_{out}C_{out}}{Q_{in}C_{in}} \times 100$$

C_i and C_{out} are inlet and outlet concentrations of CH_4 in g/m^3 ,

Q_{in} and Q_{out} are the flow rate of CH_4 entering at the column's base and flow rate of the column's effluent, respectively. (Nikiema et al., 2007).

4.10 Laboratory batch experiments for determination of reaction kinetics parameters

Since the main concept behind aeration of biofilter columns was to distribute oxidation through the full thickness of bed, one might expect to observe close-to-equal maximum CH_4 oxidation rates at different portions of the biofilter columns. Therefore, after the columns were run and dismantled, batch experiments were conducted to compare CH_4 oxidation capacity at different depths of each column to evaluate the effects of active aeration on microbial activities.

For these, experiments air tight bottles (1000 ml) sealed with rubber septa for gas sampling were filled with samples taken from different portions of operated columns. The moisture content of all samples was held consistent. The weight of the compost sample placed in the bottles was pre-determined. The samples were incubated under varying initial headspace CH_4 concentrations of 3-6%, and the changes in headspace concentration were tracked over time. To develop a graph for changes of concentrations, measurements should be done for a minimum of three different time points.

Once concentration changes are plotted against time, the rate of oxidation is determined based on the slope of the graph. Oxidation rates obtained for different initial headspace

concentration were then plotted with respect to the initial concentration to determine the maximum oxidation rate. The relationship between reaction rate and the maximum reaction rate in CH₄ oxidation is described by the single substrate Michaelis-Menten reaction kinetics as shown in Equation 4-5.

Equation 4-5
$$\frac{V}{V_{\max}} = \left(\frac{S}{K_m + S} \right)$$

V is reaction rate,

V_{max} is the maximum reaction rate,

S is the substrate concentration,

K_m is the Michaelis-Menten constant (determined as substrate concentration at half of the maximum oxidation rate).

Since the direct plot of CH₄ oxidation rates with respect to initial concentration is nonlinear, the data were linearized to simplify calculation of kinetic parameters. The three most commonly used linearization method of the Michaelis-Menten reaction kinetics are :

1. Lineweaver-Burk plot (Lineweaver and Burk, 1934): This method is also known as the double reciprocal plot. A plot of the reciprocal of the measured concentration (S) versus the calculated reaction rate (V) yields the simplest method. This plot is a straight line whose slope and intercept coordinate determine K_m and V_{max}.
2. Eadie (Eadie, 1942) and Hofstee (Hofstee, 1952) plot : In this method a V is plotted against V/S. Needless to say, in this method the reaction rate appears in both coordinates.

3. Hanes (Hanes, 1932)plot : This method plots initial S versus S/V. the slope of the line generated will directly yield 1/Vmax. However, care should be taken when applying this method as at points diverge from the straight line at low concentrations.

The graphical comparison of these three methods is illustrated in Figure 4-8.

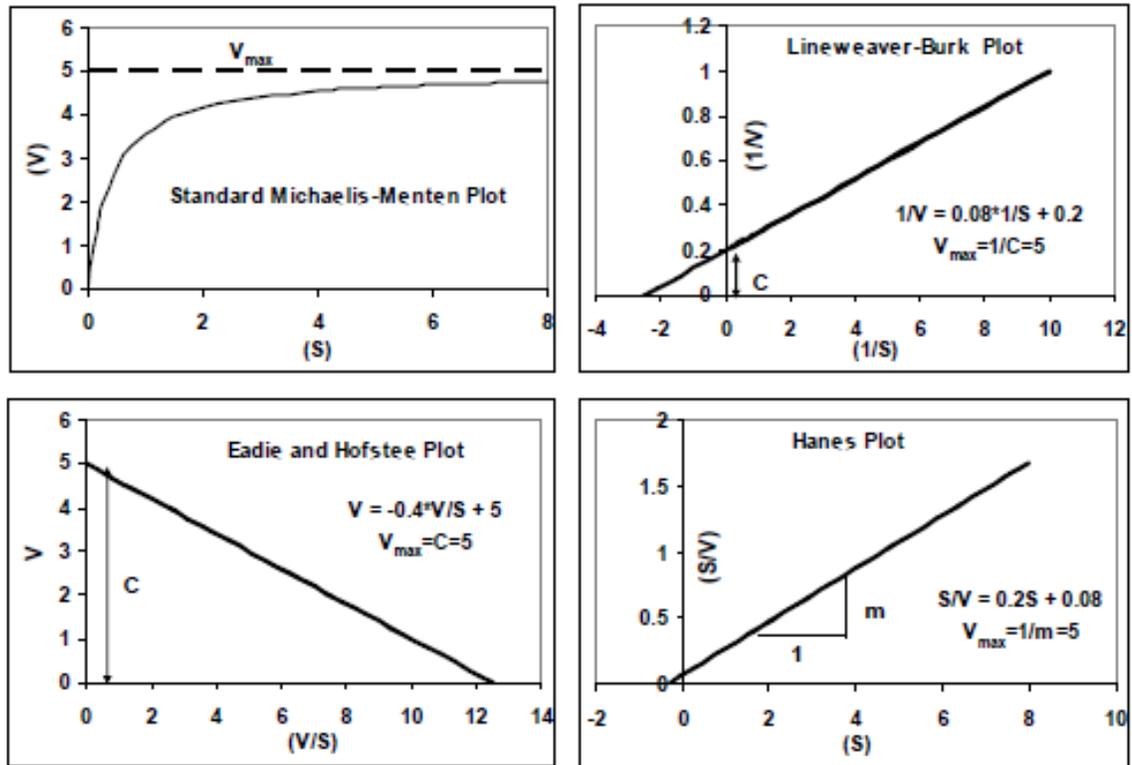


Figure 4-8 Graphical representation of different forms of the Michaelis-Menten equation (reprinted with permission from (Pokhrel, 2006))

These three methods are then compared together and the most reliable method is used for the analysis of results.

Chapter Five: Experimental Results and Discussion

5.1 Overview

Prior to starting experiments towards meeting the objectives mentioned in Section 1.2, a set of column biofilters were run to compare performances of similar biofilters aerated passively and actively, and to evaluate whether or not multiple level aerated biofilters offer an advantage in CH₄ treatment. The results of this preliminary set of experiments are presented and discussed in Section 5.2.

Next, the influence of CH₄ gas flow rate into the biofilter and levels of aeration were evaluated by measuring CH₄ removal rate and CH₄ removal efficiency in the model biofilters. Biofilters were subjected to 99.99% pure methane at flow rates of 6, 9, 12, 15, and 18 mL/min. Air is introduced at a flow rate 10 times higher than that of CH₄s calculated based on stoichiometry. Air is injected via perforated tubes. The first biofilter column is subjected to air injected at three points positioned evenly through the 70 cm height of the packing media. The second column receives air at two levels positioned 35 cm apart (the first probe is injected at the very bottom, and the second one is located 35 cm above). The third column is aerated at one level via the air probe positioned at the bottom of the column. A duplicate of the column aerated at two levels is also run in parallel for the purposes of error analysis when running ANOVA. The flow rates correspond to empty bed retention times (EBRT)s of 270.5, 180.3, 135.2, 108.2, and 90.1 min, respectively for the three-level air injection column. Corresponding EBRTs of the second column (with air injection levels) are 231.1, 154.1, 115.6, 92.5, and 77.1 min, respectively. The last columns aerated at one level has EBRT values of 163.2, 108.8, 81.6, 65.3, and 54.4 min corresponding to the five operating flow rates.

In Section 5.3, The overall performance of biofilter columns over 195 days of operation are discussed and compared. This comparison is based on the achieved removal rates and efficiencies. In Section 5.4, performances of biofilters are evaluated according to their retention times. A discussion on how residence times change over the three different columns according to their levels of aeration, and how they change over the course of the study as flow rates are increased leads to a better evaluation of biofilters performances.

In Section 5.6, the results of the ANOVA are presented. In running the analysis, oxidation rate is set as the response variable, number of aeration levels as the between-subjects factor, and flow rate as the within-subjects factor. A mixed ANOVA with repeated measures (as measurements were done at several points in time for each column) is run and the effects of different factors on the response variable are evaluated. A response surface is then plotted to provide a better understanding on the optimum region of operation for aerated biofilters of this study.

Section 5.7 includes the results obtained from batch experiments run after the columns were disassembled. Samples were extracted from the top, middle, and bottom sections of each column with minimum disturbance. These samples were analyzed for their maximum oxidation rate (V_{\max}), results of which are presented in Section 5.7.1. A fraction of each sample taken was sent to the biology lab for analysis of the variation in the microbial community as discussed in Section 5.7.2.

In Section 5.8, the results of the second set of columns aerated at varying air to CH_4 flow rates are presented and discussed.

5.2 Preliminary Results

Prior to starting column experiments, a set of experiments were performed to compare the effects of passive and active aeration on CH₄ oxidation. These experiments were intended to confirm the proposition that multiple aeration points enhance oxidation activities in MBFs.

The preliminary experiments consisted of two passive columns and two active columns, 0.6 and 1m thick. Active columns were aerated at one point at the bottom of the columns. The active column with a thickness of 0.6 m was replicated to test the effects of multiple aeration levels. Column biofilters were subjected to varying flow rates over the course of 120 days. Figure 5-1 shows how loading rates change over time for each column at each stage. A-60, A-100, P-60, P-100, and A-60* represent active columns of 0.6 and 1 m thickness, passive columns of 0.6 and 1 m thickness, and active column of 0.6 m thickness replication, respectively. The values of loading rates at each stage are also presented in Table 5-1.

Table 5-1 Varying loading rates in g/m³/day for preliminary experiments

Stage	Loading rate (g/m ³ /day)				
	A-60	A-100	P-60	P-100	A-60*
I	667	400	333	200	667
II	833	500	417	250	833
III	1042	625	521	313	1042
IV	1302	781	651	391	

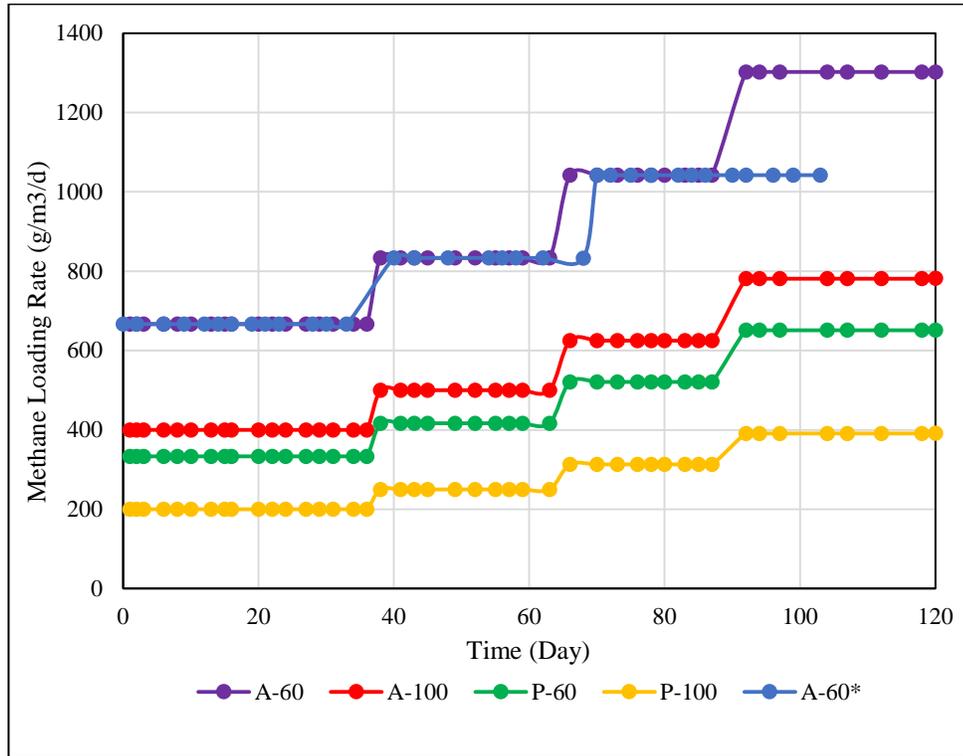


Figure 5-1 Varying loading rates for preliminary experiments

Figure 5-2 shows CH₄ oxidation rates in g/m³/day for each column. As can be seen in Figure 5-2, after the second stage started at day 40, performances of columns A-60, A-100, P-60, P-100, oxidation rates of all four columns leveled out, despite the increase in loading rates at each stage. This translates into decrease in the columns efficiencies as illustrated in Figure 5-3.

The exception is with column A-60 replicate. After oxidation rates dropped during stage II, this column was aerated through two additional inlets. This was to confirm that multiple aeration

points in active biofilters improves their performance. Figure 5-2 shows how oxidation rates increased after the column was subjected to two additional aeration points.

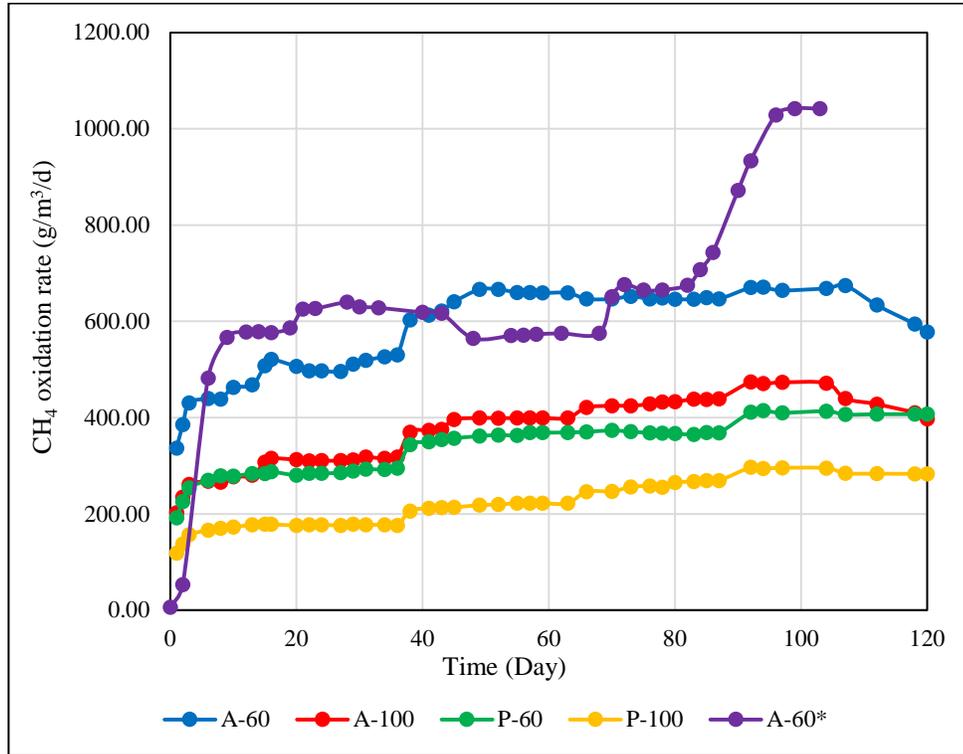


Figure 5-2 CH₄ oxidation rate for preliminary experiments

Table 5-2 presents average oxidation rates for preliminary column experiments. The second and third columns of this table compare the oxidation rates of the two active columns of different thicknesses. As the columns were loaded at different rates, we will use their oxidation efficiencies, instead of oxidation rates, for the purposes of comparison of performances.

Table 5-2 Average CH₄ oxidation rates in g/m³/day for preliminary experiments

Stage	Average oxidation rate (g/m ³ /day)				
	A-60	A-100	P-60	P-100	A-60*
I	475	289	274	169	592
II	645	391	360	217	583
III	647	431	369	259	808
IV	644	446	410	290	

Figure 5-3 shows oxidation efficiencies for the preliminary column experiments. This graph suggests that aerating the 0.6 m active column at three points, as opposed to one, increases the systems efficiency, until it reaches 100%.

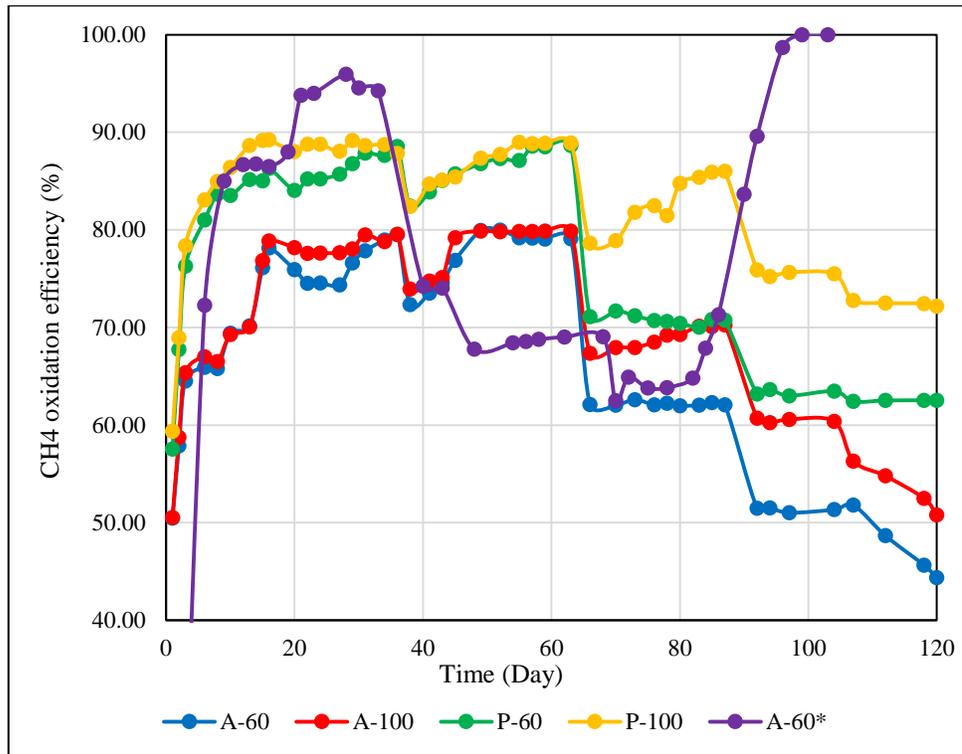


Figure 5-3 CH₄ oxidation efficiency for preliminary experiments

Table 5-3 shows average oxidation efficiencies over the course of each stage for each of the preliminary columns. We draw our attention to 0.6 m aerated column and its replicate aerated at three points, as presented in columns labelled A-60 and A-60*. As suggested by the values in Table 5-3, efficiencies drop during the second stage after which column A-60* is subjected to two additional aeration points. This is followed by an increase in the systems efficiency during the third stage. Therefore, it is deduced that multiple aeration levels can enhance MBFs performances. With this conclusion, we intend to find the effects of number of aeration levels and their

interactions with inlet flow rates and resulting residence times on active biofilters, as expanded in the remaining of this Chapter.

Table 5-3 Average CH₄ oxidation efficiencies (%) for preliminary experiments

Stage	Average oxidation efficiency (%)				
	A-60	A-100	P-60	P-100	A-60*
I	71.2	72.4	82.2	84.5	88.9
II	77.4	78.2	86.4	86.8	70.0
III	62.2	69.0	70.8	82.8	77.6
IV	62.2	68.8	70.8	82.4	

5.3 Methane Oxidation as a function of time: Experimental results

Figure 5-4 to Figure 5-9 present the oxidation rate values in g/m³/day and oxidation efficiencies in % for the three columns over time, respectively. Biofilter columns were operated at 5 different stages. Table 5-4 lists the averaged oxidation rates in g/m³/day and oxidation efficiencies in % for the three columns over the 5 stages.

As listed in the second column of Table 5-4, CH₄ loading rate started at 529 g CH₄/ m³/day (370 g CH₄/m²/day). The lag period for methanotrophic activities in biofilter columns was close to 29 days during which oxidation rates fluctuate as seen in the first stage of Figure 5-4, Figure 5-6, and Figure 5-8. After this period performances of all three columns start to more or less level out for the first stage. The average oxidation rate for columns with one, two and three injection points in the first stage were 420 g /m³/day (294 g/m²/day), 422 g /m³/day (295 g/m²/day), and 501 g /m³/day (350 g/m²/day), respectively.

Table 5-4 Average oxidation rates and efficiencies of aerated columns over time

Stage	Loading rate (g/m ³ /d)	Three-level aeration oxidation rate (g/m ³ /d)	Two-level aeration oxidation rate (g/m ³ /d)	One-level aeration oxidation rate (g/m ³ /d)	Three-level aeration efficiency (%)	Two-level aeration efficiency (%)	One-level aeration efficiency (%)
I	529	501	423	420	95	77	78
II	794	704	671	519	89	84	65
III	1059	931	1025	716	84	92	65
IV	1324	1083	1217	600	80	89	45
V	1588	633	1309	563	40	82	35

The second stage started with introducing 794 g CH₄/ m³/day (555 g CH₄/m²/day) to all columns. A slow and steady increase in performances of biofilters was observed during this stage. The average oxidation rate for columns with one, two and three injection points in the first stage were 538 g/m³/day (377 g/m²/day), 660 g/m³/day (462 g/m²/day), and 721 g/m³/day (505 g/m²/day), respectively. As can be seen in Figure 5-5 and Figure 5-7, performances of columns with two and three injection levels fluctuate around the maximum capacity and reach 100% efficiency in the period between days 58 to 63 after start-up. The one-injection level biofilter, however, never reached 100% efficiency and operated at an average 65% efficiency with a maximum of efficiency of 84% corresponding to an oxidation rate of 669 g/m³/day (468 g/m²/day) which occurred at the end of this stage.

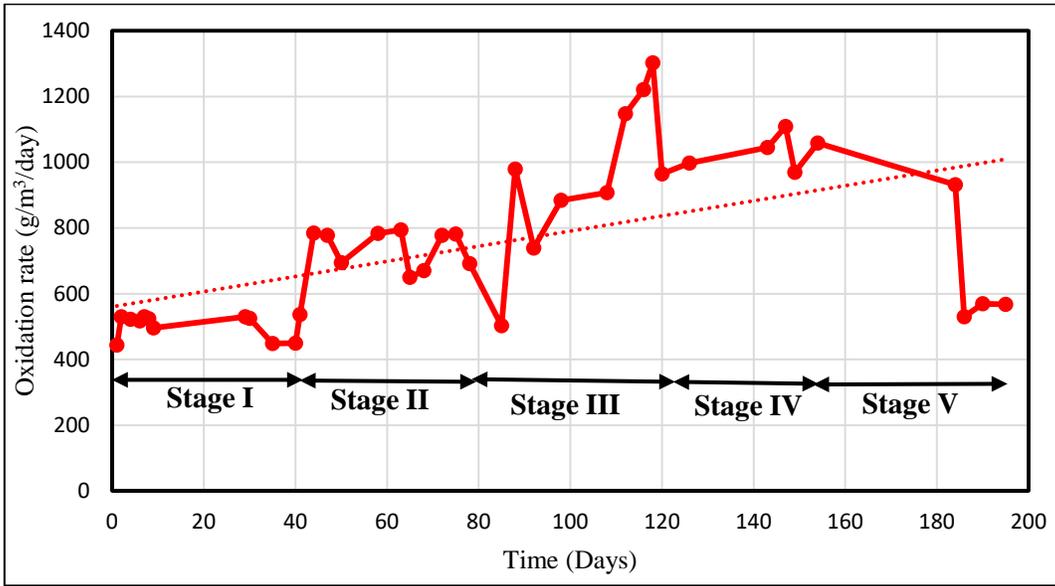


Figure 5-4 Three-level aerated column oxidation rate (g/m³/day) over time

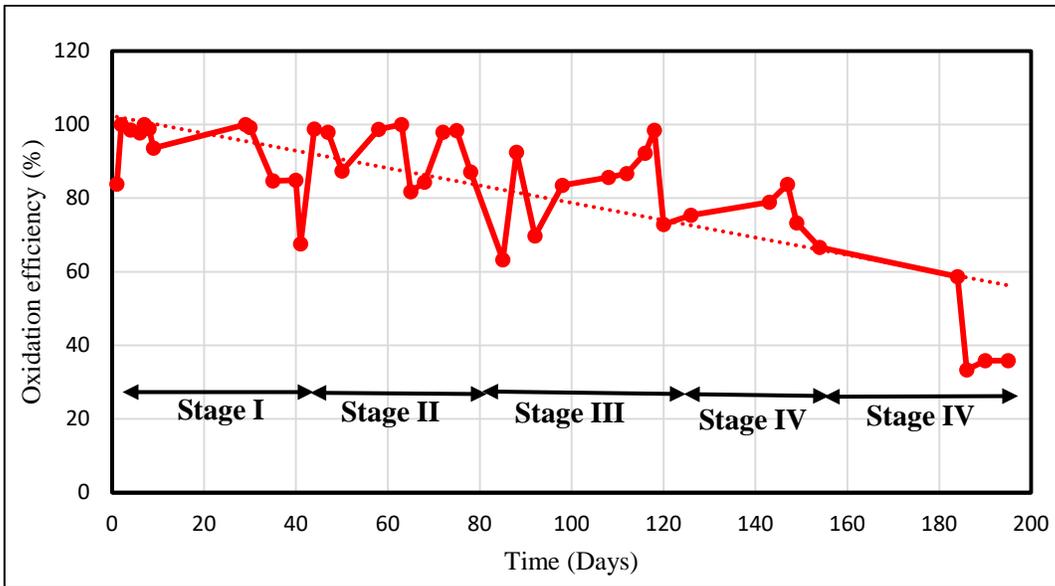


Figure 5-5 Three-level aerated column oxidation efficiency (%) over time

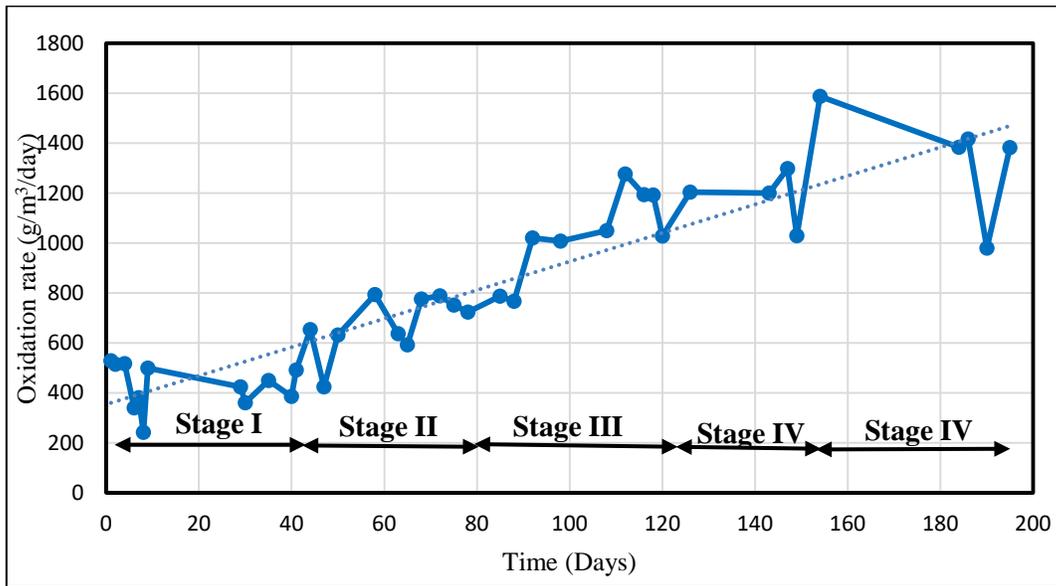


Figure 5-6 Two-level aerated column oxidation rate ($\text{g}/\text{m}^3/\text{day}$) over time

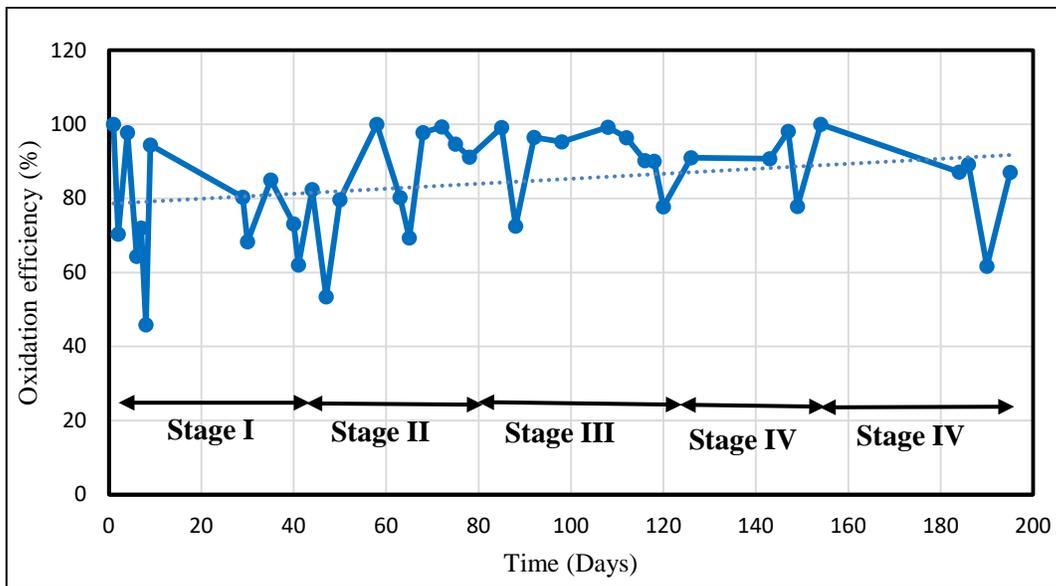


Figure 5-7 Two-level aerated column oxidation efficiency (%) over time

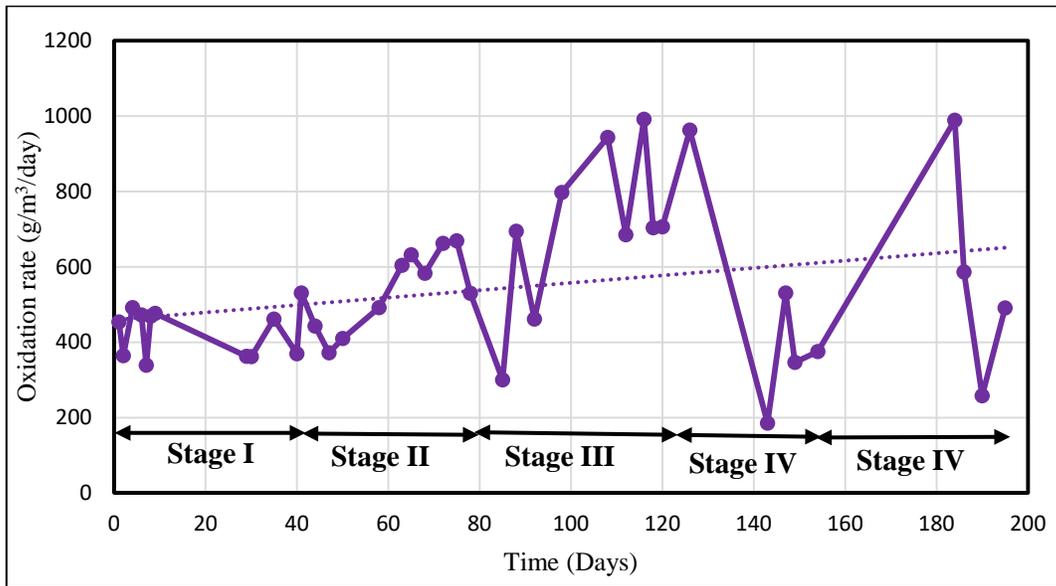


Figure 5-8 One-level aerated column oxidation rate (g/m³/day) over time

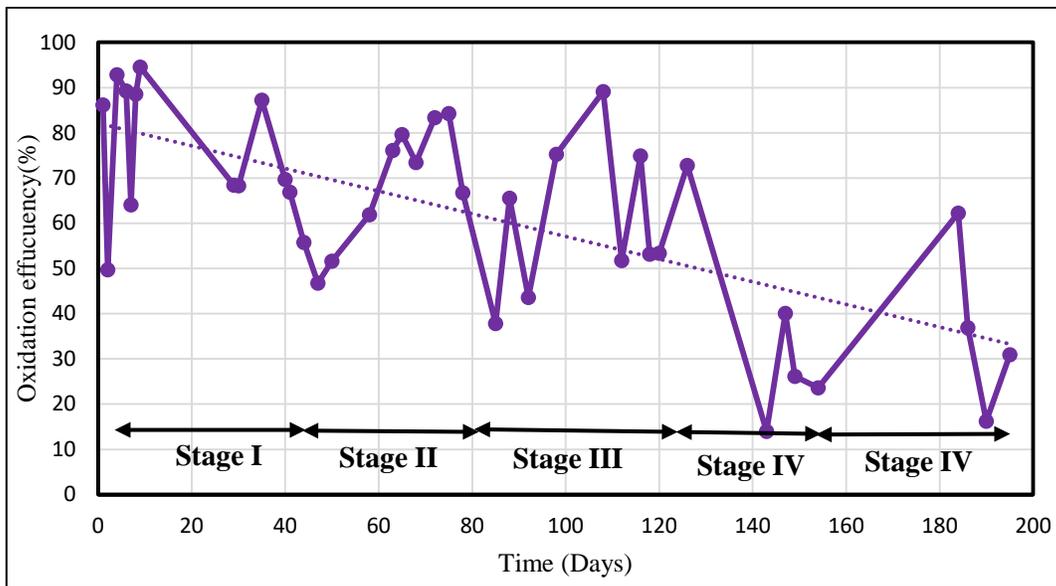


Figure 5-9 One-level aerated column oxidation efficiency (%) over time

The third stage started three months after start-up of the columns and involved feeding the columns with 1058 g/m³/day (741 g/m²/day) of CH₄. During this stage, the two air injection level operated at higher oxidation rates compared to the other two designs. The average oxidation efficiencies of columns was 65%, 92%, and 84% for one, two, and three aeration levels,

respectively. These values correspond to oxidation rates of 716 g/m³/day (501 g/m²/day), 1025 g/m³/day (718 g/m²/day), and 931 g/m³/day (652 g/m²/day), respectively. As the performance of all the three biofilter design were stable to some extent at this stage, flow rates were increased after 20 days of operation leading to stage four of this set of experiments.

Increasing the feeding rate to 1323 g/m³/day (926 g/m²/day) during stage four, a more noticeable difference in performance of different aeration designs was observed. With an average oxidation rate of 1217 g/m³/day (852 g/m²/day) and a maximum of 1323 g/m³/day (925 g/m²/day), the biofilter with two aeration levels had the best performance. The average oxidation rate is twice as that of the column with one air injection level with an average of 600 g/m³/day (420 g/m²/day). The biofilter with three aeration levels operated at an average oxidation rate of 1083 g/m³/day (758 g/m²/day), although never reaching full oxidation capacity.

The last stage involved increased loading rates of 1588 g/m³/day (1112 758 g/m²/day). During this stage performance start to drop significantly hinting that the oxidation capacities of the biofilter columns were exhausted. The oxidation rates were found to decrease after day 184 until day 195 on which the columns were dismantled for microbial community counts and V_{max} analysis.

The fact that columns were run for a longer period of time compared to similar studies, gives us a better understanding of the long-term behaviour of actively-aerated CH₄ biofilters. As suggested by the results of this study actively-aerated biofilters can be operated at loading rates up to 5 times higher than those of passive aeration. This is in fact of help especially when operating biofilters for treatment of solution gas in which flow rates fluctuate regularly with values usually higher than those of landfills. However, operating such systems under stoichiometric air to CH₄ flow rates may not always be economically practical in large-scale replicas. Therefore, a second

set of columns were set up and operated at different air to CH₄ flow rate ratios as discussed further in Section 5.8.

As evident from average oxidation rates of all three designs, after stage three the two-injection level design seems to have a better performance compared to the three-injection level column.

5.4 Residence time

Fluid retention time is defined as the length of time that the gas stream (mixture of CH₄ and air) is in contact with the bed material. Fluid residence time is defined as the volume of the bed times the porosity of the material divided by the gas flow rate. Residence time is a good estimate of the time it takes for gas to travel through bed thickness, assuming that transport is mainly advection-derived and that methanotrophic activities are present throughout the entire volume. As the bed porosity changes over time with self-compaction of the packing material, some researches have used the term Empty bed Residence Time (EBRT) instead. The term is defined as the bed volume divided by the gas flow rate. However, the actual residence time, rather than the frequently used EBRT, is used for the purposes of this study.

As the retention time governs the substrate-methanotroph contact period, small values result in less interaction. As our earlier definition of residence time suggests, higher flow rates lead to a decrease in retention time. Residence times for the biofilter columns operated in this study are calculated and presented in Table 5-5 . The assumption here is that because the biofilters of the current research are actively vented, oxidation most likely occurs through the entire bed thickness. This assumption is checked in Section 5.7 where we determine oxidation reaction kinetics parameters for the three designs introduced.

Table 5-5 Residence times of biofilter columns under varying flow rates and aeration levels

Three aeration levels						
CH ₄ flow rate (ml/min)	Air flow rate (ml/min)	Total flow rate (ml/min)	Flow rate in each section (ml/min)	Flow rate in m ³ /min	Residence time (min)	Total residence time (min)
6	60	66	26	0.00002	69.04	135.26
			46	0.00004	39.02	
			66	0.00006	27.20	
9	90	99	39	0.00003	46.03	90.173
			69	0.00006	26.01	
			99	0.00009	18.13	
12	120	132	52	0.00005	34.52	67.63
			92	0.00009	19.51	
			132	0.00013	13.60	
15	150	165	65	0.00006	27.62	54.11
			115	0.00011	15.61	
			165	0.00016	10.88	
18	180	198	78	0.00007	23.01	45.08
			138	0.00013	13.01	
			198	0.00019	9.07	
Two aeration levels						
CH ₄ flow rate (ml/min)	Air flow rate (ml/min)	Total flow rate (ml/min)	Flow rate in each section (ml/min)	Flow rate in m ³ /min	Residence time (min)	Total residence time (min)
6	60	66	36	0.00003	74.79	115.59
			66	0.00006	40.80	
9	90	99	54	0.00005	49.86	77.06
			99	0.00009	27.20	
12	120	132	72	0.00007	37.40	57.8
			132	0.00013	20.40	
15	150	165	90	0.00009	29.92	46.23
			165	0.00016	16.32	
18	180	198	108	0.00010	24.93	38.53
			198	0.00019	13.60	

One aeration level						
CH ₄ flow rate (ml/min)	Air flow rate (ml/min)	Total flow rate (ml/min)	Flow rate in each section (ml/min)	Flow rate in m ³ /min	Residence time (min)	Total residence time (min)
6	60	66	66	0.00006	81.59	81.59
9	90	99	99	0.00009	54.39	54.39
12	120	132	132	0.00013	40.80	40.79
15	150	165	165	0.00016	32.64	32.64
18	180	198	198	0.00019	27.20	27.20

The first and second columns of Table 5-5 list different CH₄ and air flow rates tested. The total flow rates exiting the columns at the top are presented in column 3. In carrying out the residence time calculations, each column is divided into one, two, or three sections based on the number of aeration levels. Residence time is calculated for each section according to the flow rate that passes through that particular section. The total residence time for each operating flow rate is then estimated as the sum of the individual sections of each column as presented in column 7.

CH₄'s low water solubility (Henry's constant of 27) can be a limiting factor when operating biofilters at high flow rates. Table 5-5 suggests a residence times of 45 and 38 min for the three level aerated column and the two-level aerated one at their maximum flow rate, respectively. As seen in Figure 5-5 and Figure 5-7 (Three-level aerated column oxidation rate (g/m³/day) over time), at this flow rate the column aerated at two levels has an average oxidation rate 106 % higher than that of the three-level aerated column. Therefore, it is concluded that the retention time (the time needed for CH₄ to be dissolved in the biofilm, be accessible to methanotrophs and assimilated) required for CH₄ oxidation is met even in the two-level air injection system.

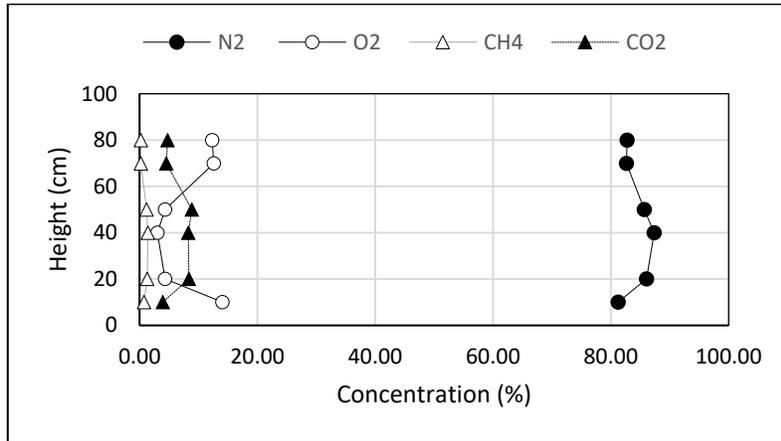
Although there has not been sufficient work on the minimum required residence time for CH₄ oxidation, based on the values presented in Table 5-5 for the column aerated at one level, the decline in biofilter performance at high flow rates can be justified to some degree by the residence times of less than 30 min.

5.5 Concentration Profiles

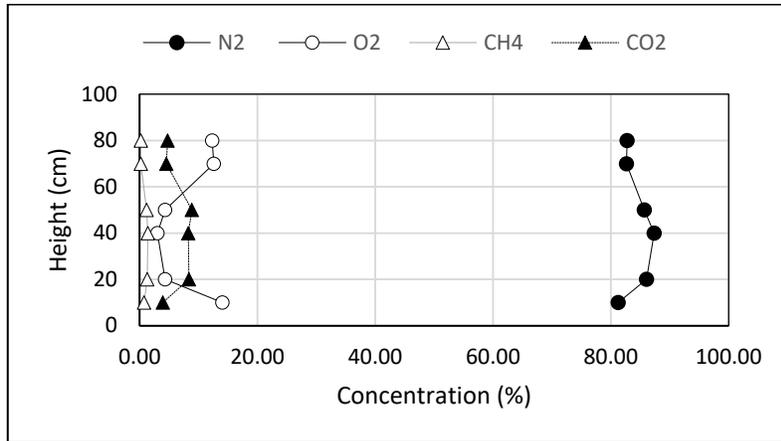
Vertical gas concentration profiles in each of the biofilter columns are presented in this section for a single day at each of the five stages. The complete set of graphs for the sampled days over 195 days of operation will be presented in Appendix A.

5.5.1 Concentration Profiles for the first stage

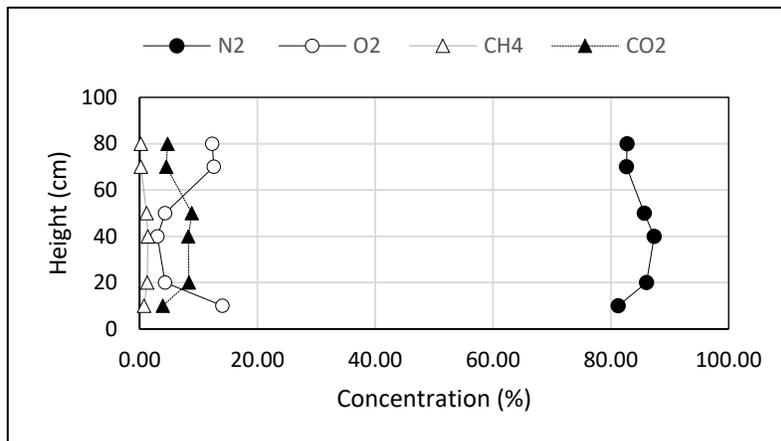
Vertical gas profiles for day 6 of the first stage operated at a flow rate of 6 ml/min (370.6 g/m²/day or 529.4 g/m³/day) are presented in Figure 5-10. Readings for height 80 cm represent the measurements taken at the outlet sampling port. As the figure suggests, O₂ concentration for the three columns decrease at a height of 10 cm until they increase again at 40 cm. This decrease in O₂ concentration is accompanied by an increase in CO₂ concentration, inferring that most oxidation activities occur in this height for a flow rate of 6. As previously seen in Figure 5-4, Figure 5-6, and Figure 5-8, there is no significant variation between the columns' oxidation rates at this stage.



a) Concentration Profiles-Column 3- Day 6



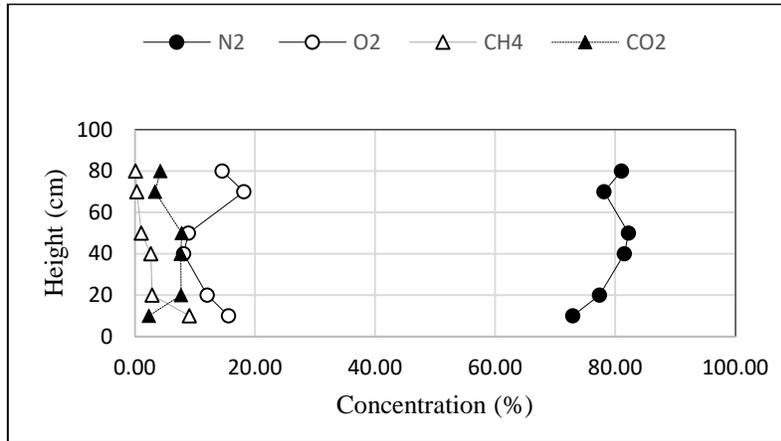
b) Concentration Profiles-Column 2- Day 6



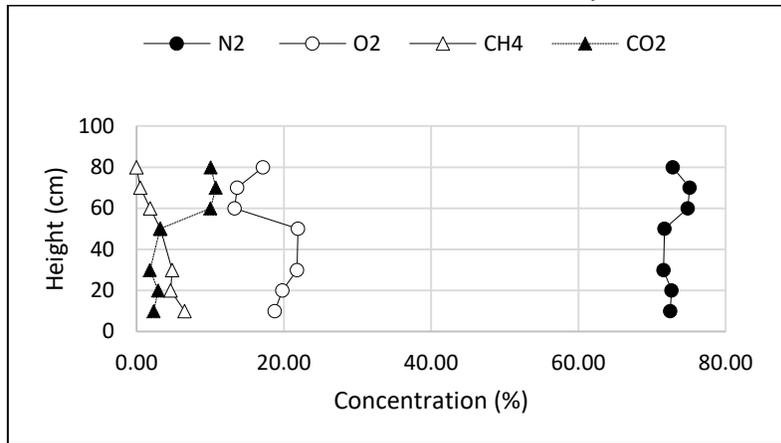
c) Concentration Profiles-Column 1- Day 6

Figure 5-10 Gas profiles for day 6 (first stage) for a) Column aerated at three levels b) Column aerated at 2 levels c) Column aerated at one level

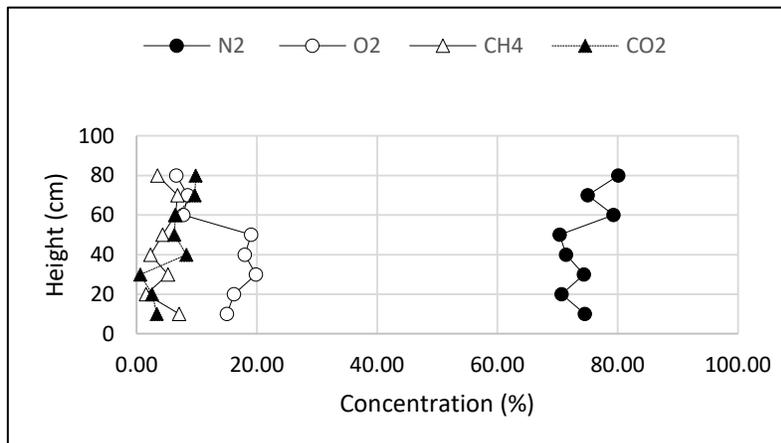
Figure 5-11 presents the vertical profiles for day 58 of the operation, columns run at the second stage with a flow rate of 9 ml/min (556 g/m²/day 794 g/m³/day). As seen in Figure 5-11, the area in which O₂ concentration decrease and CO₂ concentrations increase is shifted to a height of 20-40 cm for the column aerated at three levels, 50-80 cm for the one aerated at two levels, and 30-60 cm for the column aerated at one level. Therefore, the bottom portion have less contribution with regards to CH₄ oxidation, and the oxidation zones are shifted upwards as flow rates increase.



a) Concentration Profiles-Column 3- Day 58



b) Concentration Profiles-Column 2- Day 58

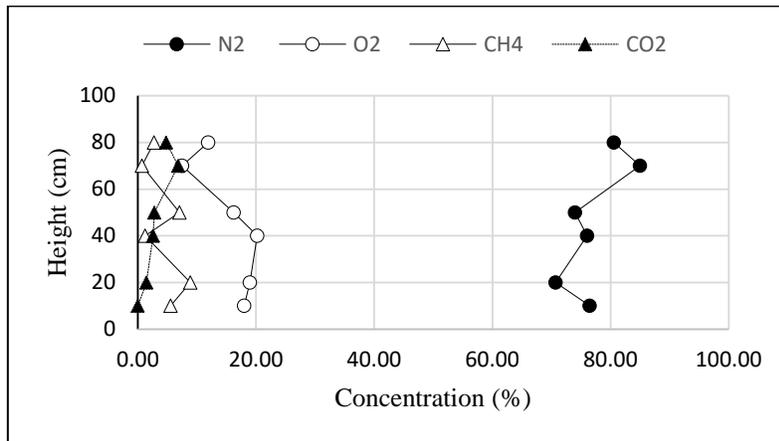


c) Concentration Profiles-Column 1- Day 58

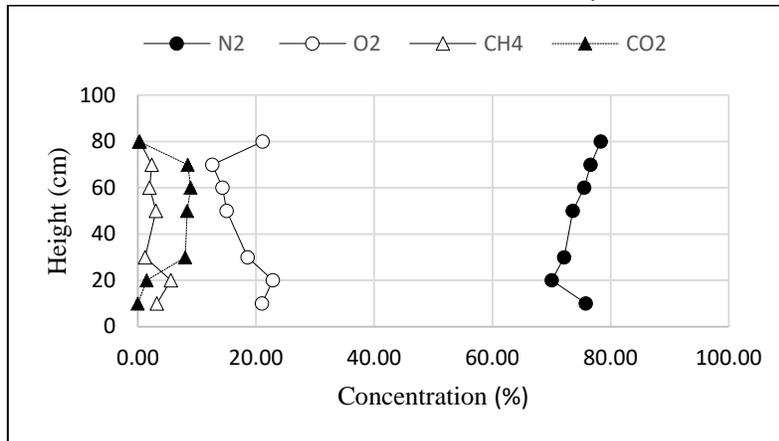
Figure 5-11 Gas profiles for day 58 (second stage) for a) Column aerated at three levels b) Column aerated at 2 levels c) Column aerated at one level

Vertical profiles for day 92 operated in the third stage of the columns operation are given in Figure 5-12. Biofilter columns are run at a flow rate of 12 ml/min (741 g/m²/day or 1058 g/m³/day). It can be seen that the oxidation zones have shifted vertically upwards as the flow rate has increased. O₂ concentration decreases are observed at a height of 40-70 cm, 30-60 cm, and 40-70 cm for three, two, and one air injection levels respectively. Therefore, oxidation occurs to some degree at the bottom portions of the columns, especially because CH₄ has a higher retention times in these sections as suggested by Table 5-5. However, as the loading rate increases, not all CH₄ can be assimilated in the lower sections, and the remaining undigested stream flows through upper levels with more O₂ concentration available. Hence, oxidation zones will expectedly move towards the higher heights.

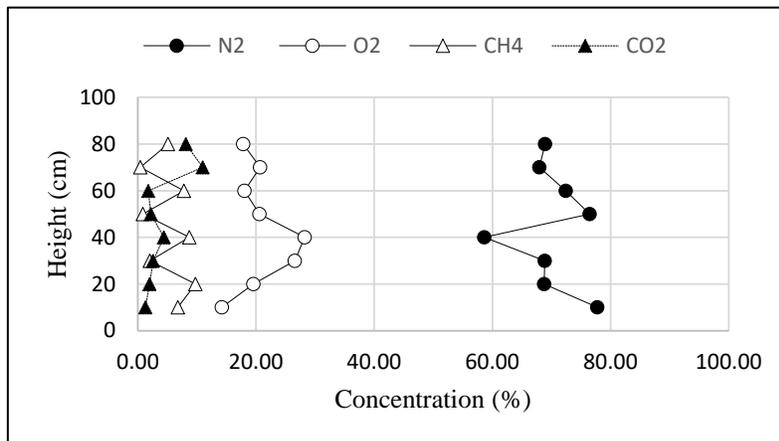
One interesting observation in Figure 5-12 is the rather uniform distribution of O₂ in the column aerated at two level compared to its other two counterparts.



a) Concentration Profiles-Column 3- Day 92



b) Concentration Profiles-Column 2- Day 92



c) Concentration Profiles-Column 1- Day 92

Figure 5-12 Gas profiles for day 92 (third stage) for a) Column aerated at three levels b) Column aerated at 2 levels c) Column aerated at one level

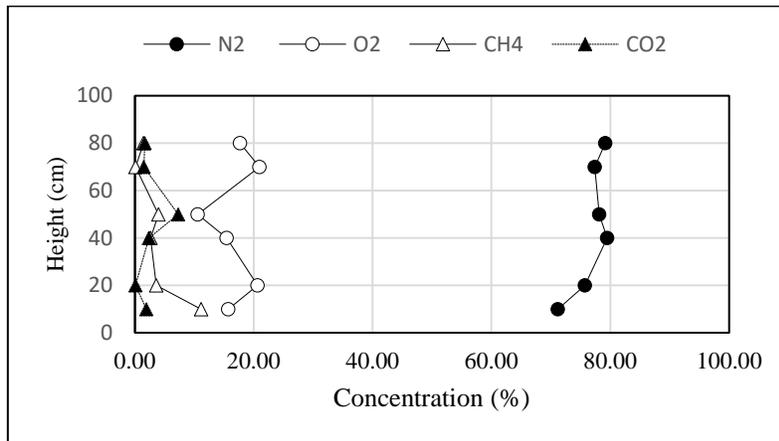
Gas profiles for 147 days after the start-up of the biofilter columns are provided in Figure 5-13.

Columns are run at a flow rate of 15 ml/min (926 g/m²/day or 1323 g/m³/day).

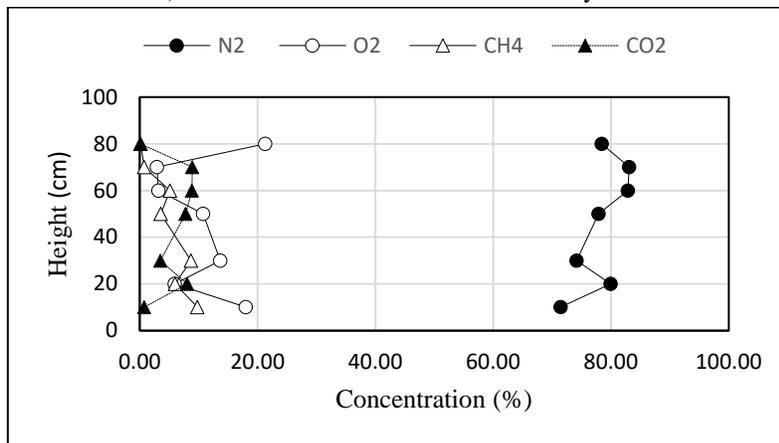
As seen in Figure 5-13 graph (a) for the column aerated at three levels, O₂ concentration increases to atmospheric levels at a height of approximately 25 cm, and slowly reduces to value of 10.5% at a height of 50 cm, where it starts increasing again. Evidently, the rise in O₂ concentration is due to air injections at heights of 25 cm and 50 cm. O₂ concentrations decrease between the two aeration levels as CH₄ is oxidized, as confirmed by the decrease in CH₄ concentrations and increase in CO₂ concentrations between these two levels.

The same phenomenon occurs for the column aerated at two levels, as inferred from graph (b) of Figure 5-13, as O₂ concentration increase at 35 cm due to air injection and slowly decrease to values as low as 3%.

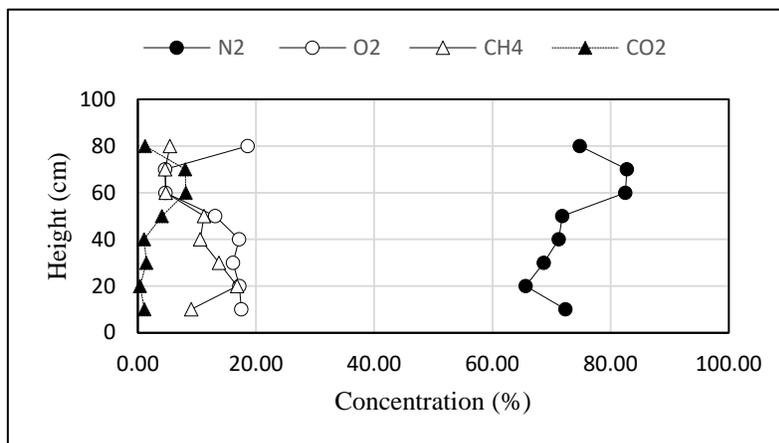
Part (c) of the same graph, suggests that for the column aerated at the bottom the maximum oxidation zone occurs between 40-70 cm, which is where O₂ concentration drop and CO₂ concentrations increase. As argued before, as the CH₄ feeding rates increase, a portion of CH₄ which is not oxidized moves through upper levels where available O₂ concentrations is still found. O₂ is diffused from the headspace interface with compost is used by microorganisms for CH₄ digestion in this region.



a) Concentration Profiles-Column 3- Day 147



b) Concentration Profiles-Column 2- Day 147



c) Concentration Profiles-Column 1- Day 147

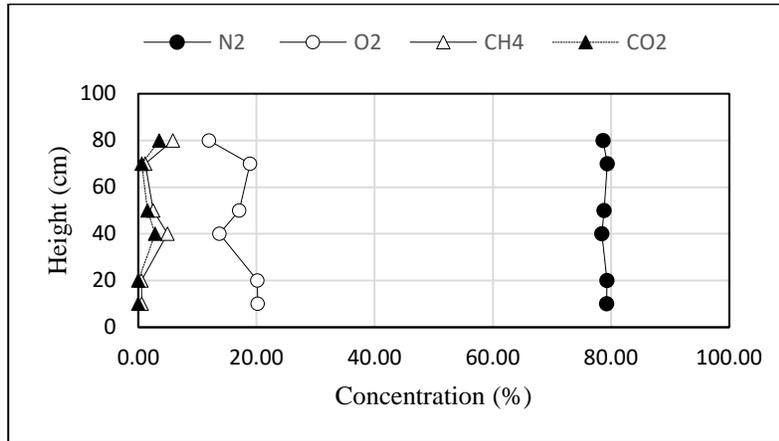
Figure 5-13 Gas profiles for day 147 (fourth stage) for a) Column aerated at three levels b) Column aerated at 2 levels c) Column aerated at one level

Vertical profiles day 190 of the biofilter columns operation provided in Figure 5-14. Columns are run at a maximum flow rate of 18 ml/min (1112 g/m²/day or 1588 g/m³/day) at this final stage.

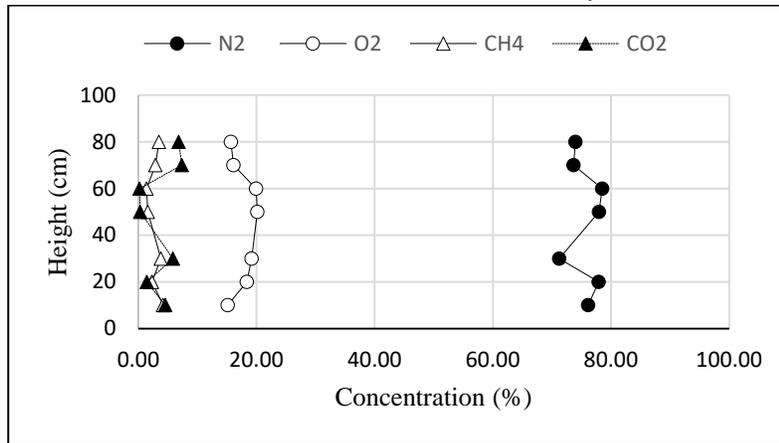
Graph (a) in Figure 5-14 shows a slight increase in CH₄ concentration as it travels up the bed thickness, although some oxidation occurs between 20-40 cm. This suggests that most activities at this flow rate occur in the bottom section (the area between the first and second aeration levels) where the residence time is 23 min. Compared with a 13 min residence time for the volume between the second and third aeration levels, and the 9 min residence time for the top section (as calculated in Table 5-5), this will allow more time for microbial reactions to take place. Low residence times at the top can mean that the remaining unassimilated CH₄ is flushed out and hence concentrations are higher at top ports.

The rather uniform distribution of O₂ concentration in the two-level aerated column, can be evidence to its better performance in this stage as suggested by average oxidation rate values listed in Table 5-4. Compared to the column with three air injection levels, residence times are more distributed between the two sections of column 2, having a residence time of 25 min at the bottom section, and 14 min at the top portion.

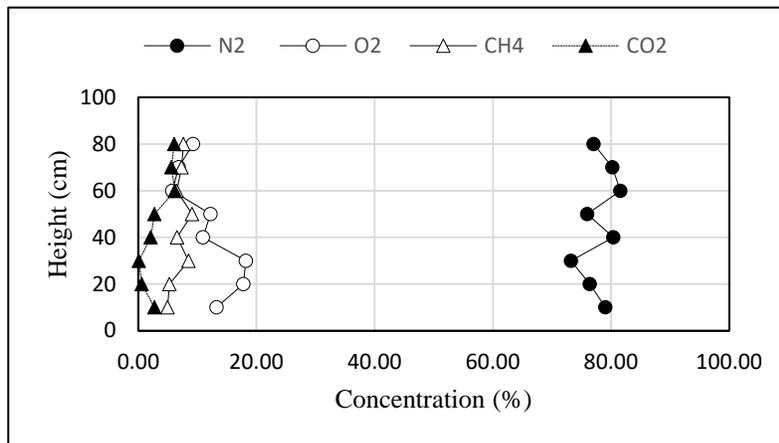
The relatively uniform profile of CH₄ in the column aerated at one level infers that the column has reach its maximum oxidation capacity and has been exhausted. This is confirmed in Section 5.7.1 where batch experiments are done to measure maximum oxidation rate at the end of columns' operation.



a) Concentration Profiles-Column 3- Day190



b) Concentration Profiles-Column 2- Day190



c) Concentration Profiles-Column 1- Day190

Figure 5-14 Gas profiles for day 190 (fifth stage) for a) Column aerated at three levels b) Column aerated at 2 levels c) Column aerated at one level

As the discussion above implies, a combination of residence times, flow rates, and injection levels control the biofilter performance in the experiments done. This leads to a different maximum oxidation zone in each stage of the 195-day operation. Therefore, a mixed ANOVA is performed to evaluate the extent of the effects and interactions of these factors on the system performance.

5.6 Analysis of Variances (ANOVA)

To analyze the effects of number of injection points and inlet CH₄ flowrates, a mixed ANOVA has been performed using SPSS Statistics. A mixed ANOVA tests differences between two or more independent groups while the participants are subjected to repeated measures. In other words, it incorporates a mixture of between-group and repeated measures variables. The purpose of a mixed ANOVA is to understand whether there is an interaction between the within-group and between-groups variables on the dependent variable.

In this study, the number of injection points is taken as the between-groups factor. Time and flow rate are set as the repeated measures (within-groups) variable, as all columns are measured repeatedly over time for different flowrates.

5.6.1 Assumptions

There are seven underlying assumptions for a mixed ANOVA test to hold valid. These assumptions are listed below.

- 1) The dependent variable should be measured at a continuous level. Because our response variable, oxidation rate, is easily calculated based on the outlet CH₄ concentrations and inlet and outlet flowrates, this assumption is met.
- 2) The within-subjects factor(s) should consist of at least two categorical related groups, indicating that the same subjects are present in both groups. This means that each subject

is measured on two occasions on the dependent variable, whether that is two different time points or two different experimental conditions. The within-subjects variable in this study flow rate, has five different levels for all of which all the subjects have been measured at the same time points.

- 3) The between-subjects factor (i.e. the independent variable) should consist of at least two independent groups. In this study, we have three different configurations for the number of injection levels.
- 4) There should be no significant outliers in any group of within-subjects or between-subjects factors. Outliers are single data points which do not follow the usual pattern and therefore will affect the analysis negatively and reduce the accuracy of the results. When using SPSS to run a mixed ANOVA, these outliers can be easily detected and removed.
- 5) The dependent variable should be approximately normally distributed for each combination of the between-subjects and within-subjects factors groups. The data used in this study are tested for normality using SPSS, results of which will be presented later in Section 5.6.2.
- 6) Variances for each of the groups of the within-subjects and between-subjects variables should be homogeneous. This assumption is tested using Levene's test of homogeneity of variances in SPSS.
- 7) The variances of differences between the related groups (levels) of the within-subjects factors for all groups of the between-subjects factor must be equal. Repeated measure ANOVAs are usually susceptible to the violation of this assumption. Sphericity in such analyses is similar to homogeneity of variances in a between-Subjects ANOVA. This

assumption is known as sphericity of the covariance matrix and is tested using Mauchly's Test of Sphericity in SPSS Statistics. Fortunately, if this assumption is violated, corrections have been developed to compensate for the increase in error. These corrections are applied to the degrees of freedom for the F-distribution based on the degrees to which sphericity has been violated. This will result in a more conservative F-value and decrease the error incorporated.

5.6.2 Results

Table 5-6 indicates mean values and standard deviation of oxidation rates for columns with one, two, or three injections subjected to flow rates of 6 to 18 mL/min.

Table 5-6 SPSS Output 1-Descriptive Statistics

Flow rate (ml/min)	No. Injection Levels	Mean	Standard Deviation	N
6	1	377.7800	97.25773	5
	2	382.1240	94.19645	5
	3	520.7020	14.25274	5
	Total	426.8687	100.07964	15
9	1	580.6980	110.60546	5
	2	727.0860	78.76752	5
	3	714.0800	61.47768	5
	Total	673.9547	105.05730	15
12	1	683.6640	197.72258	5
	2	878.5840	218.43808	5
	3	820.3680	154.64216	5
	Total	794.2053	196.94317	15
15	1	618.0380	286.89191	5
	2	1185.0680	97.49778	5
	3	1083.6700	133.91661	5
	Total	962.2587	310.91440	15
18	1	602.1100	409.43940	5
	2	1350.4460	223.93956	5
	3	731.3200	245.31656	5
	Total	894.6253	440.12260	15

As discussed earlier, at lower flow rate values, the column with three aeration levels has the highest average oxidation rate compared to the other two. But as flow rates increase, the two-level injection design presents a better performance. This is counterintuitive as one would normally assume higher O₂ levels will lead to better oxidation results. Also, as values in Table 5-5 for residence time of gas in biofilter columns suggest, as the number of injection points increase, so does the retention time for the thickness between the aeration levels. So based on the results of Table 5-6 only, it can be deduced that the contact time required for CH₄ to travel through the filter media, be absorbed into the biofilm and ingested by bacteria is not fully met at high flow rates for three aeration levels.

Table 5-7 shows SPSS output on Mauchly's test of sphericity. The null hypothesis is that the error covariance matrix of the orthonormalized transformed dependent variable is proportional to an identity matrix. In other words, the hypothesis is that the variances of differences between levels are significantly different. If the p-value is smaller than the significance level (.05), as in this case, the null hypothesis different is accepted. In other words, the assumption of sphericity has been violated. If Mauchly's test is significant, the Greenhouse-Heisser or Huynh-Feldt corrected degrees of freedom are used to assess the significance of F-factors for different variables. Corrected tests are displayed in the tests of within-subjects effects Table 5-8. If the assumption is violated, and the epsilon value for Green-Geisser estimate of sphericity is lower than 0.75 then Green-Geisser correction factor will be used, otherwise the Hyunh-Feldt correction is applied. In this case, the Greenhouse-Geisser factor is used to adjust the F-distribution.

Table 5-7 SPSS Output 2-Mauchly's Test of Sphericity

Within-Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Flow rate	.088	25.352	9	.003	.549	.790	.250

Table 5-8 shows the results of the “repeated measures” part or the within-subjects part of the ANOVA. The output contains sections that refer to each of the effects in the model and the error terms associated with these effects.

Looking at the significance value obtained for CH₄ flow rate, it can be seen that the p-value with the Greenhouse-Geisser correction applied is less than the significance value of 0.05. Therefore, inlet flow rate values have a statistically significant effect on oxidation rate. This is understandable as changing the inlet flow rate directly changes the resulting residence time of CH₄ in each filter, as discussed in the Equation 2-23, and consequently affects oxidation rates. Needless to say, feeding methanotrophs with more CH₄ leads to more CH₄ oxidation until the system reaches its maximum oxidation capacity and breaks through after a flow rate value of 18 ml/min.

Moreover, the table suggests that with a p-value of 0.003, the interaction effects of flow rate and number of injection points have a statistically significant effect on oxidation rates. This means that simultaneous influence of these two parameters is not necessarily additive. So in order to develop an aeration design that has the highest performance we must refer to the response surface to define an optimum range of values for the combination of these two factors.

Table 5-8 SPSS Output 3- Tests of Within Subjects Effects

Factors	Tests	Type III sum of squares	df	Mean square	F	Sig.	Partial Eta Squared
Flow rate	Sphericity met	2671805.396	4	667951.349	20.151	.000	.627
	Greenhouse- Geisser	2671805.396	2.197	1215992.872	20.151	.000	.627
	Huynh-Feldt	2671805.396	3.158	846012.251	20.151	.000	.627
	Lower-bound	2671805.396	1.000	2671805.396	20.151	.001	.627
Injection* Flow rates levels	Sphericity met	1345787.074	8	168223.384	5.075	.000	.458
	Greenhouse- Geisser	1345787.074	4.394	306247.508	5.075	.003	.458
	Huynh-Feldt	1345787.074	6.316	213067.979	5.075	.001	.458
	Lower-bound	1345787.074	2.000	672893.537	5.075	.025	.458
Errors	Sphericity met	1591053.880	48	33146.956			
	Greenhouse- Geisser	1591053.880	26.367	60343.410			
	Huynh-Feldt	1591053.880	37.897	41983.193			
	Lower-bound	1591053.880	12.000	132587.823			

Levene's test of homogeneity of variance tests the null hypothesis that the error variance of the dependant variable is equal across all levels of the within-subject factor. The results of SPSS analysis on Levene's test are presented in Table 5-9. As the p-values in the table are all greater than 0.05, it is indicated that variances are homogeneous for all levels of the repeated measures and the homogeneity assumption is met.

Table 5-9 SPSS Output 4- Levene's Test of Equality of Error Variances

Flow rate (ml/min)	F	df1	df2	Sig.
6	3.053	2	12	0.085
9	1.187	2	12	0.339
12	0.493	2	12	0.623
15	2.218	2	12	0.151
18	0.663	2	12	0.533

The main effects of the independent variable, number of injection levels, are listed separately in Table 5-10. The results reveal that at a significant level of 0.05 the number of injection points do not affect the oxidation rate significantly. However, a more precise understanding of the main effects of said factor is possible through one-on-one comparisons of different column configurations as presented in Table 5-11.

Table 5-10 SPSS Output 5- Tests of Between Subjects Effects

Variable	Type III sum of squared	df	Mean square	F	Sig.	Partial Eta Squared
Intercept	42230545.975	1	42230545.975	895.797	.000	.987
Injection Levels	1400456.952	2	700228.476	14.853	.001	.712
Error	565715.661	12	47142.972			

The first column of Table 5-11 indicates the design to which the other two configurations are compared two. The third column presents the mean difference between the two designs of comparison. Cells marked by an asterisk (*) suggest that the mean difference is statistically significant at a 0.05 significance level. Bonferroni corrected post hoc tests shows that configurations with two and three air injection points do not significantly differ in performance, whereas there is a statistically significant difference between the two former designs and the column with one air injection point at the bottom.

Table 5-11- SPSS Output 6- Pairwise Comparisons

No. of Injection Level (i)	No. of Injection Levels (j)	Mean difference (i-j)	Std. Error	Sig.	95% Confidence interval for difference	
					Lower bound	Upper bound
1	2	-332.204*	61.412	.000	-502.897	-161.511
	3	-201.570*	61.412	.020	-372.263	-30.877
2	1	332.204*	61.412	.000	161.511	502.897
	3	130.634	61.412	.164	-40.059	301.327
3	1	201.570*	61.412	.020	30.877	372.263
	2	-130.634	61.412	.164	-301.327	40.059

5.6.3 Response Surface

Figure 5-15 illustrates the 3D response surface for CH₄ oxidation in biofilters when operated at stoichiometric air to CH₄ flow rate ratios. As evident from the plot, at flow rates lower than 12 ml/min (corresponding to loading rates of 1058.92 g/m³/day or 741.25 g/m²/day), the performance of the three designs are not significantly different. However, as CH₄ flow rates increase differences are more distinct. The graph peaks at the maximum flow rate of 18 ml/min (corresponding to 1588 g/m³/day or 1112 g/m²/day) for the design aerated at two levels. This is the point where the other two designs' performances drop and the experiments are terminated.

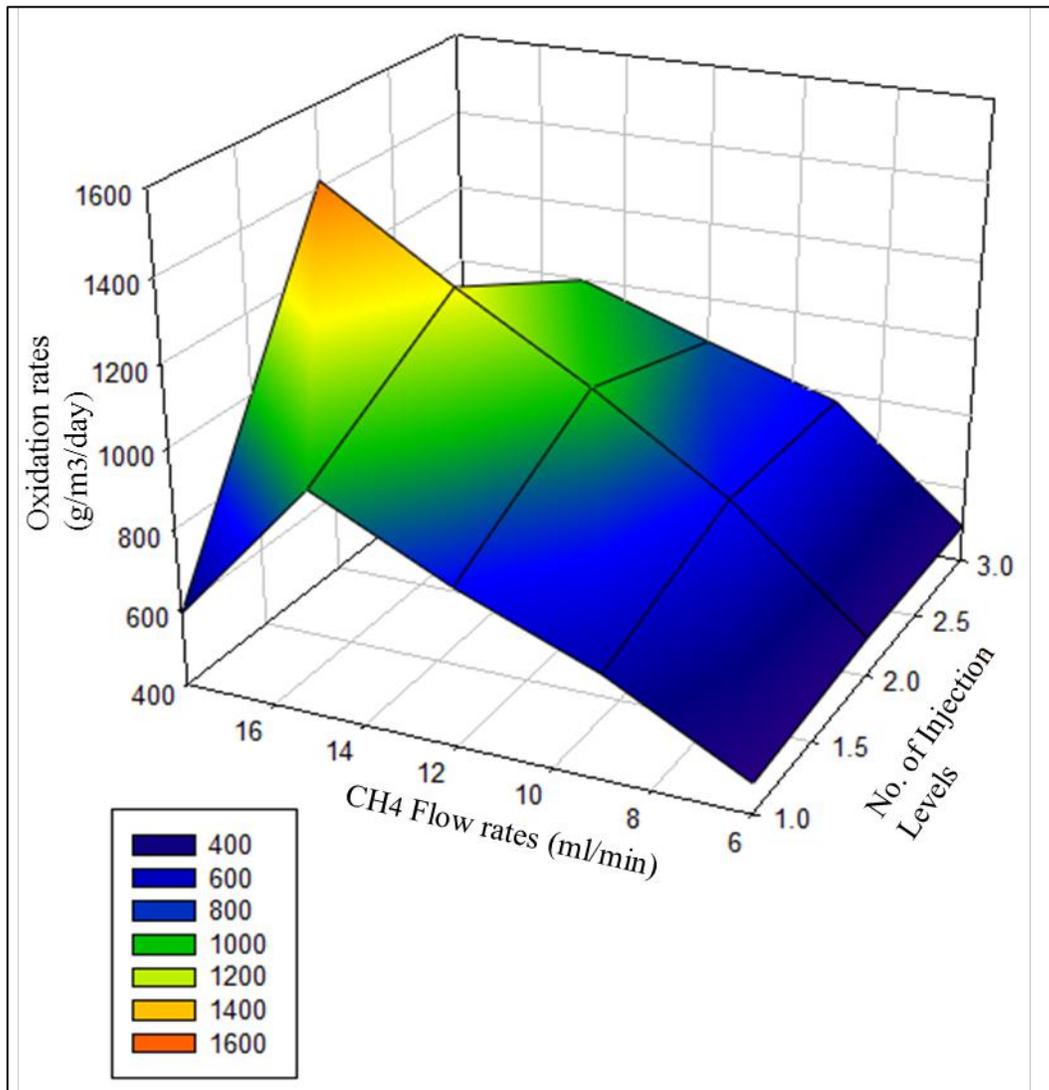


Figure 5-15 3D response Surface for columns run at stoichiometric air flow rates (the x-axis indicates flow rates measured in ml/min, the y-axis is the No of aeration levels (1,2, or 3), and the z-axis is the oxidation rate in g/m3/day)

As verified by ANOVA results presented in Table 5-11 (- SPSS Output 6- Pairwise Comparisons) , the differences between performances of columns with two and three aeration levels is not statistically significant ($p\text{-value} = 0.164 > \text{significance level} = 0.05$). The divergence between performances of these two columns at the final stage, as seen in Figure 5-15, can be explained based on CH_4 residence time in different sections of the columns as bounded by the

aeration probes. With a residence time of 23 min (as calculated in Table 5-5) for the bottom section of the column aerated at three levels, it is expected that the majority microbial activities occur in this area. CH_4 oxidation leads to production of H_2O , which is subsequently pushed upwards by the flow of air injected. Therefore, more activities produce more H_2O , of which a portion is driven towards the outlet. This leads to a non-uniform distribution of moisture content along the vertical profile, with expected saturation levels at higher heights and material drying at the bottom portion. As the space between compost particles is filled with H_2O , at the top of the column, there is less effective contact area between CH_4 and microorganism decreases and so does the system's efficiency. This is more apparent at high air flow rates, as more water is pushed from the bottom areas towards the top of the columns. The droplets formed on the top cap of the column aerated at three levels by the end of the operation are shown in Figure 5-16.



Figure 5-16 Droplets formed on the top of the three-level aerated biofilter column

Based on the O_2 profiles presented in Figure 5-10 to Figure 5-14 and gas residence times listed in Table 5-5, it can be inferred that the column aerated at two levels has a more uniform

distribution of O_2 , microbial reactions, and therefore a more homogenous performance over a wide range of CH_4 feeding rates.

As for the biofilter column aerated at one level, integrating the gas profiles of Figure 5-11 to Figure 5-14 and the 3D response surface of Figure 5-15, it can be concluded that the majority of microbial activities occur in the area between 40-60 cm. Having the majority of oxidation concentrated at a thin layer leads to an uneven distribution of EPS formation (complexes formed as a side product of CH_4 microbial oxidation reaction). With EPS compressed into a 20-cm layer, occupying pore spaces between compost particles in that region, CH_4 movement to the upper layers is impeded. Furthermore, as oxidation reactions continue more EPS is built up in the mentioned layer over time, which subsequently blocks CH_4 interaction with the methanotrophic bacteria. Therefore, oxidation rates decline with time as previously plotted in Figure 5-8. A picture of the EPS formed in the biofilter configuration with one air injection level is shown in Figure 5-17.



Figure 5-17 EPS formation in the column aerated at one level

Figure 5-18 depicts contour plots for average oxidation rates for the 5 loading rates and three designs tested. In developing the contour plots, results from multiple measurements during each stage have been averaged over time. The superior performance of the two-level aerated biofiltration design is clearly inferred from the contour plots.

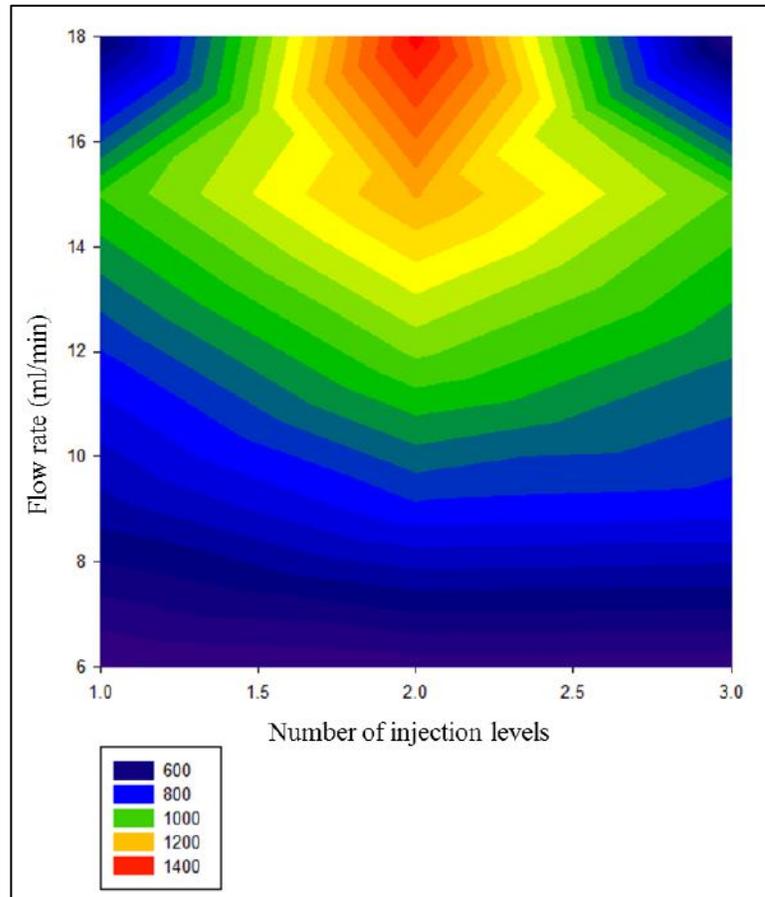


Figure 5-18 Contour plot for average oxidation rates over time at varying flow rates and number of aeration levels.

Figure 5-19 to Figure 5-23, present oxidation rate contour plots at various times for the three design tested at constant flow rates of 6, 9, 12, 15, and 18 mL/min, respectively.

As seen in Figure 5-19, when run at the minimum flow rate studied, the performances of the three configuration are not significantly different from one another, and high oxidation efficiencies are achieved in all three.

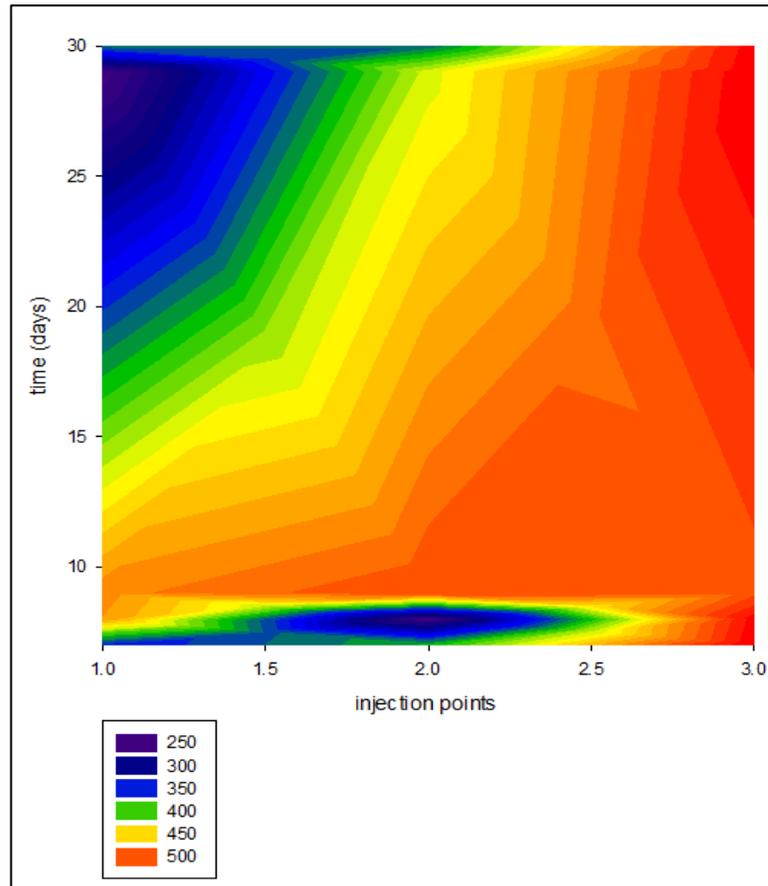


Figure 5-19 Oxidation rate contour plots for constant flow rate of 6 mL/min

As flow rates increase to 9 mL/min the maximum oxidation rates shift towards the multiple air injection designs as inferred from Figure 5-20.

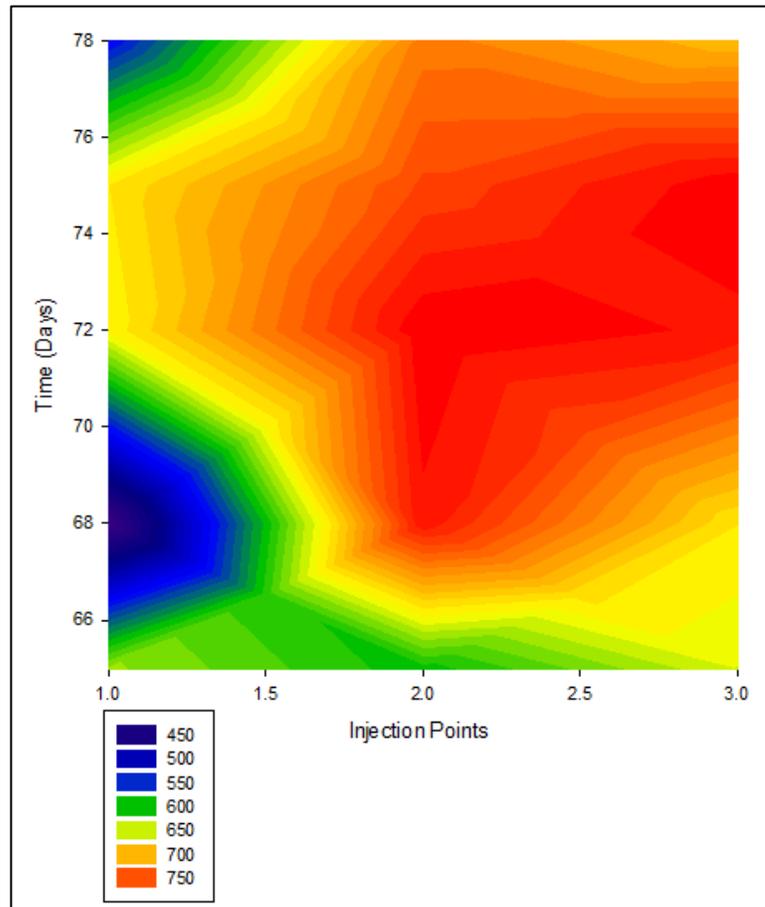


Figure 5-20 Oxidation rate contour plots for constant flow rate of 9 mL/min

Further increase of inlet flow rates to 12 mL/min, shrinks high oxidation rate zones to the column aerated at two levels as presented in Figure 5-21.

The gap between days 100 to 107 is caused by the breakdown of the Gas Chromatography system.

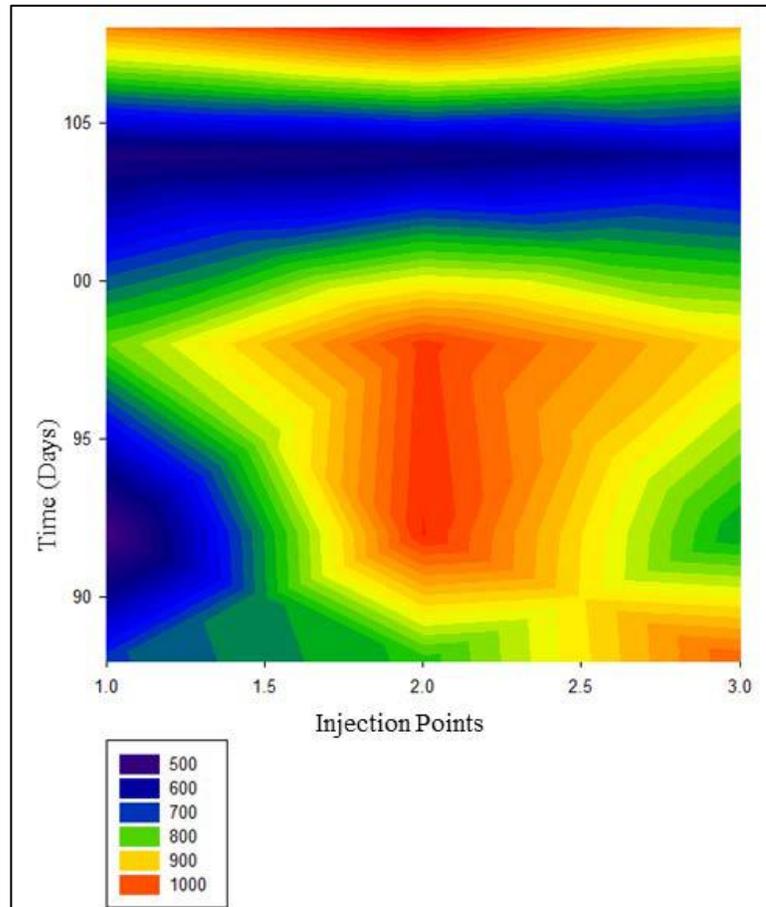


Figure 5-21 Oxidation rate contour plots for constant flow rate of 12 mL/min

It can be seen in Figure 5-22 that at an inlet flow rate of 15 mL/min, the divergence between columns with two and three air injection levels becomes less significant. This conclusion was also confirmed by the pairwise comparisons in ANOVA results. However, the design aerated at the bottom fails in performance after day 130. This decline is suggested to be a result of EPS accumulation and limitation of gas transport as discussed in Section 5.6.3.

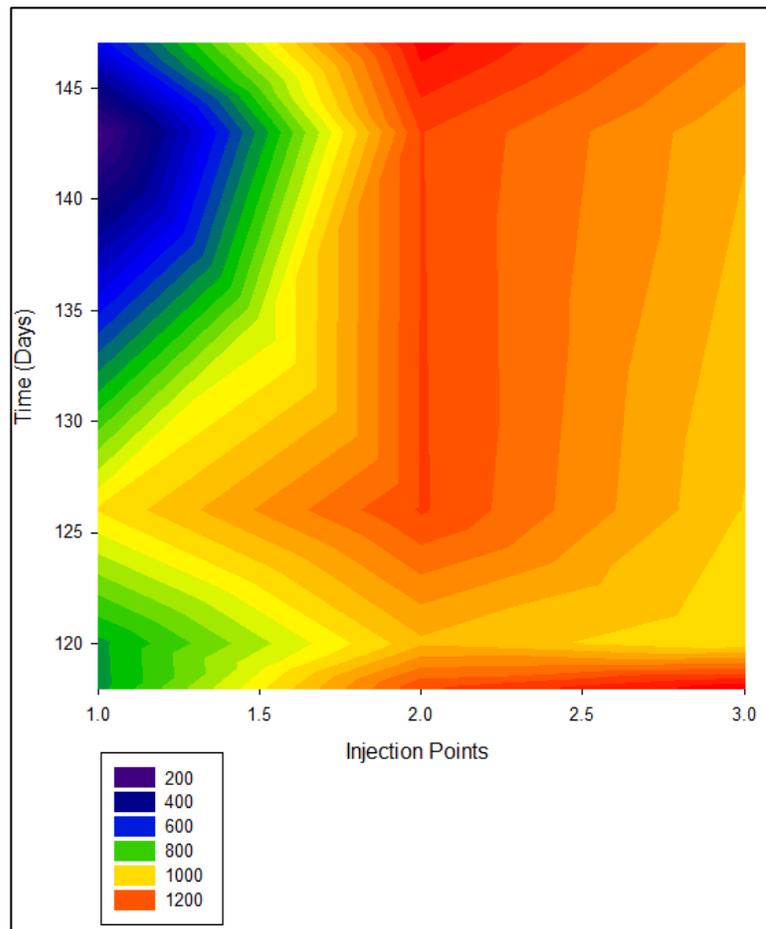


Figure 5-22 Oxidation rate contour plots for constant flow rate of 15 mL/min

The final stage of the experiments, highlights the divergence between columns with two and three aeration points as observed in Figure 5-23. The failure of the three-level aerated design is due to the uneven distribution of residence time between the three units of this column. The majority of microbial activities take place at the bottom portion of column which yields a higher retention time, Therefore, more H₂O is produced in this region as a result of oxidation reactions. The H₂O produced is subsequently pushed upwards leading to its accumulation on the column walls and saturation of the packing bed. This phenomenon limits gas transport in the top sections of the column leading to a decline in the system's performance.

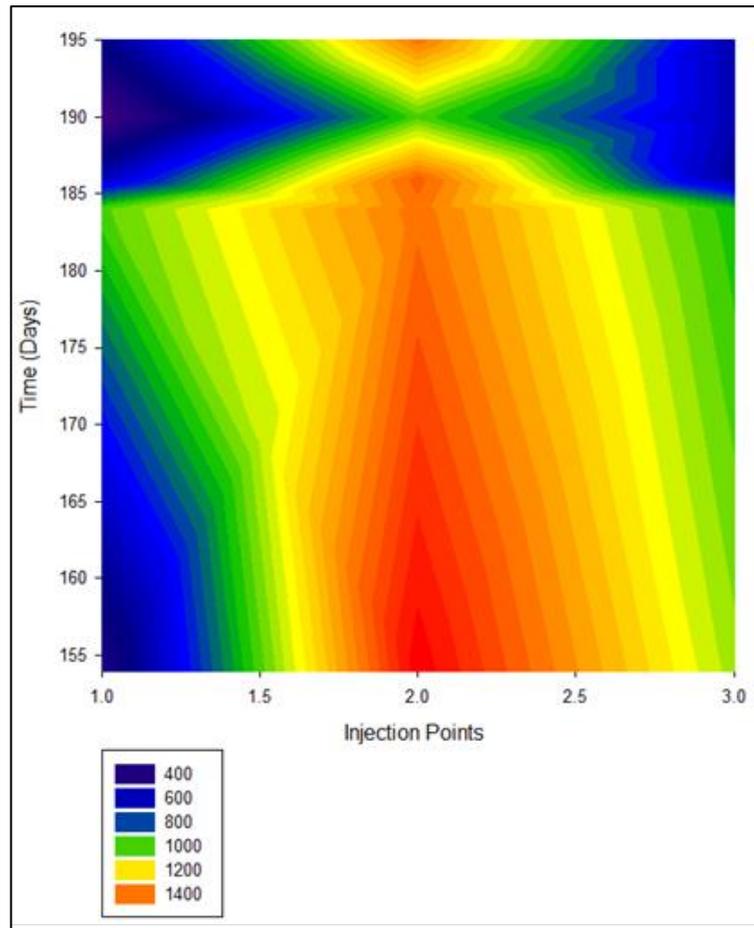


Figure 5-23 Oxidation rate contour plots for constant flow rate of 18 mL/min

5.7 Batch experiment Experiments: Operated under stoichiometric aeration rates

After having run for 195 days, the columns were dismantled. Samples were collected from top, middle and bottom portions of the columns and analyzed for oxidation kinetics parameters and the microbial community count. The results of which are presented in sections 5.7.1 and 5.7.2.

5.7.1 Methane Oxidation Kinetics

In order to measure oxidation kinetics parameters, it is essential to make sure microbial growth has reached a steady state condition. For this reason, in most V_{\max} studies, pre-incubation is necessary as the preliminary step. Pre-incubation is the process through which the media is microbially activated to ensure a steady state methanotrophic growth. Because the samples analyzed in this section have been taken from the previously run collected, it is assumed that the microbial community is active already and that there is no need for pre-incubation.

Samples were taken from the top, middle and bottom portions of each biofilter column. Moisture content is a dominating factor in the level of oxidation activities. To make sure the samples taken from all three columns were comparable, moisture content of the samples was determined beforehand and was set to a constant value of 60% dw. Samples were incubated under 6, 5, 4, 3% of CH_4 . Samples were analyzed for CH_4 concentration changes over time at 4 different times. Figures present the changes in concentrations of CH_4 as a function time for Column 1 top, middle, and bottom portions, respectively.

Three different linearization methods derived from the Michaelis-Menten model are compared. Values obtained for each of these methods as well as the averaged parameters are presented in Table 5-12, Table 5-13, and Table 5-14 for columns with three, two and one aeration level, respectively.

Table 5-12 presents the results of V_{\max} batch experiments for the biofilter with three aeration levels. As the V_{\max} values obtained using all three linearization methods are approximately similar, their values are averaged. Maximum CH_4 oxidation activity is therefore reported as 1.46, 13.03, and 7.4 $\mu\text{g/g dw/h}$ for the top, middle and bottom portions, respectively. Maximum activity occurs in the middle section, where O_2 is expected to be more prevalent due to diffusion from the top and bottom aeration probes. The top portion has the least V_{\max} value, likely because most the majority of CH_4 fed to the column are already assimilated before they reach the top portion. Therefore, the methanotrophic community are not nourished as much compared to the bottom and middle sections of the same column.

Table 5-12 Michaelis-Menten reaction parameters for the three-level aerated biofilter column

Three-level aerated biofilter column- Top Portion				
	E-H	L-B	Hanes	Average
Slope:	-590.65	507.64	0.79	-27.41
Intercept:	1.16	0.87	540.80	180.94
V_{\max} ($\mu\text{g/g dw/h}$)	1.16	1.14	1.27	1.19
K_m ($\mu\text{g/L}$)	590.65	580.63	687.22	619.5
Three-level aerated biofilter column-Middle Portion				
	E-H	L-B	Hanes	Average
Slope:	-2.77	3.81	0.08	0.37
Intercept:	11.90	0.07	0.95	4.31
V_{\max} ($\mu\text{g/g dw/h}$)	11.90	14.87	12.31	13.03
K_m ($\mu\text{g/L}$)	2.77	56.74	11.75	23.75
Three-level aerated biofilter column-Bottom Portion				
	E-H	L-B	Hanes	Average
Slope:	194.46	-9.00E-05	0.08	64.85
Intercept:	4.07	0.17	0.00	1.41
V_{\max} ($\mu\text{g/g dw/h}$)	4.07	6.04	12.09	7.40
K_m ($\mu\text{g/L}$)	-194.46	-5.43E-04	0.00	-64.82

Table 5-13 lists V_{max} values for the two-level air-injection biofilter systems. The average V_{max} values for top, middle, and bottom sections is calculated as 65.27, 25.95, and 23.48 $\mu\text{g/g dw/h}$, respectively, using the three linearization methods. It can be observed that methanotrophic activity is distributed evenly through the biofilter thickness. Compared to the biofilter system with three injection levels, V_{max} values were found to be 5.24 times higher on average. The results here are comparable to the overall superior performance of the two-level aerated column compared to its three-level equivalent as discussed in Section 5.3.

Table 5-13 Michaelis-Menten reaction parameters for the two-level aerated biofilter column

Two-level aerated biofilter column- Top Portion				
	E-H	L-B	Hanes	Average
Slope:	-2819.88	6.34E+01	0.02	-918.82
Intercept:	49.35	0.01	60.86	36.74
V_{max} ($\mu\text{g/g dw/h}$)	49.35	82.1	64.38	65.28
K_m ($\mu\text{g/L}$)	2819.88	5.20E+03	3917.94	3980.69
Two-level aerated biofilter column-Middle Portion				
	E-H	L-B	Hanes	Average
Slope:	-3255.75	1.69E+02	0.04	-1028.93
Intercept:	22.12	0.03	158.33	60.16
V_{max} ($\mu\text{g/g dw/h}$)	22.12	30.19	25.56	25.96
K_m ($\mu\text{g/L}$)	3255.75	5.10E+03	4046.78	4133.95
Two-level aerated biofilter column-Bottom Portion				
	E-H	L-B	Hanes	Average
Slope:	-900.49	4.18E+01	0.04	-286.22
Intercept:	22.9	0.04	39.632	20.86
V_{max} ($\mu\text{g/g dw/h}$)	22.9	24.44	23.1	23.48
K_m ($\mu\text{g/L}$)	900.49	1.03E+03	915.28	947.95

Results from the batch Vmax measurements on the biofilter system aerated at a single point at the bottom are presented in Table 5-14. Average Vmax values for the top, middle, and bottom samples are 19.74, 11.57, and 7.82 $\mu\text{g/g dw/h}$.

Table 5-14 Michaelis-Menten reaction parameters for the one-level aerated biofilter column

One-level aerated biofilter column- Top Portion				
	E-H	L-B	Hanes	Average
Slope:	-31.51	8.02E+01	0.041	16.25
Intercept:	9.52	0.04	78.83	29.46
Vmax ($\mu\text{g/g dw/h}$)	9.52	25.26	24.46	19.75
Km ($\mu\text{g/L}$)	2819.88	1.03E+03	1928.25	1924.79
One-level aerated biofilter column-Middle Portion				
	E-H	L-B	Hanes	Average
Slope:	-661.02	9.16E+01	0.08	-189.77
Intercept:	9.88	0.08	100.05	36.67
Vmax ($\mu\text{g/g dw/h}$)	9.88	11.93	12.89	11.57
Km ($\mu\text{g/L}$)	661.03	1.09E+03	1289.69	1014.71
One-level aerated biofilter column-Bottom Portion				
	E-H	L-B	Hanes	Average
Slope:	-171.46	3.55E+01	0.12	-45.27
Intercept:	7.32	0.13	48.48	18.64
Vmax ($\mu\text{g/g dw/h}$)	7.32	7.76	8.38	7.82
Km ($\mu\text{g/L}$)	171.46	2.76E+02	406.58	284.65

5.7.2 Microbiological analysis

After the biofilter columns were disassembled, samples were extracted from the top, middle and bottom portions of each column and prepared. 500 mg of the extracted samples was mixed and analyzed in order to identify and count the diversity of the microbial community. The results are presented in Table 5-15, in which C1T, C1M, C1B stand for top, middle, and bottom section samples of the biofilter design with one aeration level, respectively. C2T, C2M, C2B are top, middle, and bottom section samples of the column with two aeration levels, respectively. C3T, C3M, C3B represent the analysis for top, middle, and bottom sections of the biofilter with three aeration levels.

Table 5-15 Summary of the microbial population in column biofilters

#OTU ID	C3T	C3M	C3B	C2T	C2M	C2B	C1T	C1M	C1B
Archaea	0.040	0.038	0.045	0.137	0.042	0.059	0.090	0.061	0.071
Acidobacteria	4.615	3.655	4.390	5.453	3.423	3.701	4.416	3.586	3.986
Actinobacteria	7.410	5.810	5.389	7.818	4.443	4.142	4.692	4.911	4.323
Armatimonadetes	0.126	0.151	0.090	0.242	0.236	0.130	0.216	0.218	0.166
Bacteroidetes	4.310	4.492	5.085	6.523	6.606	7.510	7.631	5.961	6.077
Bacteroidetes; Chryseolinea	1.288	1.299	1.528	0.932	0.801	1.441	1.342	0.849	0.710
Bacteroidetes; uncultured Cytophagaceae	0.714	0.525	0.774	1.089	0.699	0.824	0.849	0.542	0.508
Bacteroidetes; uncultured Saprospiraceae	0.229	0.257	0.130	1.144	0.733	0.568	1.201	0.522	0.688
Candidate division OD1	1.054	0.649	0.874	2.812	1.044	0.565	1.508	0.989	0.704
Chlamydiae	0.340	0.157	0.275	0.327	0.146	0.145	0.226	0.164	0.361
Chlorobi	0.406	0.693	0.380	1.027	0.547	0.594	0.490	0.399	0.564
Chloroflexi	9.431	7.436	7.292	8.484	6.606	8.401	5.352	6.459	7.351
Chloroflexi; Anaerolineaceae	1.843	2.194	1.938	2.519	2.117	3.924	1.394	2.211	3.413
Chloroflexi; Caldilineaceae	2.228	1.462	1.468	1.618	1.230	1.262	1.265	1.247	1.111
Cyanobacteria	0.018	0.075	0.050	0.050	0.056	0.033	0.041	0.022	0.034
Firmicutes	2.311	1.807	1.419	1.761	2.143	1.743	1.935	2.012	1.818
Gemmatimonadetes	2.206	1.380	1.404	2.184	1.306	1.053	1.687	1.212	1.309
Planctomycetes	17.35	12.83	14.17	12.16	12.88	14.12	13.25	12.92	11.14
Planctomycetes; Blastopirellula	1.415	0.772	0.954	1.099	0.639	0.919	0.726	0.804	0.855
Planctomycetes; Planctomyces	5.555	3.965	4.186	2.594	3.808	4.506	3.204	3.380	2.872
Alphaproteobacteria	16.88	15.83	13.71	12.55	19.85	17.83	15.38	16.29	15.14
Alphaproteobacteria; Woodsholea	8	4	1	2	5	5	0	5	6
Alphaproteobacteria; Woodsholea	1.342	0.956	0.884	0.655	1.398	1.824	1.393	2.080	1.771
Alphaproteobacteria; Methylocapsa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
Alphaproteobacteria; Methylocella	0.000	0.003	0.000	0.000	0.000	0.001	0.000	0.001	0.000
Alphaproteobacteria; uncultured Beijerinckiaceae	0.827	0.198	0.375	0.009	0.723	1.900	0.143	0.532	1.402
Alphaproteobacteria; Hyphomicrobiaceae	2.826	2.429	2.417	2.936	4.000	3.446	5.062	5.801	3.448
Alphaproteobacteria; Methylobacterium	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Alphaproteobacteria; Methylocystis	1.969	3.965	0.914	0.010	6.060	1.315	0.409	0.546	0.230
Alphaproteobacteria; Methylosinus	0.004	0.003	0.000	0.003	0.008	0.004	0.003	0.000	0.002
Alphaproteobacteria; other Methylobacteriaceae	0.525	0.324	0.255	0.757	0.278	0.362	0.650	0.559	0.572
Alphaproteobacteria; other Methylocystaceae	1.328	2.497	2.188	0.218	1.637	2.081	0.954	0.471	0.632
Alphaproteobacteria; Phyllobacteriaceae	1.543	0.810	0.964	0.552	1.004	1.473	0.970	1.019	1.192
Betaproteobacteria	3.172	2.726	4.016	4.313	5.469	4.891	5.458	4.518	4.527
Betaproteobacteria; Methylophilaceae	0.278	0.822	1.189	1.085	1.356	0.883	2.347	1.832	1.097
Betaproteobacteria; uncultured Nitrosomonadaceae	1.011	0.515	1.009	1.227	1.082	0.940	1.077	0.719	0.821
Deltaproteobacteria	4.613	3.137	4.930	4.466	3.011	3.712	3.160	2.910	3.912

Epsilonproteobacteria	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gammaproteobacteria	19.42	35.01	31.77	23.79	26.98	27.08	28.84	33.01	34.64
Gammaproteobacteria; Methylobacter	10.63	27.00	23.06	13.88	15.05	17.81	17.94	22.60	25.39
Gammaproteobacteria; Methylocaldum	0.608	0.609	0.649	0.617	0.599	0.566	0.680	0.567	1.010
Gammaproteobacteria; Methylococcus	0.000	0.003	0.000	0.001	0.000	0.002	0.000	0.000	0.000
Gammaproteobacteria; Methylochromium	3.517	4.486	4.850	5.236	7.474	5.405	6.553	7.095	5.137
Gammaproteobacteria; Methylomonas	0.034	0.003	0.010	0.007	0.006	0.012	0.001	0.001	0.016
Gammaproteobacteria; Methylosarcina	0.025	0.031	0.010	0.015	0.014	0.007	0.028	0.020	0.020
Gammaproteobacteria; Methylovulum	0.001	0.003	0.000	0.001	0.000	0.001	0.000	0.000	0.001
Gammaproteobacteria; Other Methylococcales	0.044	0.043	0.005	0.016	0.000	0.016	0.007	0.010	0.008
Gammaproteobacteria; Xanthomonadales	3.714	1.954	2.487	2.727	3.069	2.577	2.838	2.089	2.306
Verrucomicrobia	3.031	1.957	2.198	1.675	2.692	2.195	2.298	2.054	1.385
Verrucomicrobia; Methyloacidiphilum	0.098	0.010	0.040	0.001	0.052	0.060	0.001	0.000	0.038
Others	3.252	2.156	2.512	4.216	2.506	2.084	3.325	2.288	2.468
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 5-15 is summarized in Table 5-16 with special attention to methanotrophic community count. Columns are labelled as previously defined. Values in the table are percentages of the total microbial population. For instance, in column C3T (Top section of the three-level aerated biofilter) 18.79% of the microbial community is comprised of methanotrophs, out of which 3.83% are alpha-methanotrophs (Type IIs) and 14.87 are gamma-methanotrophs (Type Is).

Table 5-16 Methanotroph community in the biofilter columns

#OTU ID	C3T	C3M	C3B	C2T	C2M	C2B	C1T	C1M	C1B
Methanotrophs	18.79	38.98	31.98	20.77	31.18	27.64	27.23	31.88	33.06
Others	81.21	61.02	68.02	79.23	68.82	72.36	72.77	68.12	66.94
alpha-methanotrophs	3.83	6.79	3.36	0.99	7.98	3.76	2.02	1.58	1.44
gamma-methanotrophs	14.87	32.18	28.58	19.78	23.15	23.82	25.21	30.30	31.59
verruco-methanotrophs	0.10	0.01	0.04	0.00	0.05	0.06	0.00	0.00	0.04
others	81.21	61.02	68.02	79.23	68.82	72.36	72.77	68.12	66.94

Methanotrophs metabolize CH_4 by combining it with O_2 to form formaldehyde. Formaldehyde is then incorporated into organic compounds via either the serine or the ribulose monophosphate (RuMP) pathway. These pathways are illustrated in Figure 5-24 and Figure 5-26.

Type I methanotrophs are a class of the Gammaproteobacteria which utilize the RuMP pathway for carbon assimilation. RuMP pathway combines formaldehyde with sugar ribulose (A monosaccharide with chemical formula of $\text{C}_5\text{H}_{10}\text{O}_5$) which is then broken down to glyceraldehyde (monosaccharide with chemical formula of $\text{C}_3\text{H}_6\text{O}$). Glyceraldehyde is further broken down into other organic compounds as shown in Figure 5-24. Type I methanotrophs are more prevalent in zones where O_2 is present sufficiently (Hanson and Hanson, 1996; Holmes et al., 1999).

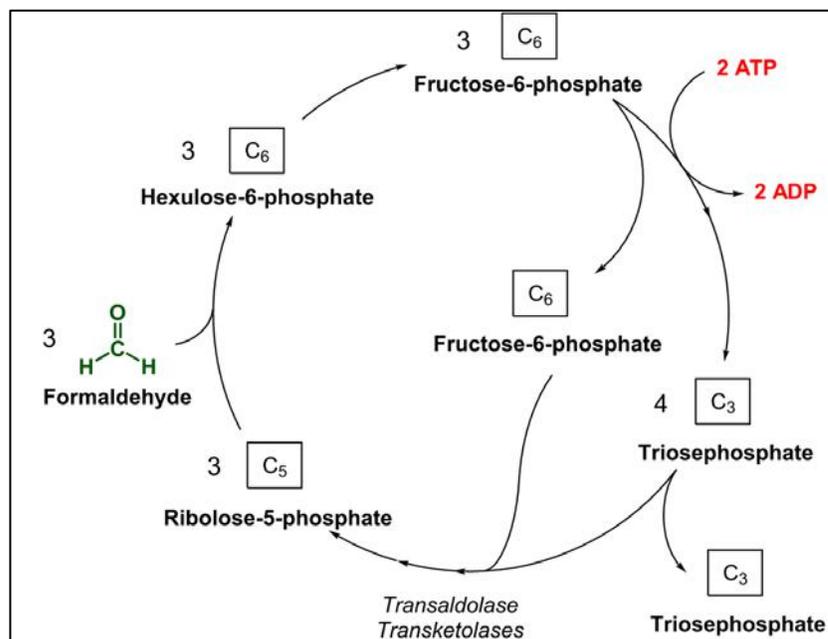


Figure 5-24 RuMP pathway in type I methanotrophs (reprinted under the Creative Commons Attribution-Share alike license)

Type II methanotrophs, on the other hand, are a group of Alphaproteobacteria which assimilate carbon through the Serine pathway. The pathway combines formaldehyde and Glycine (an amino acid with chemical formula of $\text{C}_2\text{H}_5\text{NO}_2$) to form Serine (an amino acid used in the

biosynthesis of proteins with chemical formula of $C_3H_7NO_3$). Serine is further converted into other organic molecules as illustrated in Figure 5-25 . Type II methanotrophs can fixate Nitrogen and unlike type I methanotrophs can be present in O_2 deficient environments. Other methanotrophs are found in the Verrucomicrobiae (Hanson and Hanson, 1996; Holmes et al., 1999) .

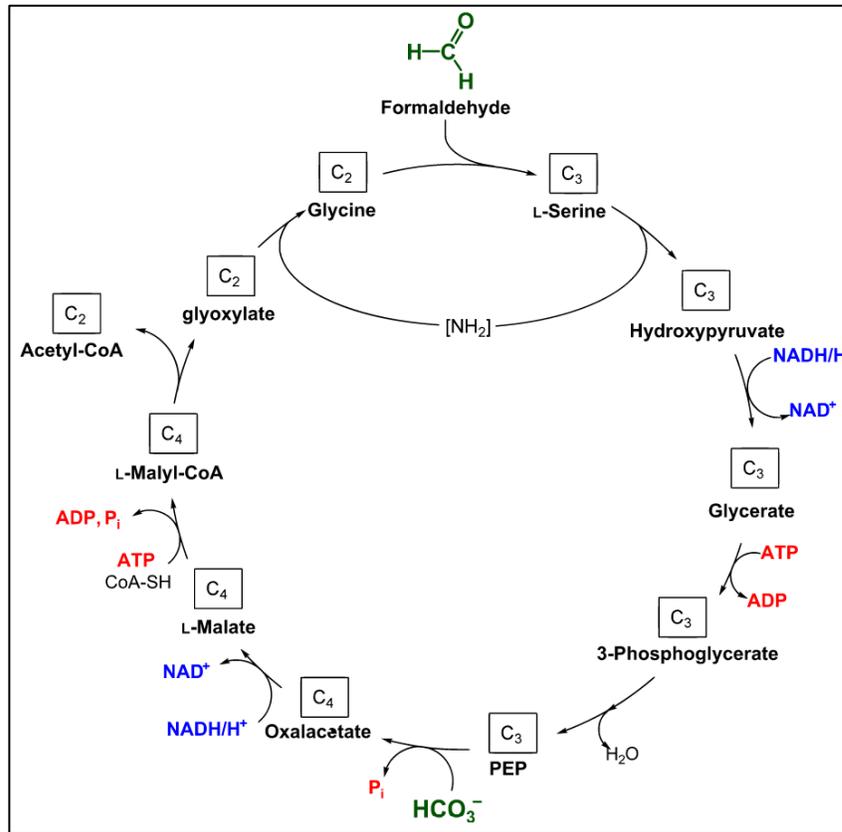


Figure 5-25 Serine pathway in type II methanotrophs (reprinted under the Creative Commons Attribution-Share Alike license)

Table 5-16 suggests that with respect to the total methanotrophic population the biofilter with three air injection levels has the highest population in the middle section. This is justified especially as calculated in 5.7.1, this biofilter design also has the highest V_{max} value in the middle section. Also, the column is aerated at three points along the vertical length of the column, O_2 concentrations are expected to be higher in the middle portion due to O_2 diffusion from the bottom

and top air probes. Type I methanotrophs, therefore, are expected to be more prevalent in the middle section as confirmed by values of Table 5-16.

The biofilter column aerated at two levels has a more or less homogeneous methanotrophic population all through the profile. This can be interpreted as having microbial activities occurring throughout the thickness, as validated by the results of V_{\max} measurements in Table 5-13.

Table 5-16 implies that the biofilter column with one air injection level at the bottom has a consistent methanotrophic population across the depth. Not surprisingly, Type II methanotrophs have the lowest population at the top portion of the column compared to other parts. As air is only introduced at the bottom of the column, lower concentration of O_2 are expected at the top section. Type II methanotrophs are sensitive to O_2 deficiencies, subsequently their population is decreased with height.

5.8 Performance of aerated biofilter columns run at various air to methane flow rate ratios

After the first set of columns were run at stoichiometric air to CH_4 flow rates for 195 days to determine the effects of number of aeration levels and their interaction with CH_4 feeding rate, a second set of columns were set up according to the experimental design presented in Table 4-5. Air to CH_4 flow rate ratios vary between 2.5 to 15 at five levels, with the stoichiometric value being the centre experimental point. Due to time constraints, air to CH_4 flow rate of 15 was not tested in this research. However, since the main objective of this experiment was to determine the minimum ratio at which column biofilters operate at the same efficiency as that of the stoichiometric flow rate, not having air to CH_4 flow rate ratio of 15 tested, does not affect our main goal. However, in order to run the ANOVA analysis, evaluate the effect of changing air flow rates

on column biofilter performance, and its interactions with aeration levels and loading rates, it is suggested to have the complete experimental region shown in Figure 4-3 tested in future studies. CH₄ flow rates were changed between 6-18 ml/min (corresponding to 370.6 to 1111.8 g/m²/day or 529.5 to 1588 g/m³/day) at seven levels.

Besides, as one of the objectives of the first set of biofilter columns run at stoichiometric ratios was to evaluate the effects of different aeration levels and monitor performance variations over time, the three columns (plus the replicate) were run simultaneously for the same CH₄ loading rate. Therefore, a plot of their performances versus time was formed as in Figure 5-4 to Figure 5-9. As time and number of aeration levels were not the main concerns of this second set of experiments, biofilter columns were run for different CH₄ loading rates as suggested by the Doehlert design to ensure maximum coverage of the experimental region in a minimum period of time.

5.8.1 Gas concentration profiles

Vertical gas concentration profiles in each of the biofilter columns are presented in sections 5.8.2 to 5.8.5 for a single day at each of the four stages.

5.8.2 Concentration Profiles for the first stage

The combination of CH₄ flow rates and air to CH₄ flow rate ratios tested in the first stage along with the average oxidation rates and efficiencies are listed in Table 5-17.

Table 5-17 First stage of the Doehlert experimental design for columns run at varying air to CH₄ ratios

CH ₄ Flow rate (ml/min)	Loading rate (g/m ³ /day)	Air to CH ₄ flow rate ratios	Number of aeration levels	Average Oxidation rates over time (g/m ³ /day)	Average Oxidation efficiencies over time (%)
10	882.43	5:1	One	790.46	93.83
6	529.46	5:1	Two	468.79	84.84
8	705.95	10:1	Three	651.95	92.35

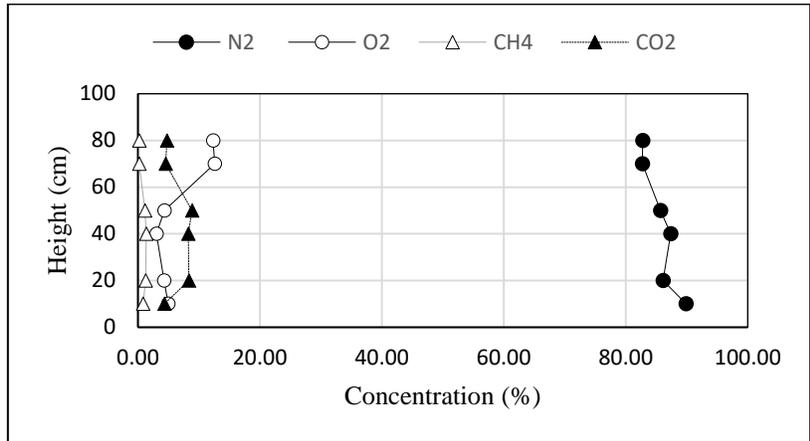
Figure 5-26 plots vertical gas profiles 24 days after the biofilter columns were run for the flow rates shown in Table 5-17.

As graph (a) for the column aerated at three levels suggests, the majority of the oxidation activities occur at a height of 20-50 cm, where O₂ concentrations decrease slightly and CO₂ concentration increase. After this point, O₂ concentration increase as air is injected at a height of 50 cm. The average O₂ concentration along the height of the biofilter is 6.9%. However, an oxidation efficiency of 100% was achieved even at an air to CH₄ flow rate of.

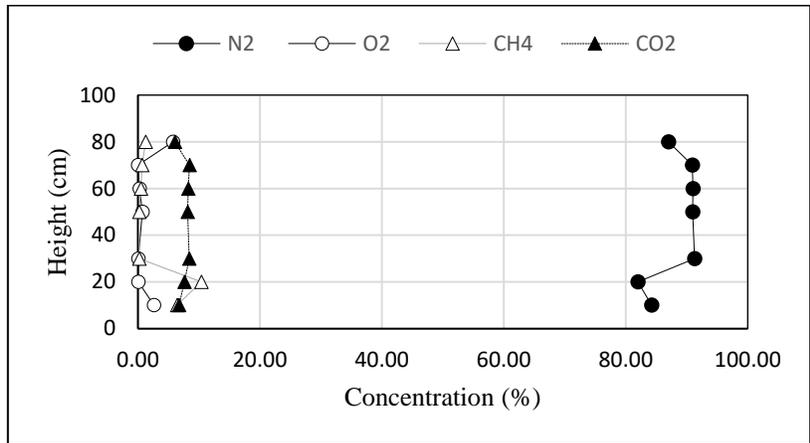
Graph (b) of the same figure presents concentration profiles for the column aerated at two levels. As evident from the graph, oxidation reactions occur at a thickness of 30-70 cm, dropping CH₄ concentrations to values close to zero, even though the average O₂ concentration along the vertical profile of the column is 1.3%.

Graph (c) plots vertical gas profiles in the column with one aeration level run at stoichiometric air flow rates. As previously seen in Figure 5-10, maximum oxidation reactions take place in the thickness of 30-60 cm where O₂ is consumed (decrease in O₂ concentrations) and CO₂ is produced

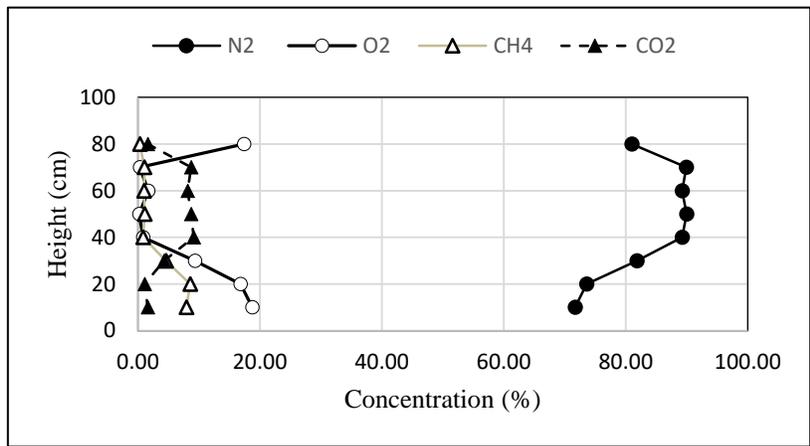
(increase in CO₂ concentrations). Based on the results obtained in this stage, it is concluded that decreasing the air to CH₄ flow rate to half of the stoichiometric value, does not affect the oxidation reactions when air is introduced at two or three levels.



a) Concentration Profiles-Column 3- June 17th



b) Concentration Profiles-Column 2- June 17th



c) Concentration Profiles-Column 1- June 17th

Figure 5-26 Gas profiles for day 24 (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level

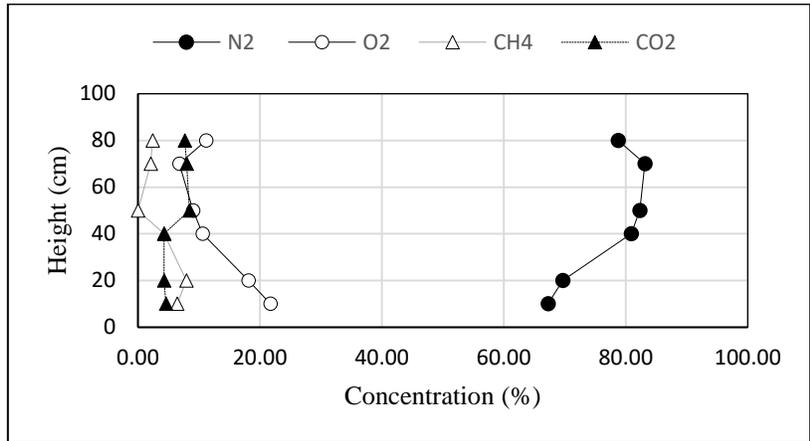
5.8.3 Concentration Profiles for the second stage

The combination of values test in the second stage experimental are listed in Table 5-18. Columns 5 and 6 of the same table list the averaged oxidation rate in g/m³/day and efficiency (%) over time. Vertical gas profiles for day 62 after the initial set up of columns are presented in Figure 5-27.

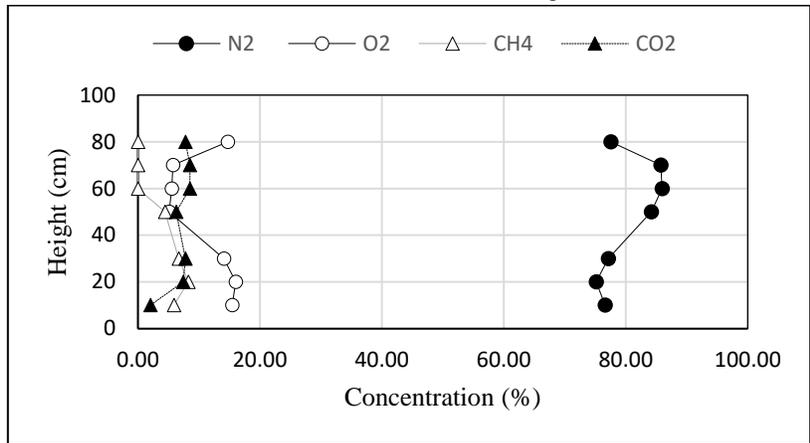
As suggested by Table 5-18, decreasing the air to CH₄ flow rate ratio to half of its stoichiometric value in the column aerated at one single point does not affect oxidation activities. For comparison reasons, we refer to Figure 5-9, in which average oxidation efficiency for the same column run at a CH₄ flow rate of 15 ml/min is 45%. Clearly, changes in properties of the packing media, formation of EPS, and blockages of pore spaces which occur over time are accountable for the resulting low average efficiency of 45%. However, the results from Table 5-18 and CH₄ profiles shown in Figure 5-27, are evidence that reducing the degree of aeration to half of its stoichiometric level does not affect the biofiltration process as far O₂ concentrations are concerned.

Table 5-18 Second stage of the Doehlert experimental design for columns run at varying air to CH₄ ratios

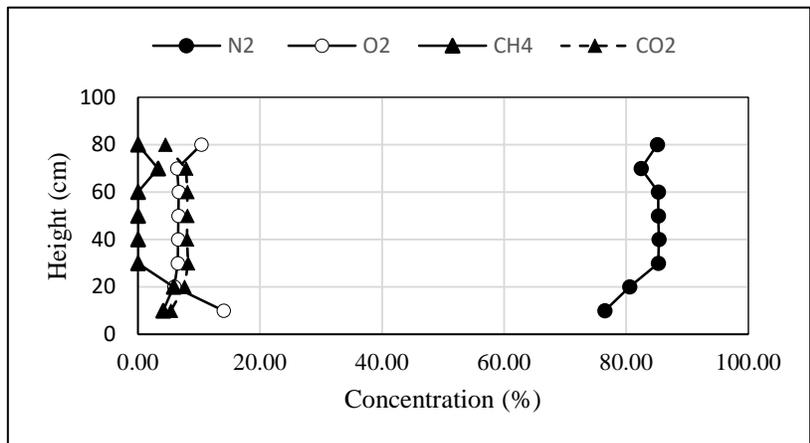
CH ₄ Flow rate (ml/min)	Loading rate (g/m ³ /day)	Air to CH ₄ flow rate ratios	Number of aeration levels	Average Oxidation rates over time (g/m ³ /day)	Average Oxidation efficiencies over time (%)
10	882.43	12.5 :1	One	853.39	96.71
6	529.46	12.5:1	Two	467.71	88.34
14	1235.41	5:1	Three	1193.99	96.65



a) Concentration Profiles-Column 3- August June 26th



b) Concentration Profiles-Column 2- June 26th



c) Concentration Profiles-Column 1- June 26th

Figure 5-27 Gas profiles for day 62 (second stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level

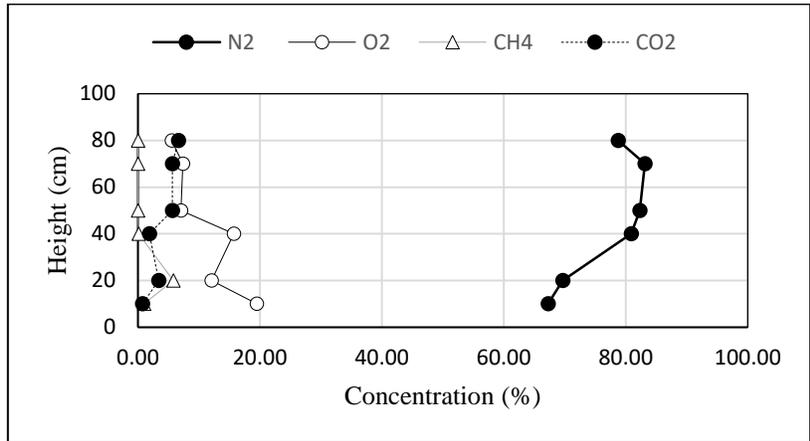
5.8.4 Concentration Profiles for the third stage

In this stage, the column aerated at two levels is subjected to an air flow rate ratio 1/4th of its stoichiometric value. Table 5-19 lists the experimental parameters, their levels, and average performances of the biofilter columns over time for the third stage.

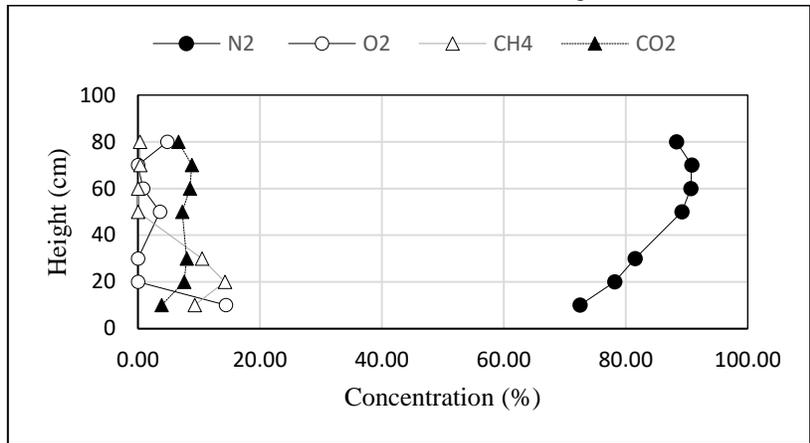
Table 5-19 Third stage of the Doehlert experimental design for columns run at varying air to CH₄ ratios

CH ₄ Flow rate (ml/min)	Loading rate (g/m ³ /day)	Air to CH ₄ flow rate ratios	Number of aeration levels	Average Oxidation rates over time (g/m ³ /day)	Average Oxidation efficiencies over time (%)
14	1235.41	12.5 :1	One	1217.45	98.55
12	1058.41	2.5:1	Two	1019.04	96.12
16	1411.90	10:1	Three	1353.35	95.85

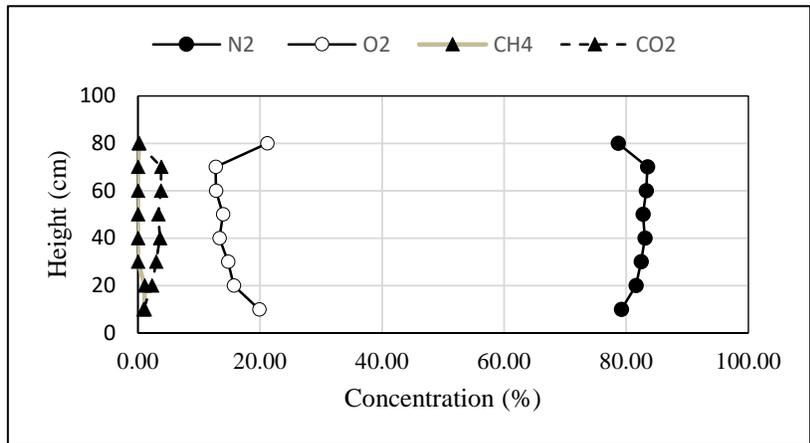
Vertical concentrations for the 76th day after start-up as plotted in Figure 5-28, in support of the values for oxidation rate and efficiency in Table 5-19, imply that even at an air to CH₄ flow rate ratio of 2.5, high oxidation efficiencies are achievable. Average O₂ concentration in the two-level aerated column are 3.2%. Noting that the accuracy of the Gas Chromatography of use is 1%, so values less than this 1% are reported as zero. The results here are comparable to those found by Wilshusen et al. (J.H. Wilshusen et al., 2004) indicating that oxidation reactions are still supported at O₂ concentrations as low as 0.2%.



a) Concentration Profiles-Column 3- August 10th



b) Concentration Profiles-Column 2- August 10th



c) Concentration Profiles-Column 1- August 10th

Figure 5-28 Gas profiles for day 76 (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level.

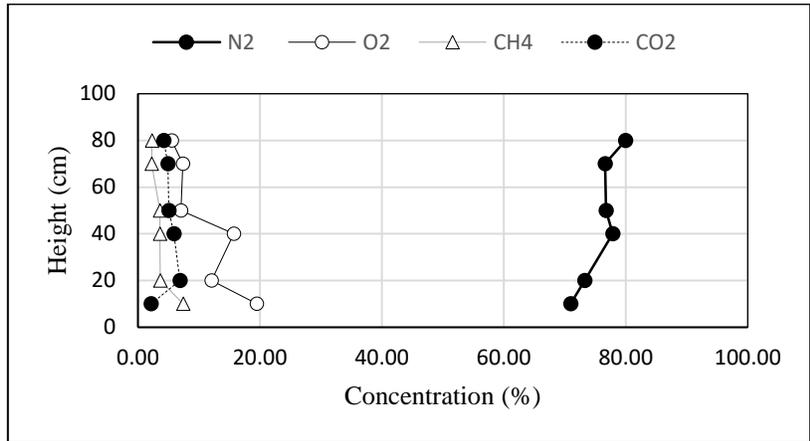
5.8.5 Concentration Profiles for the fourth stage

Increasing loading rates on day 88, initiates the final stage for which the levels of the governing parameters and the resulting oxidation rates and efficiencies are recorded in Table 5-20. As the loading rates approach the maximum value of 1588.38 g/m³/day, biofilter efficiencies decrease by 18.7%, 8.3%, and 14.5% with respect to their values in the previous stage respectively for the columns aerated at one, two, and three levels. This is interesting as the flow rates for columns with one and three air injection levels has been kept constant between the last two stages. The results of this stage highlight the influence of time, as a dominating parameter which affects porosity levels, moisture content distribution, and EPS formation on actively-aerated biofiltration.

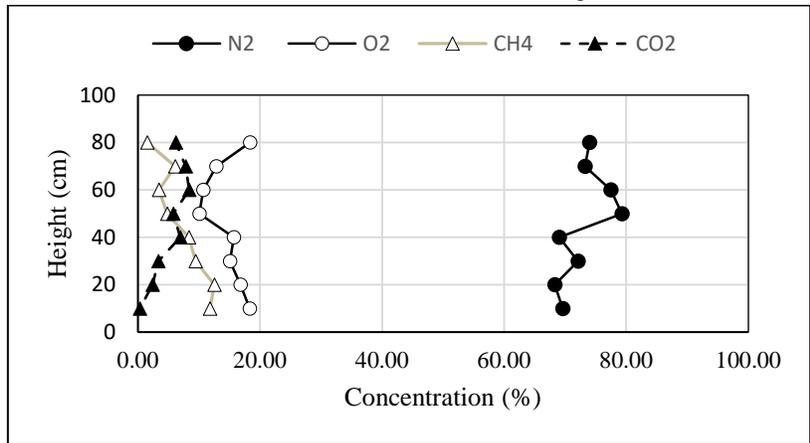
Table 5-20 Fourth stage of the Doehlert experimental design for columns run at varying air to CH₄ ratios

CH ₄ Flow rate (ml/min)	Loading rate (g/m ³ /day)	Air to CH ₄ flow rate ratios	Number of aeration levels	Average	Average
				Oxidation rates over time (g/m ³ /day)	Oxidation efficiencies over time (%)
14	1235.41	12.5:1	One	915.49	80.42
18	1588.38	5:1	Two	867.77	87.97
16	1411.90	12.5:1	Three	1157.56	81.97

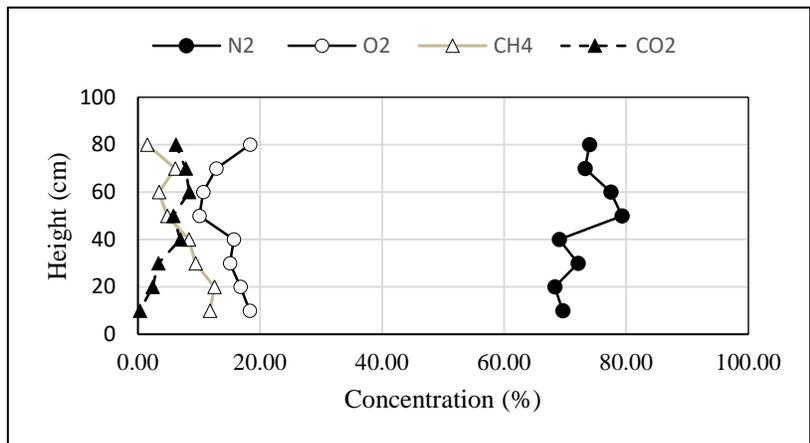
Figure 5-29 plots vertical gas concentrations after 90 days of operation. With column 2 run at an air flow rate ratio equal to half of its stoichiometric value, it is observed that CH₄ concentrations decrease rather steadily across the profiles. This implies that even at a loading rate of 1588 g/m³/day oxidation reactions still occur relatively uniformly along the height of the biofilter.



a) Concentration Profiles-Column 3- August 24th



b) Concentration Profiles-Column 2- August 24th



c) Concentration Profiles-Column 1- August 24th

Figure 5-29 Gas profiles for day 90 (fourth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level

In conclusion, based on the results discussed in this section, having actively-aerated biofiltration systems operated at values lower than the stoichiometric air flow rate grants oxidation efficiencies ranging from 80-100% depending on the feedings gas loading rate. This is particularly of interest in applying the subjects of study in large-scale as lower air requirements result in reductions in the overall cost. However, more research is required to evaluate the changes which occur as a result of long-term operation, namely, formation of EPS, reduction in pore spaces (as a result of EPS accumulation and material self-compaction), and changes in moisture content distribution (as a result of microbial activities and transport via air flows).

5.8.6 Response surface

Figure 5-30 illustrates the 3D response curve for actively-aerated biofilter columns run at varying air to CH₄ ratios. As evident from the graph, when operated at a minimum air to CH₄ flow rate ratio of 2.5, the biofilter design with three air injection levels yields the higher performance. This is due to the fact that with air being injected at low flow rates (and pressures) having O₂ injected at three levels along the thickness ensures a more evenly distributed concentration of the element.

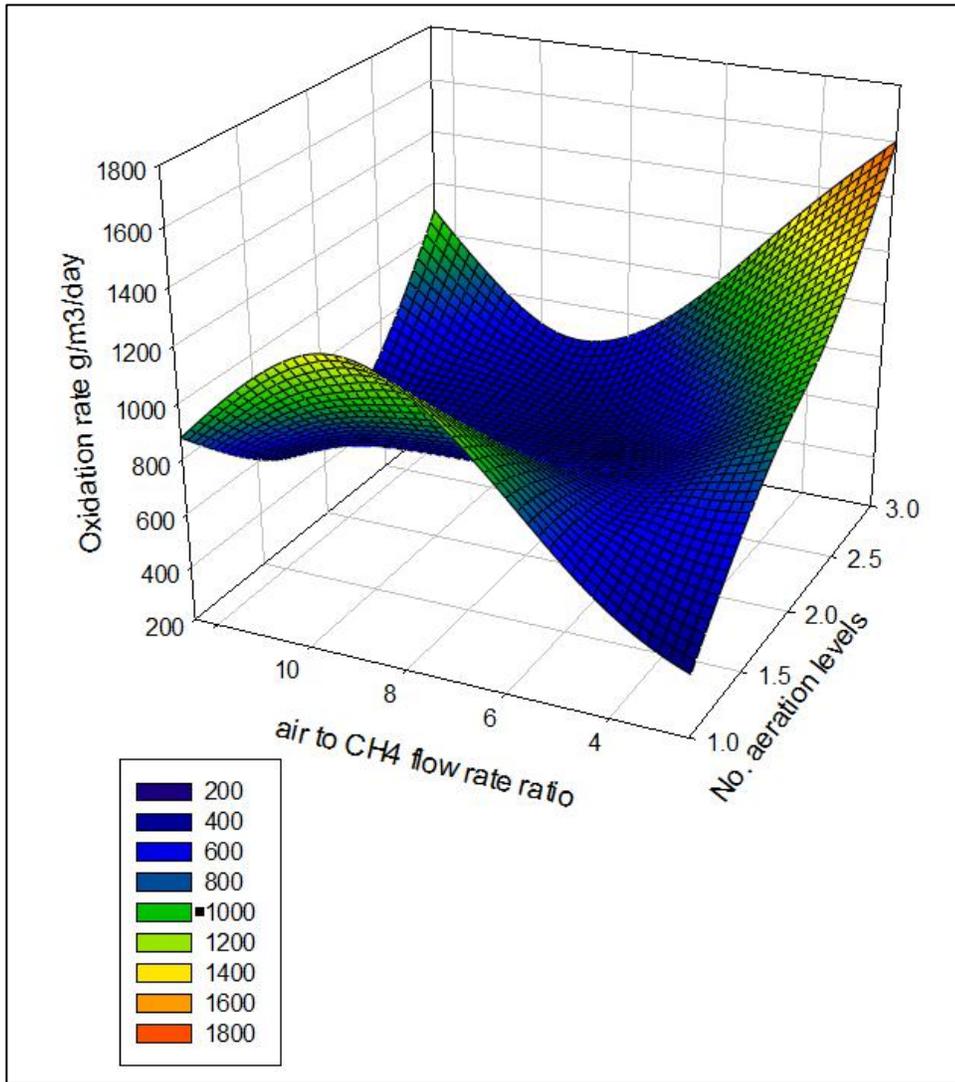


Figure 5-30- 3D response surface for biofilter columns operated at different air to CH₄ flow rate ratios

Chapter Six: Conclusions and recommendations for future research

6.1 Conclusions and Summary

This study was able to highlight the effectiveness of active aeration of CH₄ biofilters in improving the system's oxidation capacity. Three lab-scale biofilter designs were tested changing aeration levels in one, two, and three levels. The three objectives of this study have been met as detailed in Sections 6.1.1 to 6.1.3.

6.1.1 Effects of aeration levels on MBF performance

- The three design configurations were run for 195 days at flow rates changing from 6 (corresponding to 370.6 g CH₄/m²/day or 529.5 g CH₄/m³/day to 18 mL/min (corresponding to 1111.8 g CH₄/m²/day or 1588.3 g CH₄/m³/day) in 5 steps.
- The design configuration aerated at three levels yielded average oxidation efficiencies of 94.6, 88.6, 83.6, 80.2, 39.9%, respectively, during the 5 stages of study.
- Average oxidation efficiencies for the column aerated at two levels over the course of the study were 77.4, 84.1, 91.7, 89.5, 82.4 % for stages 1 to 5, respectively.
- The third column aerated via a single probe injected at the bottom of the column yielded average oxidation efficiencies of 78.1, 65.4, 65.1, 44.8, 35.4 % for the 5 inlet loading rates studied.
- ANOVA results suggest that performances of columns with two and three injection levels are not significantly different from one another. However, low oxidation

values for the three-level aeration design during the final stage justify the superior performance of the two-level aeration configuration. The two-level air aeration biofiltration system yields a more uniform performance over the entire range of flow rates tested.

- The biofilter design with a single aeration level performs reasonably well at flow rates up to 15 ml/min. But as loading rates increase, the system fails in performance as a concentrated microbial activities and EPS accumulation. Therefore, when operated at high flow rates, gas transport is impeded by EPS structures.

6.1.2 Effects of gas residence time following changes in inlet flow rates on MBF performance

- Total flow rates vary along the vertical profile of biofiltration systems with multiple aeration level as air is introduced at different sections. Therefore, gas retention time is defined separately for each section between the aeration levels. This results in different total retention times among the design configurations of the study.
- The total gas residence times of the design aerated at three levels during the 5 stages of operation are calculated as 135.2, 90.2, 67.6, 54.1, and 45.1 min, respectively.
- Having air introduced at two levels results in residence time values of 115.6, 77.1, 57.8, 46.2, and 38.5 min during the 5 stages of increasing inlet loading rates.
- Residence time values for the biofilter configuration with one aeration level are 81.6, 54.3, 40.7, 32.6, and 27.2 min for each of the 5 stages, respectively.
- Despite higher residence times for the three-level aerated configuration, the fact that there is no statistically significant difference between the removal rates of columns

with two and three injection levels, suggests that the required gas retention time is satisfied even at high flow rates.

- The failure of the three-level aerated column is justified by the distribution of residence time among the three sections of the column as divided by the aeration probes. With 1/3rd of air injected at the bottom, the lower flow rates in the bottom section result in higher gas residence time in that section. Giving more time to microbial reactions to take place, more H₂O is produced in this segment as a byproduct of the oxidation reaction. As flow rates increase, air pushes the H₂O produced upwards leading to accumulation of moisture and pore space saturation at the top sections. With gas transport limited within the top segment (a thickness of 30 cm), this section does not contribute to CH₄ removal.

6.1.3 Changes in MBF performance imposed by changing O₂ flow rates

- Air to CH₄ flow rate ratios were changed between ¼ of the stoichiometric value to 1.5 times higher than that of the stoichiometric level.
- It is concluded that reducing the inlet air flow rate to ¼ of its stoichiometric value will still result in satisfactory CH₄ removal rates. However, experiments have to be run to evaluate the long-term performance of such systems.

6.2 Recommendations

The work done in this research could be an initial step towards commercialization of active MBFs for treatment of point source emissions. However, a number of challenges should be

addressed and a number of features need to be clarified along this path. The following research directions for full implementation of the process are recommended.

- Testing alternative air delivery means e.g. inserting a vertical perforated pipe through which air is injected. The effects radius of influence, perforation configuration, and the number of vertical pipes are suggested to be investigated.
- Developing a mathematical model to enhance understanding of the actively-vented systems, gas profiles, and distribution of residence times.
- Testing the performance of multiple-level aeration MBF columns packed with a mixture of compost and a bulking agent as the filter media to compare the long-term performance with the columns operated in the present study.
- Evaluate the performance of multiple-level aeration MBFs in field. A conceptual design of the field-scale replica is presented in Figure 5-1. The biofiltration system is composed of a cartridge containing an outer housing extending vertically forming the walls, ceiling, and floor of the bioreactor. The housing contains multiple compartments, multiple air inlet ports at each level, connected to the inlet pipe positioned at one end. The housing can also contain a water reservoir for collecting waste water, and an outlet for flushing out the leachate. In operation, a source of CH_4 is passed through the inlet pipe to the ports and distributed at each level, then passing through the microorganism culture selected to degrade CH_4 . The treated gas stream is then collected by means of the outlet port disposed at the top portion of the MBF.

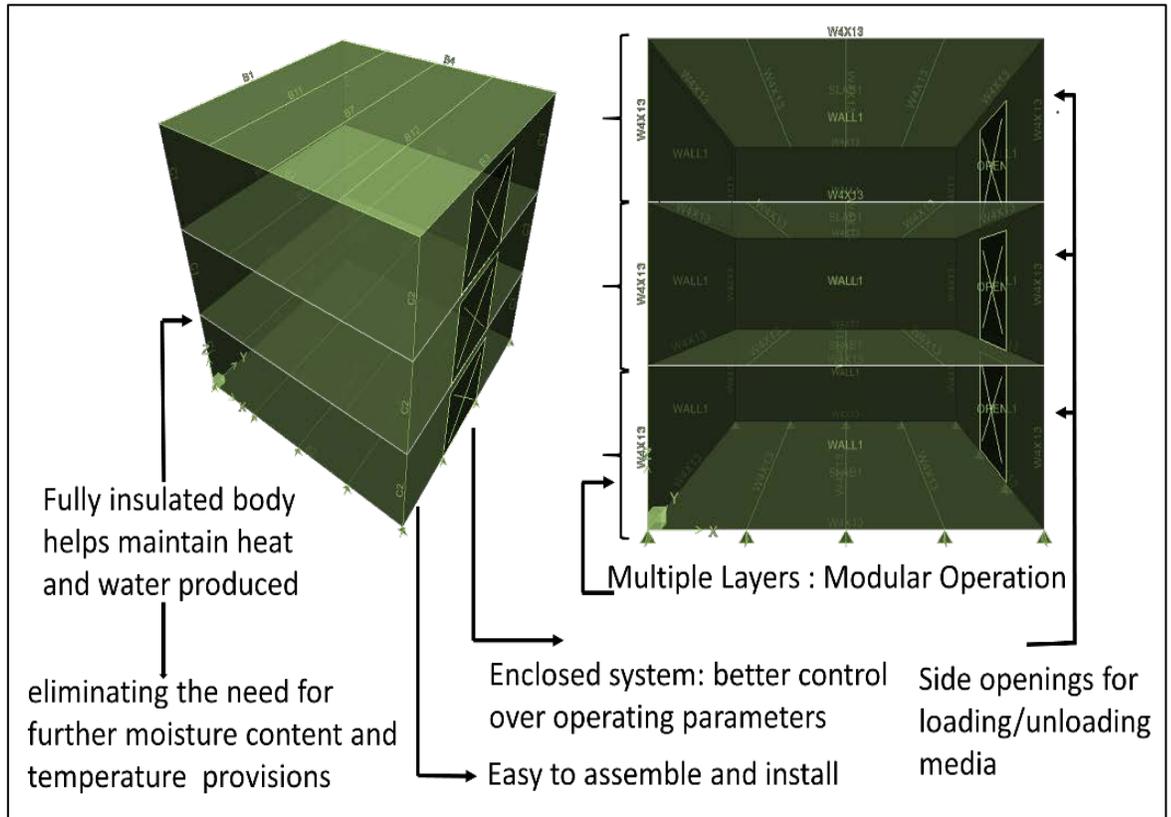


Figure 6-1 Conceptual Design of the field-scale multiple-level aerated MBF

To prevent moisture and heat loss, the housing unit can also be equipped with double covering helping to achieve the optimum temperature and moisture content conditions for the biological process. The container is equipped with a side opening through which one can access the multiple trays used to retain the filter media. Mentioned trays can be adapted to be removed and installed back in a drawer-like fashion for either cleaning purposes or media bed replacements.

References

- Abichou, T., Kormi, T., Wang, C., Chanton, J.P., Green, R., Escobar, F., 2015. A novel approach to estimate methane oxidation in interim landfill covers across the USA. *Int. J. Environ. Waste Manag.* 15, 309–326.
- Allaire, S.E., Lafond, J. a, Cabral, A.R., Lange, S.F., 2008. Measurement of gas diffusion through soils: comparison of laboratory methods. *J. Environ. Monit.* 10, 1326–36. doi:10.1039/b809461f
- Anthony, C., 1975. The biochemistry of methylotrophic micro-organisms. *Sci. Prog. (1933-)* 62, 167–206.
- Araujo, P.W., Brereton, R.G., 1996. Experimental design II. Optimization. *TrAC - Trends Anal. Chem.* 15, 63–70. doi:10.1016/0165-9936(96)80762-X
- ASTM International, 2014. ASTM D2974-14, Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils,. West Conshohocken, PA.
- ASTM International, 2007. Standard Test Methods for Laboratory Compaction Characteristics of Soil Using Standard Effort 3, 1–13. doi:10.1520/D0698-12E01.1
- Auman, A.J., Lidstrom, M.E., 2002. Analysis of sMMO-containing type I methanotrophs in Lake Washington sediment. *Environ. Microbiol.* 4, 517–24. doi:10.1046/j.1462-2920.2002.00323.x
- Auria, R., Frere, G., Morales, M., Acuña, M.E., Revah, S., 2000. Influence of mixing and water addition on the removal rate of toluene vapors in a biofilter. *Biotechnol. Bioeng.* 68, 448–455. doi:10.1002/(SICI)1097-0290(20000520)68:4<448::AID-BIT10>3.0.CO;2-2
- Bader, F.G., 1978. Analysis of Double-Substrate Limited Growth. *Biotechnol. Bioeng.* 20, 183–202.
- Bender, M., Conrad, R., 1995. Effect of CH₄ concentrations and soil conditions on the induction of CH₄ oxidation activity. *Soil Biol. Biochem.* 27, 1517–1527. doi:10.1016/0038-0717(95)00104-M
- Bender, M., Ralf Conrad, 1992. Kinetics of oxidation in oxic soils exposed to ambient air or high CH₄ mixing ratios. *FEMS Microbiol. Lett.* 101, 261–270.
- Benstead, J., King, G.M., 1997. Reposnse of methanotrophic activity in forest soil to methane availability. *FEMS Microbiol. Lett.* 23, 333–340. doi:10.1017/CBO9781107415324.004
- Bergamaschi, P., Lubina, C., Königstedt, R., Fischer, H., Veltkamp, A.C., Zwaagstra, O., 1998. Stable isotope signatures (d¹³C, d²D) of methane from European landfill sites. *J. Geophys. Res.* 103, 8251–8265.
- Bio-Logic Environmental Systems, 2008. Nova Scotia Environment Compost Maturity Study. Dartmouth, NS.
- Boeckx, P., Van Cleemput, O., 1996. Methane oxidation in a neutral landfill cover soil: influence of moisture content, temperature, and nitrogen-turnover. *J. Environ. Qual.* 25, 178–183.

- Boeckx, P., Van Cleemput, O., Villaralvo, I., 1996. Methane emission from a landfill and the methane oxidising capacity of its covering soil. *Soil Biol. Biochem.* 28, 1397–1405. doi:10.1016/S0038-0717(96)00147-2
- Bohn, H.L., 1996. Biofilter Media, in: Proceedings of the 89th Annual Meeting and Exhibition of Air and Waste Management Association. Nashville, US.
- Börjesson, G., Sundh, I., Svensson, B., 2004. Microbial oxidation of CH₄ at different temperatures in landfill cover soils. *FEMS Microbiol. Ecol.* 48, 305–312. doi:10.1016/j.femsec.2004.02.006
- Börjesson, G., Sundh, I., Tunlid, a, Frostegård, Å., Svensson, B.H., 1998. Microbial oxidation of CH₄ at high partial pressures in an organic landfill cover soil under different moisture regimes. *FEMS Microbiol. Ecol.* 26, 207–217.
- Born, M., Dorr, H., Levin, I., 1990. Methane consumption in aerated soils of the temperate zone. *Tellus, Ser. B Chem. Phys. Meteorol.* 42, 2–8.
- Bowman, J.P., Jimenez, L., Rosario, I., Hazen, T.C., Sayler, G.S., 1993. Characterization of the Methanotrophic Bacterial Community Present in a Trichloroethylene-Contaminated Subsurface Groundwater Site 59, 2380–2387.
- Brewer, L., Sullivan, D., 2001. Maturity and Stability Evaluation of Composted Yard Debris. Oregon State University.
- Brinton, W.F., Evans, E., Droffner, M.L., Brinton, R.B., 1995. A Standardized Dewar Test for Evaluation of Compost Self-Heating. *Biocycle Rep.* 36, 1–16.
- Brinton, Wi.F., 2000. Compost quality standards & guidelines. New York State Assoc. *Recycl.* 1–42, 1–42.
- Buckingham, E., 1904. Contributions to our knowledge of the aeration of soils., Bulletin 2. ed. USDA Bureau of Soils, Washington, D.C.
- Bungay, H.R., 1994. Growth rate expressions for two substrates one of which is inhibitory. *J. Biotechnol.* 34, 97–100. doi:10.1016/0168-1656(94)90170-8
- Cabral, A.R., Tremblay, P., Lefebvre, G., 2004. Determination of the diffusion coefficient of oxygen for a cover system including a pulp and paper by-product. *Geotech. Test. J.* 27, 184–197. doi:10.1520/GTJ11233
- Canadian Council of Ministers of the Environment, 2005. Guidelines for Compost Quality. Winnipeg, Manitoba.
- Caulton, D.R., Shepson, P.B., Santoro, R.L., Sparks, J.P., Howarth, R.W., Ingraffea, A.R., Cambaliza, M.O.L., Sweeney, C., Karion, A., Davis, K.J., Stirm, B.H., Montzka, S.A., Miller, B.R., 2014. Toward a better understanding and quantification of methane emissions from shale gas development. *Proc. Natl. Acad. Sci.* 111, 6237–6242. doi:10.1073/pnas.1316546111
- Chai, X., Tonjes, D.J., Mahajan, D., 2016. Methane emissions as energy reservoir: Context, scope, causes and mitigation strategies. *Prog. Energy Combust. Sci.* 56, 33–70. doi:10.1016/j.peccs.2016.05.001

- Chan, S.I., Chen, K.H.C., Yu, S.S.F., Chen, C.L., Kuo, S.S.J., 2004. Toward Delineating the Structure and Function of the Particulate Methane Monooxygenase from Methanotrophic Bacteria. *Biochemistry* 43, 4421–4430. doi:10.1021/bi0497603
- Chanton, J.P., Powelson, D.K., Green, R.B., 1995. Methane oxidation in landfill cover soils, is a 10% default value reasonable? *J. Environ. Qual.* 38, 654–663. doi:10.2134/jeq2008.0221
- Cicerone, R.J., Oremland, R.S., 1988. Biogeochemical Aspects of Atmospheric Methane. *Global Biogeochem. Cycles* 2, 299–327.
- Clearstone Engineering Ltd., 2005. A National Inventory of Greenhouse Gas (GHG), Criteria Air Contaminant (CAC) and Hydrogen Sulphide (H₂S) Emissions by the Upstream Oil and Gas Industry Volume 3 , Methodology for Greenhouse Gases. Calgary, AB.
- Compost maturity index, 2001. . Nevada City, CA.
- Conrad, R., 1996. Soil Microorganisms as Controllers of Atmospheric Trace Gases (H₂ , CO , CH₄ , OCS , N₂O , and NO) 60, 609–640.
- Cubasch, U., Wuebbles, D., Chen, D., Facchini, M.C., Frame, D., Mahowald, N., Winther, J.-G., 2013. Introduction. Cambridge, UK and New York, US. doi:10.1017/CBO9781107415324.007
- Czepiel, P.M., Mosher, B., Crill, P.M., Harriss, R.C., 1996. Quantifying the effect of oxidation on landfill methane emissions. *J. Geophys. Res. Atmos.* 101, 16721–16729. doi:10.1029/96JD00222
- De Visscher, A., Schippers, M., Van Cleemput, O., 2001. Short-term kinetic response of enhanced methane oxidation in landfill cover soils to environmental factors. *Biol. Fertil. Soils* 33, 231–237. doi:10.1007/s003740000313
- De Visscher, A., Thomas, D., Boeckx, P., Van Cleemput, O., 1999. Methane Oxidation in Simulated Landfill Cover Soil Environments. *Environ. Sci. Technol.* 33, 1854–1859. doi:10.1021/es9900961
- De Visscher, A., Van Cleemput, O., 2003. Induction of enhanced CH₄ oxidation in soils: NH₄⁺ inhibition patterns. *Soil Biol. Biochem.* 35, 907–913. doi:10.1016/S0038-0717(03)00122-6
- Delhom, M., 2005. Biofiltration of Air : A Review 53–72. doi:10.1080/07388550590935814
- Delhomenie, M.C., Nikiema, J., Bibeau, L., Heitz, M., 2008. A new method to determine the microbial kinetic parameters in biological air filters. *Chem. Eng. Sci.* 63, 4126–4134. doi:10.1016/j.ces.2008.05.020
- Devanny, J.S., Deshusses, M.A., Webster, T.S., 1999. Biofiltration for Air Pollution Control. CRC Press, pp. 43–46.
- Dispirito J. Gullidge, J. C. Murrell, A. K. Shiemke, M. E. Lidstrom, and C. L. Krema., a a, 1992. Trichloroethylene oxidation by the membrane associated methane monooxygenase in type I, type II, and type X methanotrophs. *Biodegradation* 2, 151–164. doi:10.1007/BF00124489
- Doehlert, D.H., 1970. Uniform Shell Designs. *J. R. Stat. Soc.* 19, 231–239. doi:10.2307/2346327
- Dojcinovski, M., Anakievski, G., 2013. Chromatography as a forensic method in criminalistic

- identifications. *J. Chem. Inf. Model.* 53, 1689–1699. doi:10.1017/CBO9781107415324.004
- Du Plessis, C.A., Strauss, J.M., Sebalalo, E.M.T., Riedel, K.H.J., 2003. Empirical model for methane oxidation using a composted pine bark biofilter. *Fuel* 82, 1359–1365. doi:10.1016/S0016-2361(03)00040-1
- Dunfield, P., Knowles, R., 1995. Kinetics of inhibition of methane oxidation by nitrate , nitrite , and ammonium in a humisol . These include : Kinetics of Inhibition of Methane Oxidation by Nitrate , Nitrite , and Ammonium in a Humisol 61, 3129–3135.
- Dye, R., Dallavalle, J.M., 1958. Diffusion of Gases in Porous Media. *Ind. Eng. Chem.* 50, 1195–1200.
- Eadie, G.S., 1942. The Inhibition of Cholinesterase by Physostigmine and Prostigmine. *J. Biol. Chem.* 146, 85–93.
- Environment Canada, 2014. Canada’s Sixth National Report on Climate Change 2014.
- EPA, 2010. Sources and Emissions | Methane | Climate Change | U.S. EPA [WWW Document]. epa.gov. URL <http://www.epa.gov/methane/sources.html>
- Familletti, P.C., 1987. Air lift bioreactor. US4649117 A.
- Ferreira, S.L.C., Bruns, R.E., da Silva, E.G.P., dos Santos, W.N.L., Quintella, C.M., David, J.M., de Andrade, J.B., Breikreitz, M.C., Jardim, I.C.S.F., Neto, B.B., 2007. Statistical designs and response surface techniques for the optimization of chromatographic systems. *J. Chromatogr. A.* doi:10.1016/j.chroma.2007.03.051
- Flegg, P., 1953. The effect of aggregation of diffusion of gases and vapours through soils. *J. Sci. Food Agric.* 4, 104–108.
- Fletcher, M., 1992. Bacterial Metabolism in Biofilms, in: Melo, L.F., Bott, T.R., Fletcher, M., Capdeville, B. (Eds.), *Biofilms --- Science and Technology*. Springer Netherlands, Dordrecht, pp. 113–124. doi:10.1007/978-94-011-1824-8_12
- G7, 2015. Leaders’ Declaration G7 Summit 7-8 June 2015. G7 Summit 7–8.
- Galindo, E., Pacek, A.W., Nienow, A.W., 2000. Study of drop and bubble sizes in a simulated mycelial fermentation- broth of up to four phases. *Biotechnol. Bioeng.* 69, 213–221. doi:10.1002/(SICI)1097-0290(20000720)69:2<213::AID-BIT10>3.0.CO;2-D
- Gaudin, F., Andres, Y., Cloirec, P. Le, Nantes, M. De, Cnrs, G.U.M.R., Cedex, N., n.d. Odorous emission biofiltration with new synthetic packing materials : essential nutrient release 263–271.
- Gebert, J., Groenroeft, A., 2006. Passive landfill gas emission - Influence of atmospheric pressure and implications for the operation of methane-oxidising biofilters. *Waste Manag.* 26, 245–251. doi:10.1016/j.wasman.2005.01.022
- Gebert, J., Groenroeft, A., Miehlich, G., 2003. Kinetics of microbial landfill methane oxidation in biofilters. *Waste Manag.* 23, 609–619. doi:10.1016/S0956-053X(03)00105-3
- Gebert, J., Gröngröft, a, 2006. Performance of a passively vented field-scale biofilter for the microbial oxidation of landfill methane. *Waste Manag.* 26, 399–407.

doi:10.1016/j.wasman.2005.11.007

- Girard, M., Ramirez, A.A., Buelna, G., Heitz, M., 2011. Biofiltration of methane at low concentrations representative of the piggery industry-Influence of the methane and nitrogen concentrations. *Chem. Eng. J.* 168, 151–158. doi:10.1016/j.cej.2010.12.054
- Goldman, M.B., Groffman, P.M., Pouyat, R. V., McDonnell, M.J., Pickett, S.T.A., 1995. CH₄ uptake and N availability in forest soils along an urban to rural gradient. *Soil Biol. Biochem.* 27, 281–286. doi:10.1016/0038-0717(94)00185-4
- Goldsmith, C.D., Chanton, J., Abichou, T., Swan, N., Green, R., Hater, G., 2012. Methane emissions from 20 landfills across the United States using vertical radial plume mapping. *J. Air Waste Manage. Assoc.* 62, 183–197. doi:10.1080/10473289.2011.639480
- Gonzalez, A.G., Gonzalez-arjona, D., 1999. Computational program for evaluating and optimizing response- surface curves based on central composite designs. *Talanta* 49, 433–439.
- Government of Canada, 2013. Canada ' s Emissions Trends, Environment Canada.
- Graham, D.W., Chaudhary, J.A., Hanson, R.S., Arnold, R.G., 1993. Factors Affecting Competition Between Type I and Type II Methanotrophs in Two-organism, Continuous-flow Reactors. *Microb. Ecol.* 25, 1–17.
- Grob, R.L., Barry, E.F., 2004. *Modern Practice of Gas Chromatography*, Fourth. ed. John Wiley and Sons.
- Hagan, M., 1941. Movement of Carbon Disulfide Vapor in Soils. *Hilgardia* 14. doi:10.1017/CBO9781107415324.004
- Hanes, C.S., 1932. Studies on plant amylases: The effect of starch concentration upon the velocity of hydrolysis by the amylase of germinated barley. *Biochem. J.* 26, 1406–1421.
- Hanson, R.S., Hanson, T.E., 1996. Methanotrophic bacteria. *Microbiol. Rev.* 60, 439–471. doi:10.1093/micrev/60.3.439
- Haththotuwa, C., 2005. Control of greenhouse gas emissions from oil production. University of Calgary.
- Haubrichs, R., Widmann, R., 2006. Evaluation of aerated biofilter systems for microbial methane oxidation of poor landfill gas. *Waste Manag.* 26, 408–16. doi:10.1016/j.wasman.2005.11.008
- Hayes, R.E., 2004. Catalytic solutions for fugitive methane emissions in the oil and gas sector, in: *Chemical Engineering Science*. pp. 4073–4080. doi:10.1016/j.ces.2004.04.038
- Hemond, H.F., Fechner, E.J., 2015. *Chemical Fate and Transport in the Environment*, Third. ed. Elsevier Ltd, San diego, CA.
- Henckel, T., Jäckel, U., Schnell, S., Conrad, R., 2000. Molecular Analyses of Novel Methanotrophic Communities in Forest Soil That Oxidize Atmospheric Methane. *Appl. Environ. Microbiol.* 66, 1801–1808. doi:10.1128/AEM.66.5.1801-1808.2000.Updated
- Hettiarachchi, V.C., Hettiaratchi, J.P. a., Mehrotra, a. K., 2007. *Comprehensive One-Dimensional*

- Mathematical Model for Heat, Gas, and Moisture Transport in Methane Biofilters. *Pract. Period. Hazardous, Toxic, Radioact. Waste Manag.* 11, 225–233. doi:10.1061/(ASCE)1090-025X(2007)11:4(225)
- Hettiarachchi, V.C., Hettiaratchi, P.J., Mehrotra, A.K., Kumar, S., 2011. Field-scale operation of methane biofiltration systems to mitigate point source methane emissions. *Environ. Pollut.* 159, 1715–1720. doi:10.1016/j.envpol.2011.02.029
- Hettiaratchi, P.J., Stein, V.B., Achari, G., 2000. Biofiltration: A cost-effective technique for controlling methane emissions from sub-surface sources., in: *6th Environmental Issues and Management of Waste in Energy and Mineral Production*. Balkema Rotterdam, Netherlands, pp. 291–299.
- Hilger, H.A., Cranford, D.F., Barlaz, M.A., 2000. Methane oxidation and microbial exopolymer production in land @ ll cover soil 32, 457–467.
- Hilger, H., Wollum, A.G., Barlaz, M.A., 2000. Landfill methane oxidation response to vegetation, fertilization, and liming. *J. Environ. Qual.* doi:10.2134/jeq2000.00472425002900010041x
- Hirai, M., Kamamoto, M., Yani, M., Shoda, M., 2001. Comparison of the biological NH₃ removal characteristics among four inorganic packing materials. *J. Biosci. Bioeng.* 91, 428–430. doi:10.1016/S1389-1723(01)80165-1
- Hoftsee, B.H., 1952. On the evaluation of the constants V_m and K_m in enzyme reactions. *Am. Assoc. Adv. Sci.* 116, 329–331.
- Holmes, A.J., Roslev, P., McDonald, I.R., Iversen, N., Henriksen, K., Murrell, J.C., 1999. Characterization of methanotrophic bacterial populations in soils showing atmospheric methane uptake. *Appl. Environ. Microbiol.* 65, 3312–3318.
- Howarth, R.W., 2014. A bridge to nowhere: methane emissions and the greenhouse gas footprint of natural gas. *Energy Sci. Eng.* 2, 47–60. doi:10.1002/ese3.35
- Huber-Humer, M., Gebert, J., Hilger, H., 2008. Biotic systems to mitigate landfill methane emissions. *Waste Manag. Res.* 26, 33–46. doi:10.1177/0734242X07087977
- Imandi, S.B., Bandaru, V.R., Somalanka, S.R., Garapati, H.R., 2007. Optimization of medium constituents for the production of citric acid from byproduct glycerol using Doehlert experimental design. *Enzyme Microb. Technol.* 40, 1367–1372. doi:10.1016/j.enzmictec.2006.10.012
- Jaffrin, A., Bentounes, N., Joan, A.M., Makhlof, S., 2003. Landfill biogas for heating greenhouses and providing carbon dioxide supplement for plant growth. *Biosyst. Eng.* 86, 113–123. doi:10.1016/S1537-5110(03)00110-7
- Jin, Y., Jury, W.A., 1996. Characterizing the Dependence of Gas Diffusion Coefficient on Soil Properties. *Soil Sci. Soc. Am. J.* 60, 66–71. doi:10.2136/sssaj1996.03615995006000010012x
- Johnson, M.R., Coderre, A.R., 2012a. Compositions and greenhouse gas emission factors of flared and vented gas in the Western Canadian Sedimentary Basin. *J. Air Waste Manage. Assoc.* 62, 992–1002. doi:10.1080/10962247.2012.676954
- Johnson, M.R., Coderre, A.R., 2012b. Opportunities for CO₂ equivalent emissions reductions via

- flare and vent mitigation: A case study for Alberta, Canada. *Int. J. Greenh. Gas Control* 8, 121–131. doi:10.1016/j.ijggc.2012.02.004
- Jones, H., Nedwell, D.B., 1993. Methane emission and methane oxidation in landfill cover soil. *FEMS Microbiol. Ecol.* 185–195. doi:http://femsec.oxfordjournals.org/content/11/3-4/185
- Kang, M., Kanno, C.M., Reid, M.C., Zhang, X., Mauzerall, D.L., Celia, M.A., Chen, Y., Onstott, T.C., 2014. Direct measurements of methane emissions from abandoned oil and gas wells in Pennsylvania. *Proc. Natl. Acad. Sci. U. S. A.* 111, 18173–7. doi:10.1073/pnas.1408315111
- Kightley, D., Nedwell, D.B., Cooper, M., 1995. Capacity for methane oxidation in landfill cover soils measured in laboratory-scale soil microcosms . *Capacity for Methane Oxidation in Landfill Cover Soils Measured in Laboratory-Scale Soil Microcosms* 61, 592–601.
- King, G.M., Adamsen, A.P.S., 1992. Effects of temperature on methane consumption in a forest soil and in pure cultures of the methanotroph *Methylomonas rubra*. *Appl. Environ. Microbiol.* 58, 2758–2763.
- Kirtman, B., Power, S.B., Adedoyin, J.A., Boer, G.J., Bojariu, R., Camilloni, I., Doblaz-Reyes, F.J., Fiore, A.M., Kimoto, M., Meehl, G.A., Prather, M., Sarr, A., Schär, C., Sutton, R., van Oldenborgh, G.J., Vecchi, G., Wang, H.-J., 2013. Near-term Climate Change: Projections and Predictability. *Clim. Chang. 2013 Phys. Sci. Basis. Contrib. Work. Gr. I to Fifth Assess. Rep. Intergov. Panel Clim. Chang.* 953–1028. doi:10.1017/CBO9781107415324.023
- Klute, A., 1986. *Methods of soil analysis. Part 1, physical and meneralogical methods.* Madison, Wisconsin.
- Kuriyama, K., 1998. *Stirred Tank Bioreactors.* US5728577 A.
- Lasaridi, K.E., Stentiford, E.I., Evans, T., 2000. Windrow composting of wastewater biosolids: Process performance and product stability assessment. *Water Sci. Technol.* 42, 217–226.
- Lashof, D. a, Ahuja, D.R., 1990. Relative Contributions of Greenhouse Gas Emissions to Global Warming. *Nature.* doi:10.1038/344529a0
- Lebrero, R., López, J.C., Perez, R., Quijano, G., Lebrero, R., L, J.C., 2016. Exploring the potential of fungi for methane abatement: Performance evaluation of a fungal-bacterial biofilter. *Chemosphere* 144, 97–106. doi:10.1016/j.chemosphere.2015.08.017
- Lebrero, R., Munoz, R., Villarverde, S., Hung, Y.-T., 2012. Air Biofiltration Applied to Odor Treatment, in: Hung, Y.-T., Wang, L., Shammass, N.K. (Eds.), *Handbook of Environment and Waste Management: Air and Water Pollution Control.* World Scientific.
- Lee, S.-H., Li, C., Heber, A.J., Ni, J., Huang, H., 2013. Biofiltration of a mixture of ethylene, ammonia, n-butanol, and acetone gases. *Bioresour. Technol.* 127, 366–77. doi:10.1016/j.biortech.2012.09.110
- Lelieveld, J., Crutzen, P.J., Dentener, F.J., 1998. Changing concentration, lifetime and climate forcing of atmospheric methane. *Tellus, Ser. B Chem. Phys. Meteorol.* 50, 128–150. doi:10.1034/j.1600-0889.1998.t01-1-00002.x
- Leson, G., Winer, A.M., 1991. Biofiltration: An Innovative Air Pollution Control Technology For VOC Emissions. *J. Air Waste Manage. Assoc.* doi:10.1080/10473289.1991.10466898

- levy, H., 1971. Normal Atmosphere : Large Radical and Formaldehyde Concentrations Predicted. *Am. Assoc. Adv. Sci.* 173, 141–143.
- Limbri, H., Gunawan, C., Rosche, B., Scott, J., 2013. Challenges to Developing Methane Biofiltration for Coal Mine Ventilation Air: A Review. *Water, Air, Soil Pollut.* 224, 1566. doi:10.1007/s11270-013-1566-5
- Lineweaver, H., Burk, D., 1934. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* 56, 658–666. doi:10.1021/ja01318a036
- Logan, J., Prather, M., Wofsy, S., McElroy, M., 1981. Tropospheric chemistry: A global perspective. *J. Geophys. Res.* 86, 7210–7254. doi:10.1029/2007JG000640/abstract
- Lopez, J.C., Quijano, G., Souza, T.S.O., Estrada, J.M., Lebrero, R., Muñoz, R., 2013. Biotechnologies for greenhouse gases (CH₄, N₂O, and CO₂) abatement: State of the art and challenges. *Appl. Microbiol. Biotechnol.* 97, 2277–2303. doi:10.1007/s00253-013-4734-z
- Lundstedt, T., Bergman, R., Abramo, L., Pettersen, J., Nystrom, A., Thelin, B., Seifert, E., 1998. Experimental design and optimization. *Chemom. Intell. Lab. Syst.* 42, 3–40. doi:10.1016/S0169-7439(98)00065-3
- Malashenko, I.P., Pirog, T.P., Romanovskaia, V. a, Sokolov, I.G., Gringerg, T. a, 2001. Search for methanotrophic producers of exopolysaccharides. *Prikl. Biokhim. Mikrobiol.* 37, 702–705. doi:10.1023/A:1012307202011
- Mancebo, U., Hettiaratchi, P., Jayasinghe, P., Surampalli, R., 2016. Determination of water retention capacity of granular media of methane biofilters: a simplified approach. *Environ. Earth Sci.* 75, 1–8. doi:10.1007/s12665-015-4907-4
- Mancinelli, R.L., 1995. The regulation of methane oxidation in soil. *Annu. Rev. Microbiol.* 49, 581–605. doi:10.1146/annurev.micro.49.1.581
- Marshall, T.J., 1959. the Diffusion of Gases Through Porous Media. *J. Soil Sci.* 10, 79–82. doi:10.1111/j.1365-2389.1959.tb00667.x
- Marthur, S., Owen, G., Dinel, H., Schnitzer, M., 1993. Determination of Compost Biomaturity. I. Literature Review. *Biol. Agric. Horticulture* 10, 65–85.
- Melse, R.W., Van Der Werf, A.W., 2005. Biofiltration for mitigation of methane emission from animal husbandry. *Environ. Sci. Technol.* 39, 5460–5468. doi:10.1021/es048048q
- Millington, R.J., 1959. Gas Diffusion in Porous Media Author. *Am. Assoc. Adv. Sci. Sci.* 130, 100–102.
- Millington, R.J., Quirk, J.P., 1961. Permeability of porous solids. *Trans. Faraday Soc.* 57, 1200–1207. doi:10.1039/TF9615701200
- Moiser, A., Schimel, D., Valentine, D., Bronson, K., Parton, W., 1991. Methane and nitrous-oxide fluxes in native, fertilized and cultivated grasslands. *Nature* 350, 330–332. doi:10.1038/350330a0
- Moldrup, O., Olesen, T., Yamaguchi, T., Schjonning, P., Rolston, D.E., 1999. Modeling diffusion and reation in soils: IX the Buckingham-Burdine-Campbell equation for gas diffusivity in

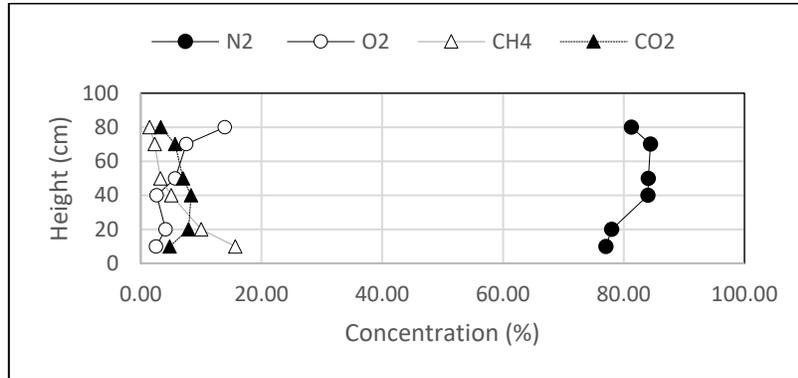
- undisturbed soil. *Soil Sci.* 164, 542–551.
- Monod, J., 1949. The Growth of Bacterial Cultures. *Annu. Rev. Microbiol.* 3, 371–394.
- Montgomery, D.C., 2001. Design and Analysis of Experiments, Design. doi:10.1198/tech.2006.s372
- Myhre, G., Shindell, D., Bréon, F.-M.F.-M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.-F.J.-F., Lee, D., Mendoza, B., Nakajima, T., Robock, A., Stephens, G., Takemura, T., Zhan, H., Zhang, H., 2013. Anthropogenic and Natural Radiative Forcing. *Clim. Chang.* 2013 Phys. Sci. Basis. Contrib. Work. Gr. I to Fifth Assess. Rep. Intergov. Panel Clim. Chang. Phys. Sci. Basis. Contrib. Work. Gr. I to Fifth Assess. Rep. 1–44. doi:10.1017/CBO9781107415324.018
- Nechar, M., Molina, M.F.M., Rodriguez, L.C., Sendra, J.M.B., 1995. The application of Doehlert designs in the optimization of experimental variables in solid phase spectrophotometry. *Anal. Chim. Acta* 316, 185–193. doi:10.1016/0003-2670(95)00351-Y
- Nielsen, a K., Gerdes, K., Murrell, J.C., 1997. Copper-dependent reciprocal transcriptional regulation of methane monooxygenase genes in *Methylococcus capsulatus* and *Methylosinus trichosporium*. *Mol. Microbiol.* 25, 399–409. doi:10.1046/j.1365-2958.1997.4801846.x
- Nikiema, J., Bibeau, L., Lavoie, J., Brzezinski, R., Vigneux, J., Heitz, M., 2005. Biofiltration of methane: An experimental study. *Chem. Eng. J.* 113, 111–117. doi:10.1016/j.cej.2005.04.005
- Nikiema, J., Brzezinski, R., Heitz, M., 2007. Elimination of methane generated from landfills by biofiltration: a review. *Rev. Environ. Sci. Biotechnol.* 6, 261–284. doi:10.1007/s11157-006-9114-z
- Nikiema, J., Payre, G., Heitz, M., 2009. A mathematical steady state model for methane bioelimination in a closed biofilter. *Chem. Eng. J.* 150, 418–425. doi:10.1016/j.cej.2009.01.032
- Omelchenko, M. V., Vasilyeva, L. V., Zavarzin, G.A., 1993. Psychrophilic methanotroph from tundra soil. *Curr. Microbiol.* 27, 255–259. doi:10.1007/BF01575988
- Ordaz, A., Lopez, J.C., Figueroa-Gonzalez, I., Munoz, R., Quijano, G., 2014. Assessment of methane biodegradation kinetics in two-phase partitioning bioreactors by pulse respirometry. *Water Res.* 67, 46–54. doi:10.1016/j.watres.2014.08.054
- Park, J.R., Moon, S., Ahn, Y.M., Kim, J.Y., Nam, K., 2005. Determination of environmental factors influencing methane oxidation in a sandy landfill cover soil. *Environ. Technol.* 26, 93–102. doi:10.1080/09593332608618586
- Park, S., Brown, K.W., Thomas, J.C., 2002. The effect of various environmental and design parameters on methane oxidation in a model biofilter. *Waste Manag. Res.* 20, 434–444. doi:10.1177/0734242X0202000507
- Penman, H., 1940. Gas and vapour movements in the soil I: the diffusion of vapour through porous solids. *J. Agric. Sci.* 30, 437–462.
- Pokhrel, D., 2006. Compost Based Biocap Performance. University of Calgary.

- Pokhrel, D., Hettiaratchi, P., Kumar, S., 2011. Methane diffusion coefficient in compost and soil-compost mixtures in gas phase biofilter. *Chem. Eng. J.* 169, 200–206. doi:10.1016/j.cej.2011.03.013
- Quigley, C., Easter, C., Burrowes, P., Witherspoon, J., 2004. Biotechnology-based odour control: Design criteria and performance data. *Water Sci. Technol.* 50, 319–326.
- Quijano, G., Rocha-Rios, J., Hernandez, M., Villaverde, S., Revah, S., Munoz, R., Thalasso, F., 2010. Determining the effect of solid and liquid vectors on the gaseous interfacial area and oxygen transfer rates in two-phase partitioning bioreactors. *J. Hazard. Mater.* 175, 1085–1089. doi:10.1016/j.jhazmat.2009.10.020
- Rettich, T., Handa, Y., Battino, R., Wilhelm, E., 1981. Solubility of gases in liquids. 13. High-precision determination of Henry's constants for methane and ethane in liquid water at 275 to 328 K. *J. Phys. ...* 717, 3230–3237. doi:10.1021/j150622a006
- Rocha-Rios, J., Kraakman, N.J.R., Kleerebezem, R., Revah, S., Kreutzer, M.T., van Loosdrecht, M.C.M., 2013. A capillary bioreactor to increase methane transfer and oxidation through Taylor flow formation and transfer vector addition. *Chem. Eng. J.* 217, 91–98. doi:10.1016/j.cej.2012.11.065
- Rocha-Rios, J., Quijano, G., Thalasso, F., Revah, S., Muñoz, R., 2011. Methane biodegradation in a two-phase partition internal loop airlift reactor with gas recirculation. *J. Chem. Technol. Biotechnol.* 86, 353–360. doi:10.1002/jctb.2523
- Rossi, C., Haupt, K., 2007. Application of the Doehlert experimental design to molecularly imprinted polymers: Surface response optimization of specific template recognition as a function of the type and degree of cross-linking. *Anal. Bioanal. Chem.* 389, 455–460. doi:10.1007/s00216-007-1484-8
- Scheutz, C., Kjeldsen, P., 2004. Environmental Factors Influencing Attenuation of Methane and Hydrochlorofluorocarbons in Landfill Cover Soils. *J. Environ. Qual.* 33, 72–79. doi:10.2134/jeq2004.7200
- Scheutz, C., Kjeldsen, P., 1997. Environmental Factors Influencing Attenuation of Methane and Hydrochlorofluorocarbons in Landfill Cover Soils.
- Scheutz, C., Kjeldsen, P., Bogner, J.E., De Visscher, A., Gebert, J., Hilger, H. a, Huber-Humer, M., Spokas, K., 2009. Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions. *Waste Manag. Res.* 27, 409–455. doi:10.1177/0734242X09339325
- Schumacher, B. a, 2002. Methods for the Determination of Total Organic Carbon in Soils and Sediments. *Carbon N. Y.* 32, 25.
- Shareefdeen, Z., Singh, A., 2005. Biotechnology for odor and air pollution control, *Biotechnology for Odor and Air Pollution Control.* doi:10.1007/b138434
- Sitaula, B.K., Bakken, L.R., Abrahamsen, G., 1995. CH₄ uptake by temperate forest soil: Effect of N input and soil acidification. *Soil Biol. Biochem.* 27, 871–880. doi:10.1016/0038-0717(95)00017-9
- Sly, L.I., Bryant, L.J., Cox, J.M., Anderson, J.M., 1993. Development of a biofilter for the removal

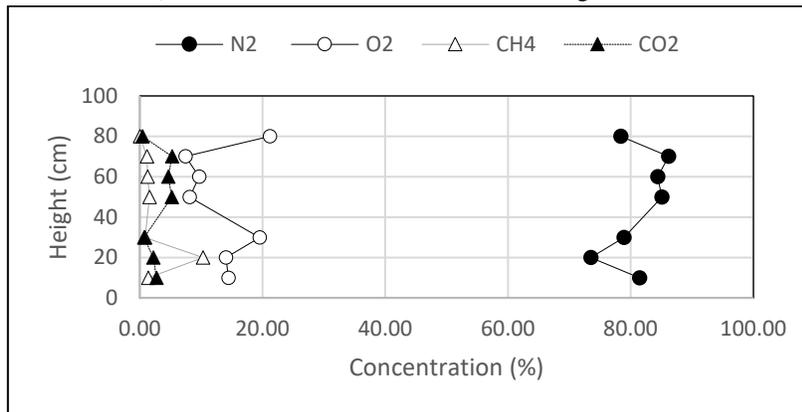
- of methane from coal mine ventilation atmospheres. *Appl. Microbiol. Biotechnol.* doi:10.1007/BF00192101
- Socolofsky, S.A., Jirka, G.H., 2005. Advective Diffusion Equation, in: CVEN 489-501: Special Topics in Mixing and Transport Processes in the Environment. p. 18.
- Stein, V.B., Hettiaratchi, J.P., 2001. Methane oxidation in three Alberta soils: influence of soil parameters and methane flux rates. *Environ. Technol.* 22, 101–111. doi:10.1080/09593332208618315
- Stein, V.B., Hettiaratchi, J.P.A., Achari, G., 2001. Numerical Model for Biological Oxidation and Migration of Methane in Soils. *Pract. Period. Hazardous, Toxic, Radioact. Waste Manag.* doi:10.1061/(ASCE)1090-025X(2001)5:4(225)
- Stuedler, P.A., Jones, R.D., Castro, M.S., Melillo, J.M., Lewis, D.L., 1996. Microbial Controls of Methane Oxidation in Temperate Forest and Agricultural Soils, in: Murrell, J.C., Kelly, D.P. (Eds.), *Microbiology of Atmospheric Trace Gases: Sources, Sinks and Global Change Processes*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 69–84. doi:10.1007/978-3-642-61096-7_5
- Streese, J., Stegmann, R., 2003. Microbial oxidation of methane from old landfills in biofilters. *Waste Manag.* 23, 573–580. doi:10.1016/S0956-053X(03)00097-7
- Sullivan, D.M., Miller, R.O., 2001. *Compost quality attributes, measurements, and variability*. CRC Press, Boca Raton, FLorida.
- Swanson, W.J., Loehr, R.C., 1997. Biofiltration: Fundamentals, Design and Operations Principles, and Applications. *J. Environ. Eng.* doi:10.1061/(ASCE)0733-9372(1997)123:6(538)
- Switzenbaum, M.S., Moss, L.H., Epstein, E., Pincince, A.B., Donovan, J.F., 1997. Defining Biosolids Stability. *J. Environ. Eng.* 123, 1178–1184.
- Tchobanoglous, G., Franklin, L.B., Stensel, D., 2003. *Wastewater engineering: treatment and reuse*, 4th ed. Metcalf and Eddi Inc., New York.
- Terry, S.C., Jerman, J.H., Angell, J.B., 1979. A gas chromatographic air analyzer fabricated on a silicon wafer. *IEEE Trans. Electron Devices* 26, 1880–1886. doi:10.1109/T-ED.1979.19791
- Tiquia, S.M., 2005. Microbiological parameters as indicators of compost maturity. *J. Appl. Microbiol.* 99, 816–828. doi:10.1111/j.1365-2672.2005.02673.x
- U.S. Environmental Protection Agency, 2001. METHOD 1683 Specific Oxygen Uptake Rate in Biosolids. *Sci. Technol.*
- Van Groenestijn, J.W., Hesselink, P.G.M., 1993. Biotechniques for air pollution control Existing biotechniques. *Biodegradation* 4, 283–301.
- vanBavel, C.H.M., 1952. Gaseous Diffusion and Porosity in Porous Media. *Soil Sci.*
- Veillette, M., Avalos Ramirez, A., Heitz, M., 2012. Biofiltration of air polluted with methane at concentration levels similar to swine slurry emissions: influence of ammonium concentration. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* 47, 1053–64. doi:10.1080/10934529.2012.667327

- Venhaus, H.L., 2012. *Designing the Sustainable Site : Integrated Design Strategies for Small Scale Sites and Residential Landscapes*. Wiley, Hoboken, US.
- Wallace, P., 2005. *Assessment of options and requirements for stability and maturity testing of composts*. The waste and Resources Action Programme, Banbury, Oxon.
- Wang, L.K., Norman, C.P., Hung, Y.-T., 2009. *Biological Treatment Processes, Journal of Chemical Information and Modeling*. Humana Press, New York, NY. doi:10.1017/CBO9781107415324.004
- Wani, A.H., Branion, R.M.R., Lau, A.K., 1997. Biofiltration: A promising and cost-effective control technology for Odors, VOCs and air toxics. *J. Environ. Sci. Heal. . Part A Environ. Sci. Eng. Toxicol.* doi:10.1080/10934529709376664
- Welty, J.R., Wicks, C.E., Rorrer, G., Wilson, R.E., 1984. *Fundamentals of momentum, heat, and mass transfer*, 3rd ed. John Wiley and Sons, New York.
- Whalen, S.C., Reeburgh, W.S., Sandbeck, K. a., 1990. Rapid Methane Oxidation in a Landfill Cover Soil. *Appl. Environ. Microbiol.* 56, 3405–3411.
- White, D., 1996. The physiology and biochemistry of prokaryotes. *Gen. Pharmacol. Vasc. Syst.* 27, 1077. doi:10.1016/0306-3623(96)90070-1
- Whittenbury, R., Dalton, H., 1981. The Methylophilic Bacteria, in: Starr, M.P., Stolp, H., Trüper, H.G., Balows, A., Schlegel, H.G. (Eds.), *The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 894–902. doi:10.1007/978-3-662-13187-9_71
- Wichuk, K.M., McCartney, D., 2010. Compost stability and maturity evaluation — a literature review. *Can. J. Civ. Eng.* 37, 1505–1523. doi:10.1139/L10-101
- Wilshusen, J.H., Hettiaratchi, J.P.A., De Visscher, A., Saint-Fort, R., 2004. Methane oxidation and formation of EPS in compost: effect of oxygen concentration. *Environ. Pollut.* 129, 305–314. doi:10.1016/j.envpol.2003.10.015
- Wilshusen, J.H., Hettiaratchi, J.P.A., Stein, V.B., 2004. Long-term behavior of passively aerated compost methanotrophic biofilter columns. *Waste Manag.* 24, 643–653. doi:10.1016/j.wasman.2003.12.006
- Woertz, J., Van Heiningen, W., Van Eekert, M., Kraakman, N., Kinney, K., Van Groenestijn, J., 2002. Dynamic bioreactor operation: Effects of packing material and mite predation on toluene removal from off-gas. *Appl. Microbiol. Biotechnol.* 58, 690–694. doi:10.1007/s00253-002-0944-5
- Xu, X., Gupta, J.L.N.S.C., 1992. Compaction Effect on the Gas Diffusion Coefficient in Soils. *Soil Sci. Soc. Am. J* 56, 1743–1750.
- Zheng, C., Bennett, G.D., 1995. *Applied Contaminant Transport Modeling: theory and practice*, second ed. ed. Van Nostrand Reinhold, New York.

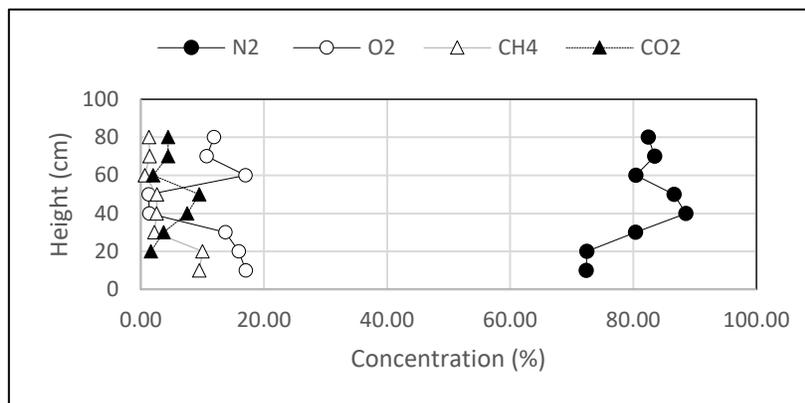
Appendix A : Concentration Profiles for columns run at stoichiometric air flow rates



a) Concentration Profiles-Column 3- August 13th

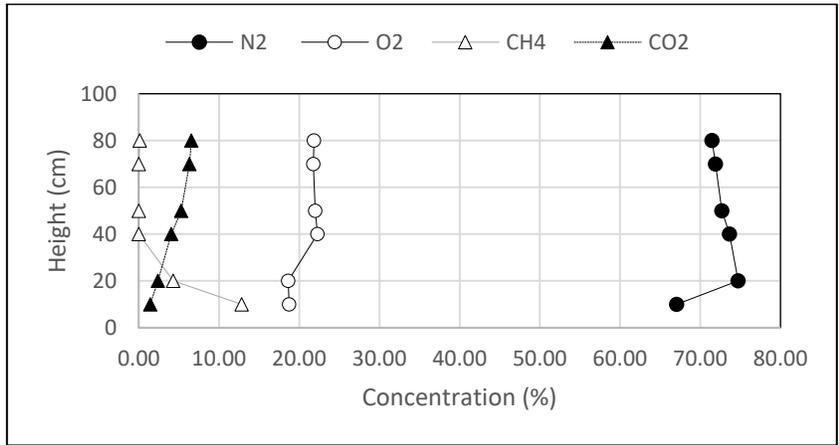


b) Concentration Profiles-Column 2- August 13th

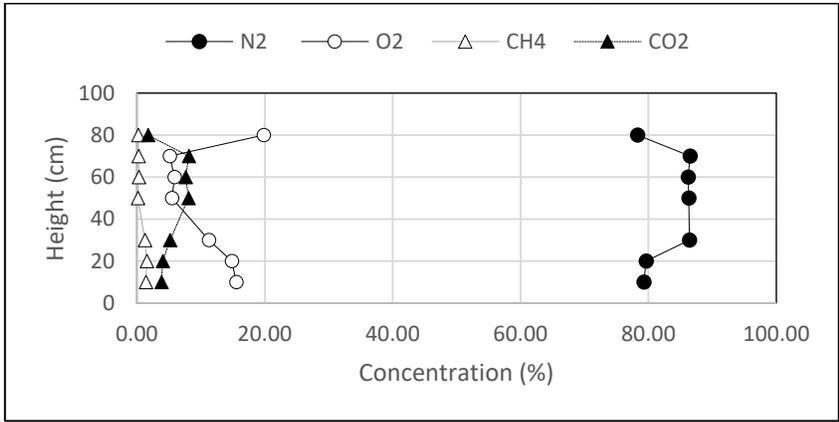


c) Concentration Profiles-Column 1- August 13th

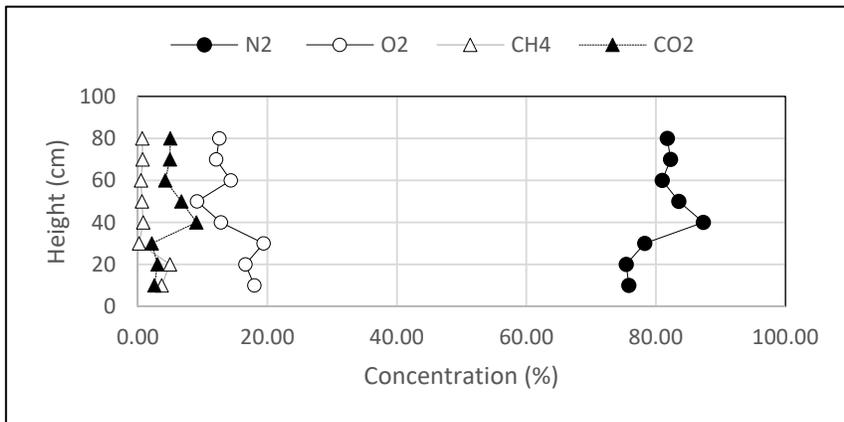
Figure A-1 Gas profiles for August 13th (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- August 16th

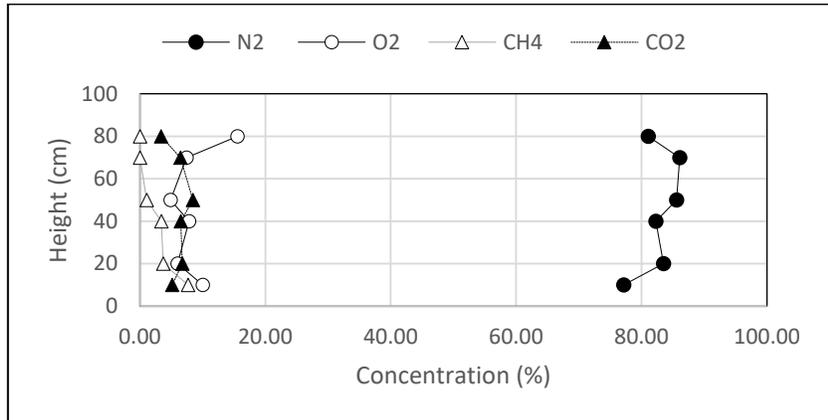


b) Concentration Profiles-Column 2- August 16th

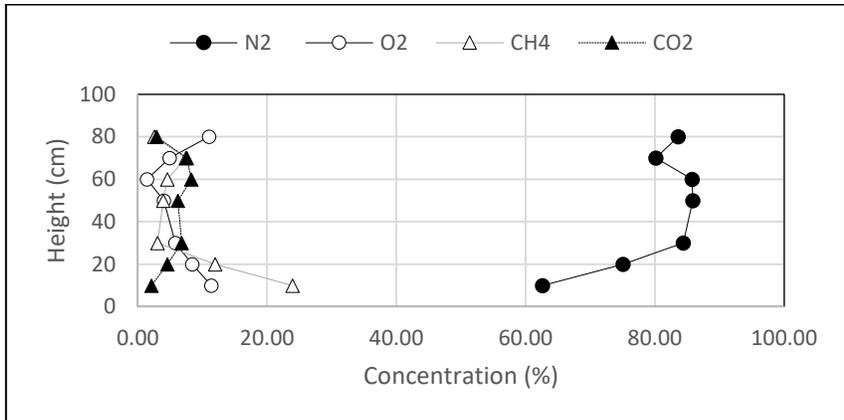


c) Concentration Profiles-Column 1- August 16th

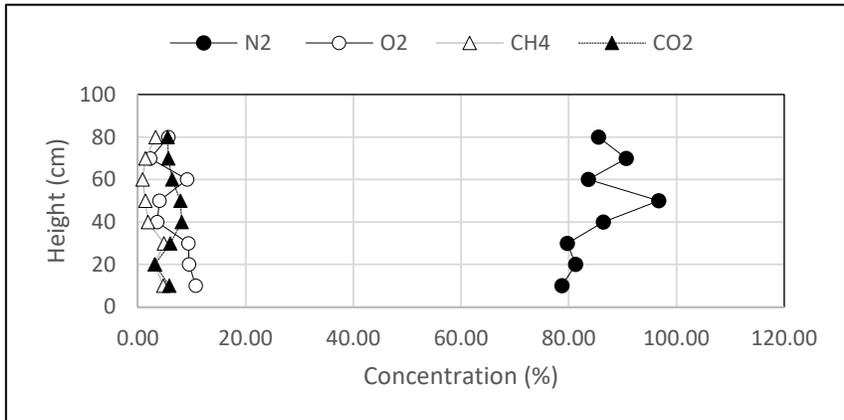
Figure A-2 Gas profiles for August 16th (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3-August 19th

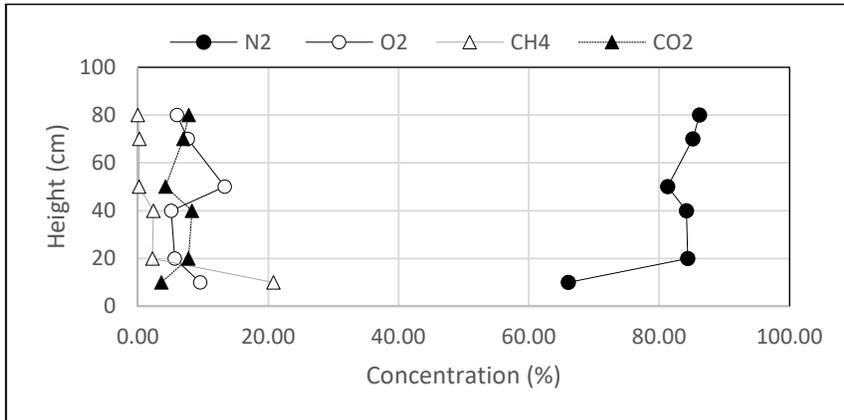


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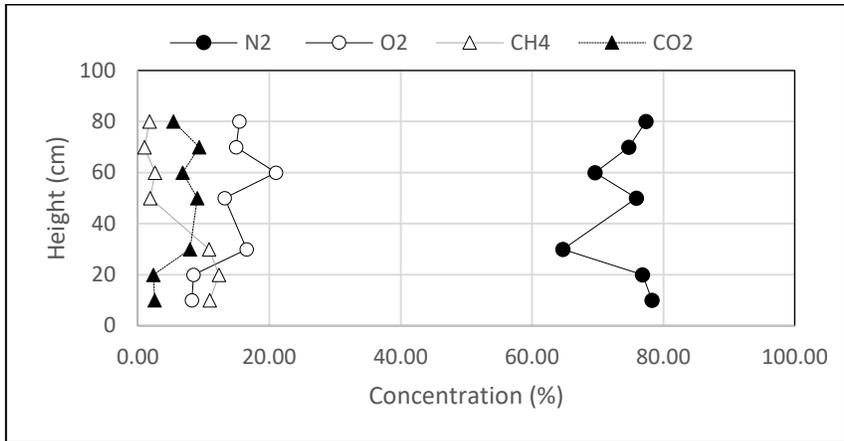


c) Concentration Profiles-Column 1- August 19th

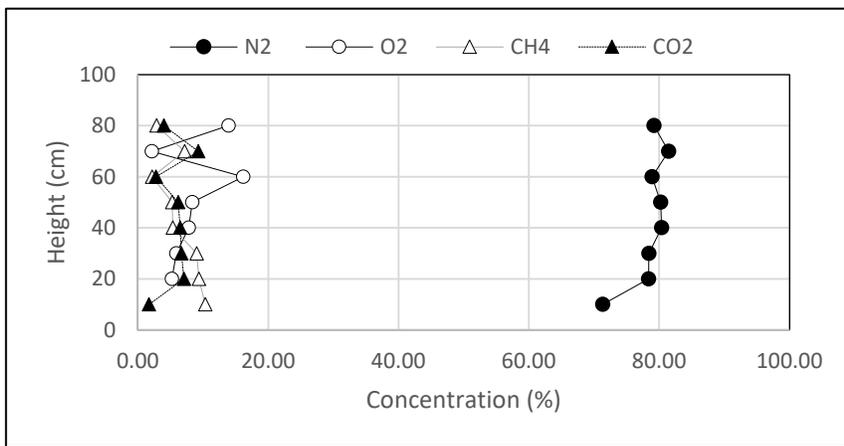
Figure A-3 Gas profiles for August 19th (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- September 10th

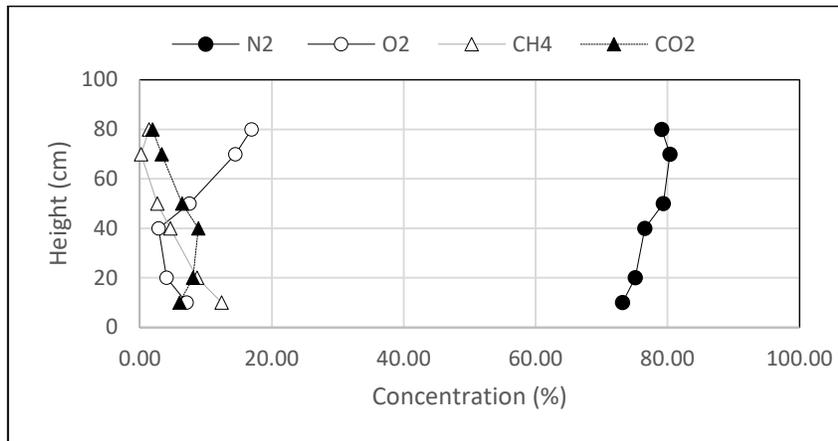


b) Concentration Profiles-Column 2- September 10th

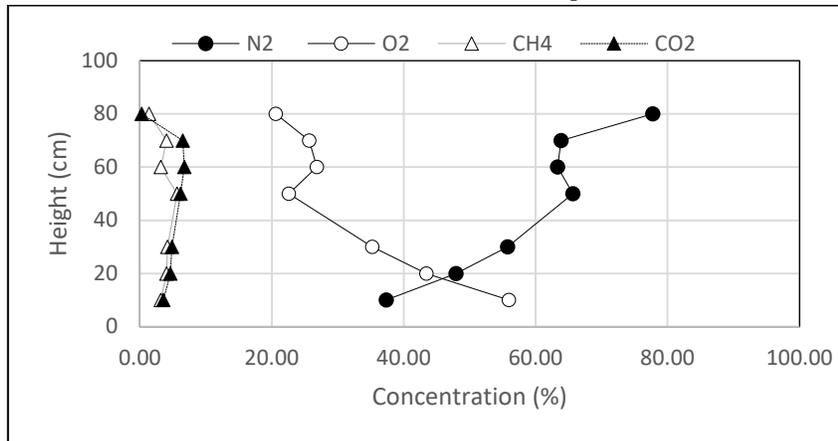


c) Concentration Profiles-Column 1- September 10th

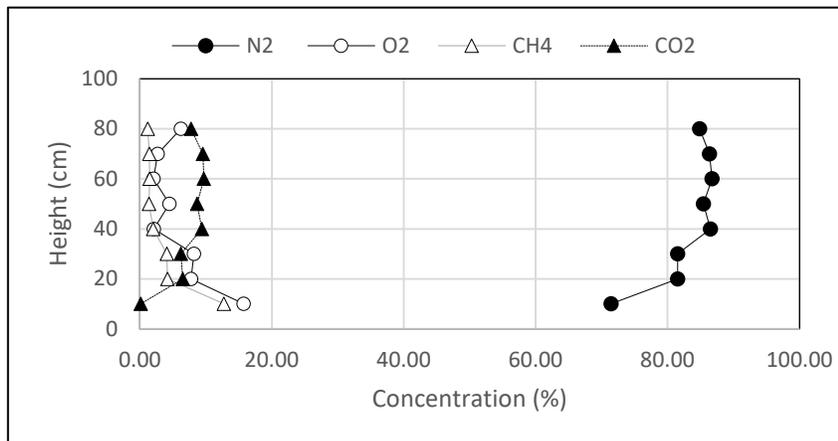
Figure A-4 Gas profiles for September 10th (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- September 16th

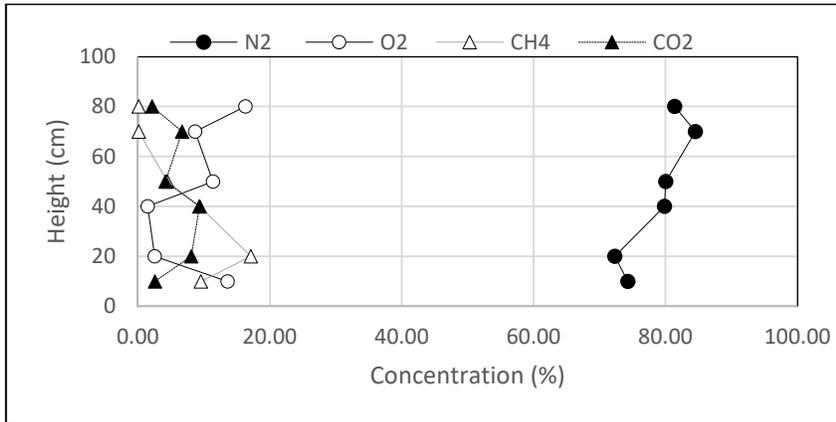


b) Concentration Profiles-Column 2-September 16th

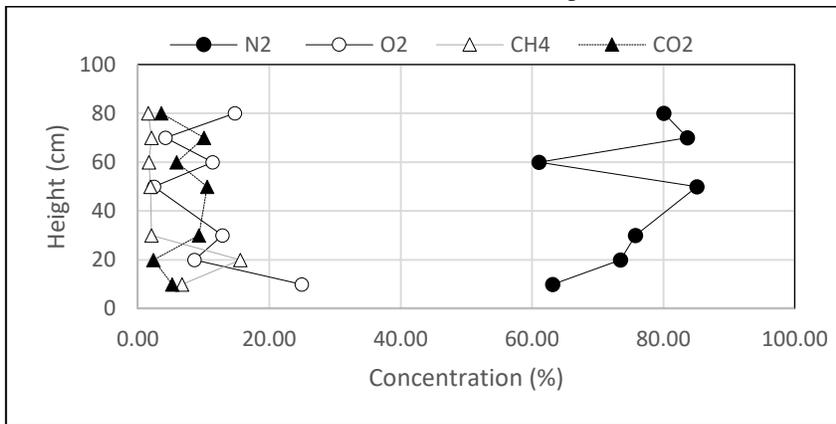


c) Concentration Profiles-Column 1- September 16th

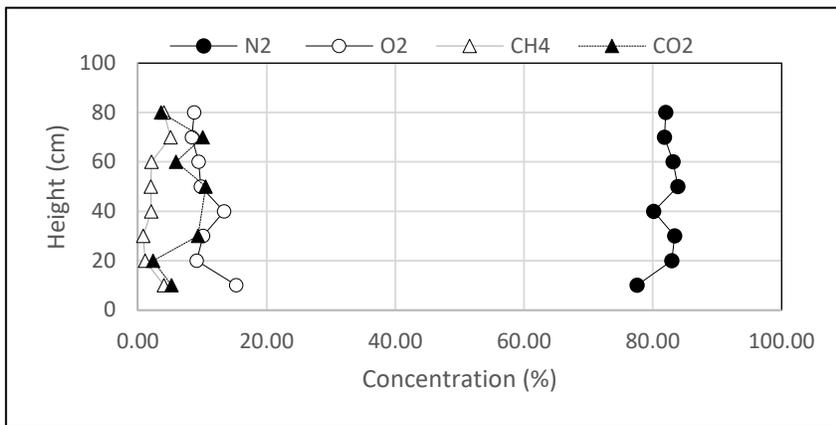
Figure A-5 Gas profiles for September 16th (first stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- September 25th

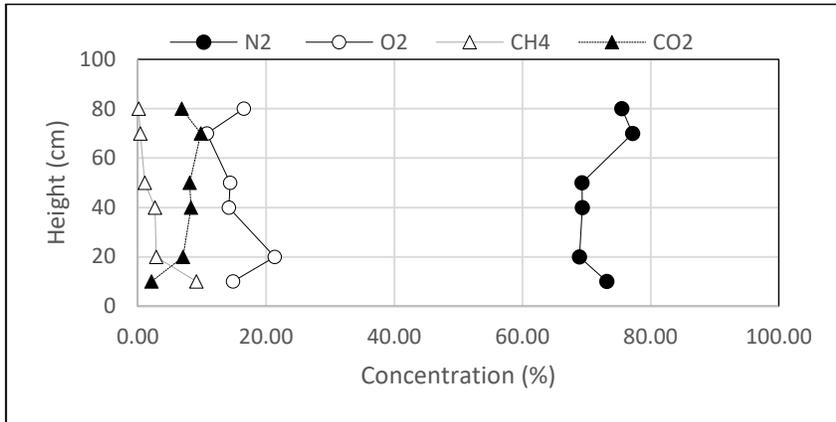


b) Concentration Profiles-Column 2- September 25th

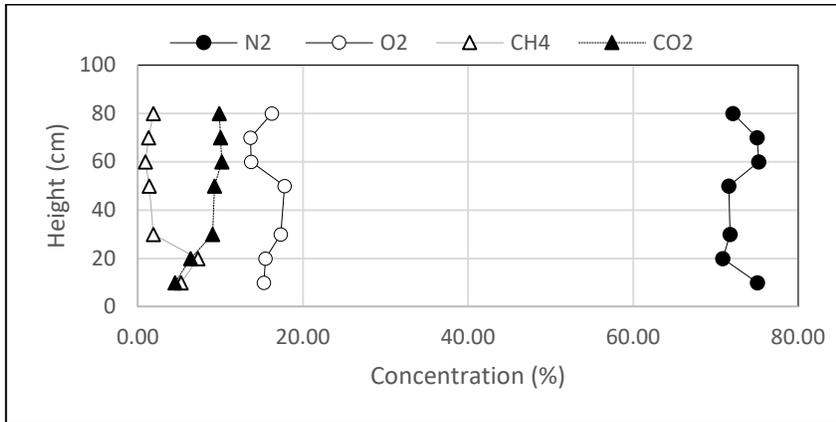


c) Concentration Profiles-Column 1- September 25th

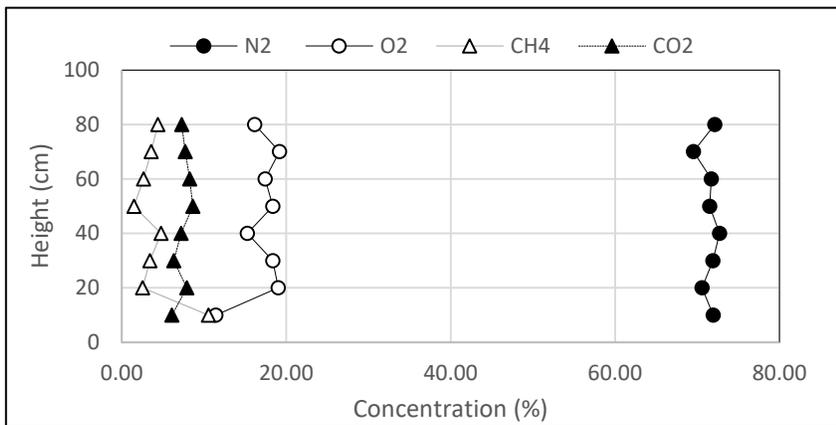
Figure A-6 Gas profiles for September 25th (second stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- October 1st

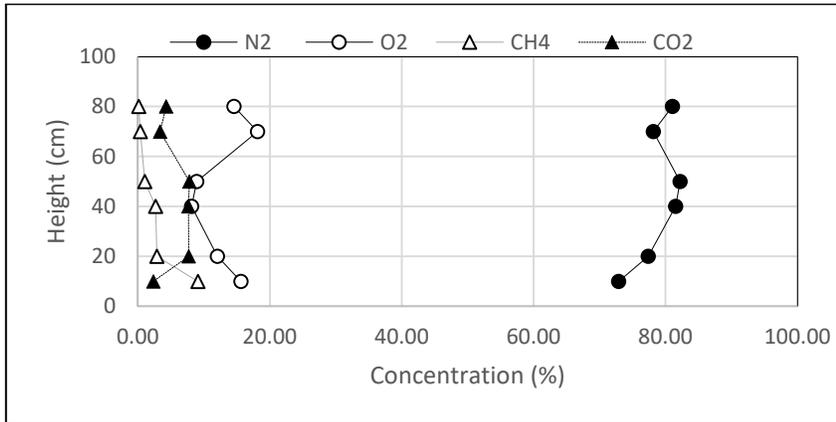


b) Concentration Profiles-Column 2- October 1st

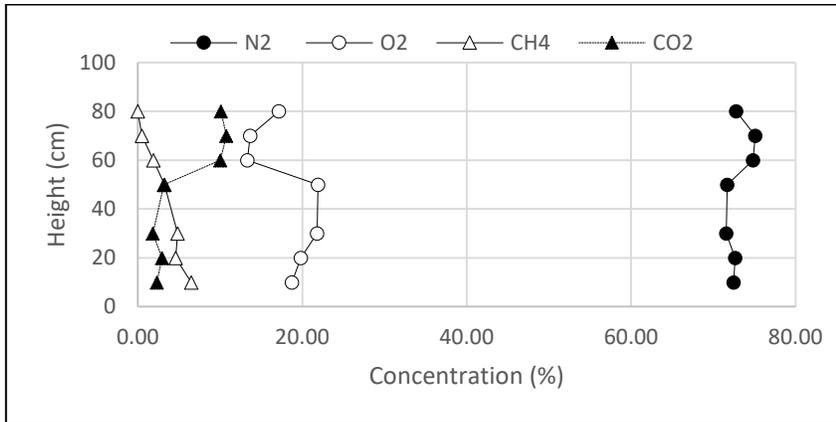


c) Concentration Profiles-Column 1- October 1st

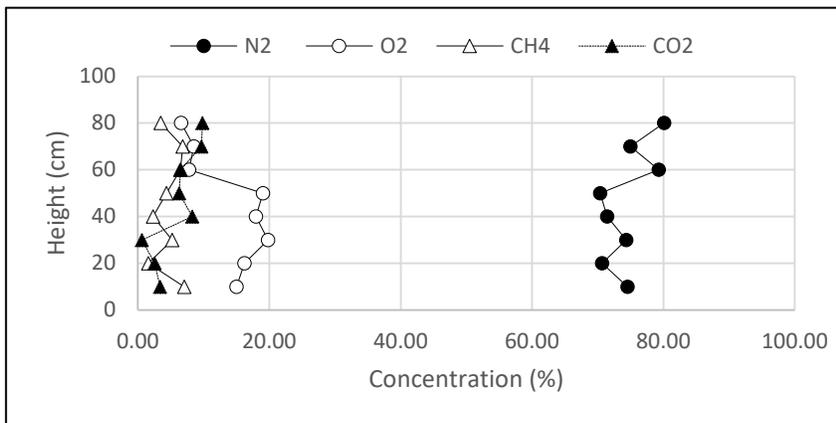
Figure A-7 Gas profiles for October 1st (second stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- October 8th

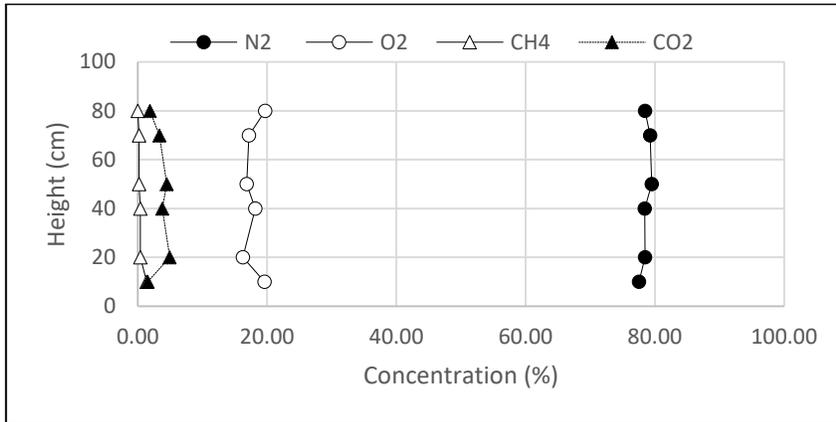


b) Concentration Profiles-Column 2- October 8th

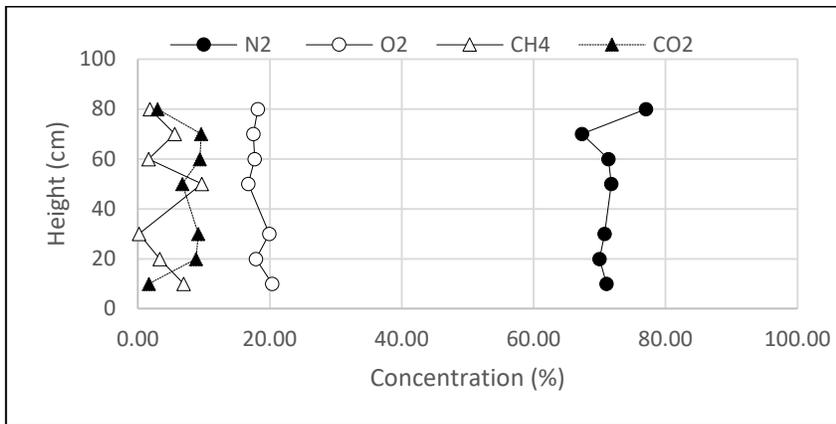


c) Concentration Profiles-Column 1- October 8th

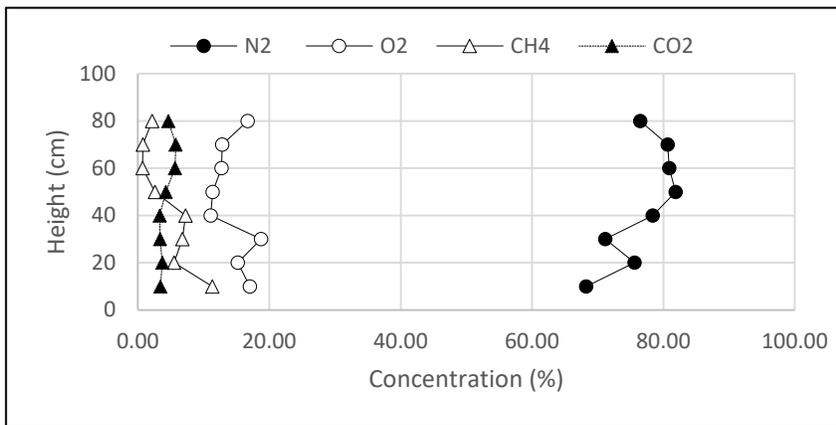
Figure A-8 Gas profiles for October 8th (second stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- October 13th

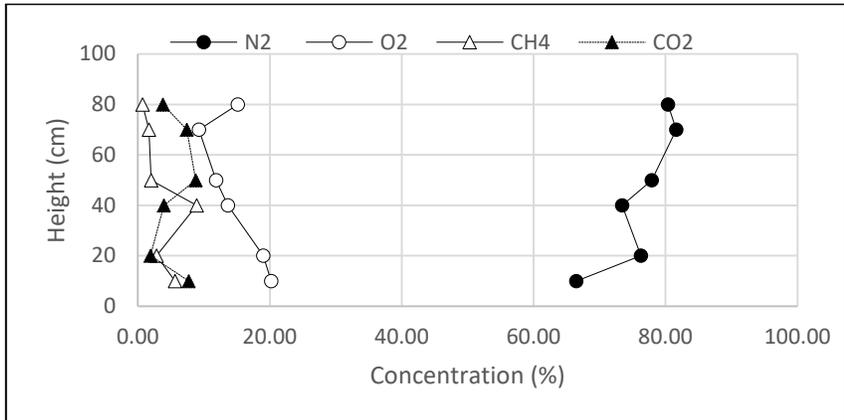


b) Concentration Profiles-Column 2- October 13th

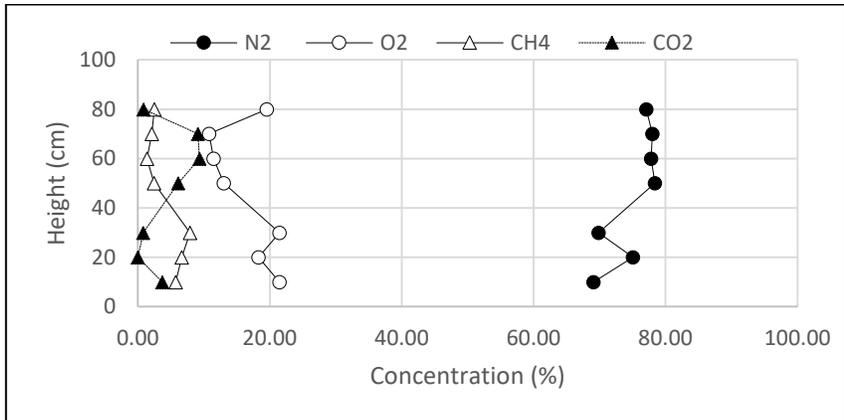


c) Concentration Profiles-Column 1- October 13th

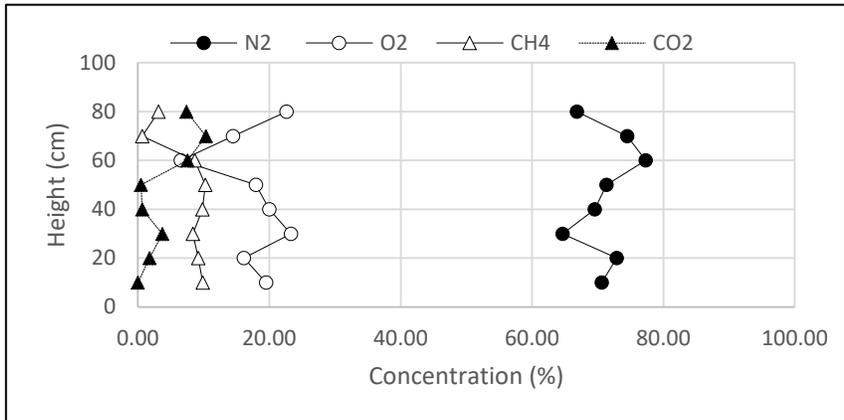
Figure A-9 Gas profiles for October 13th (second stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- November 13th

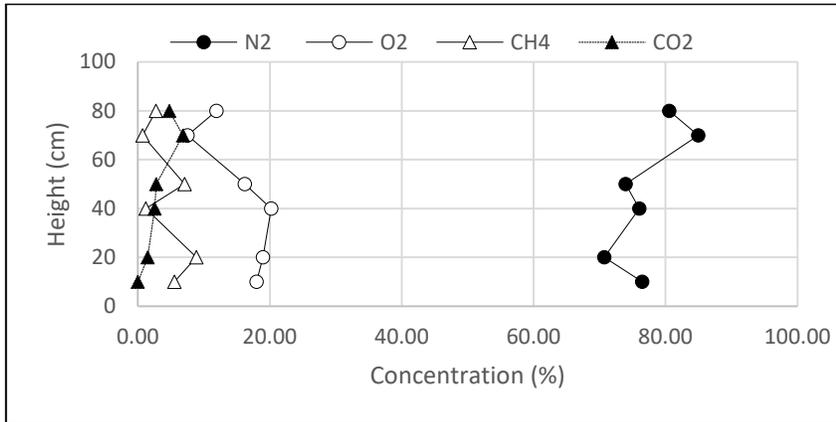


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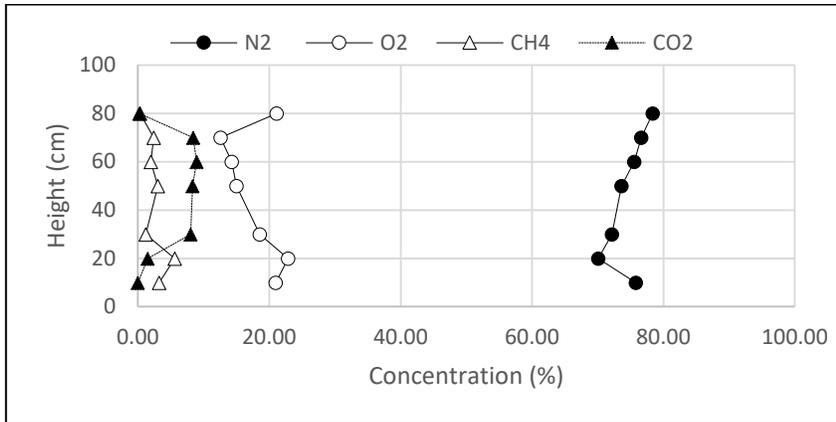


c) Concentration Profiles-Column 1- November 13th

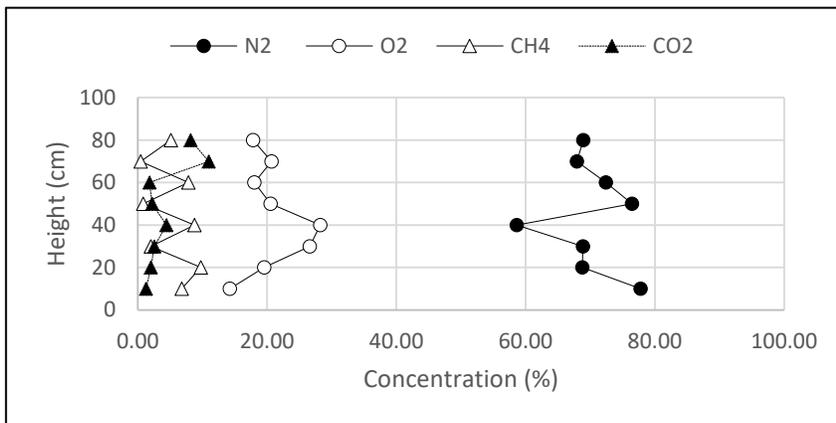
Figure A-10 Gas profiles for November 13th (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- November 18th

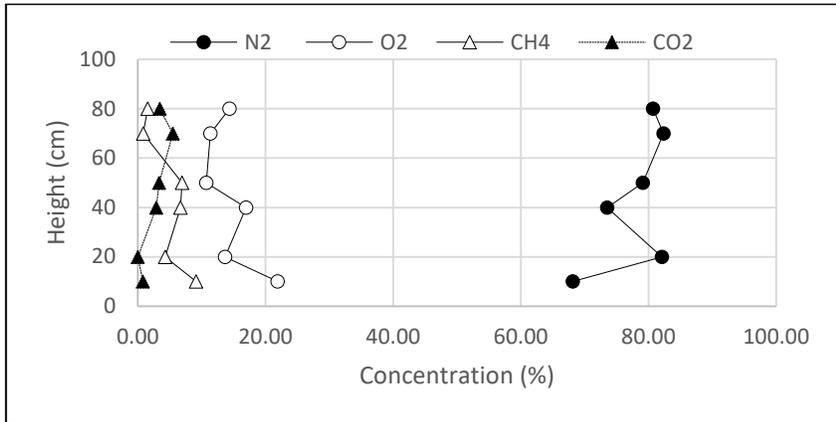


b) Concentration Profiles-Column 2- November 18th

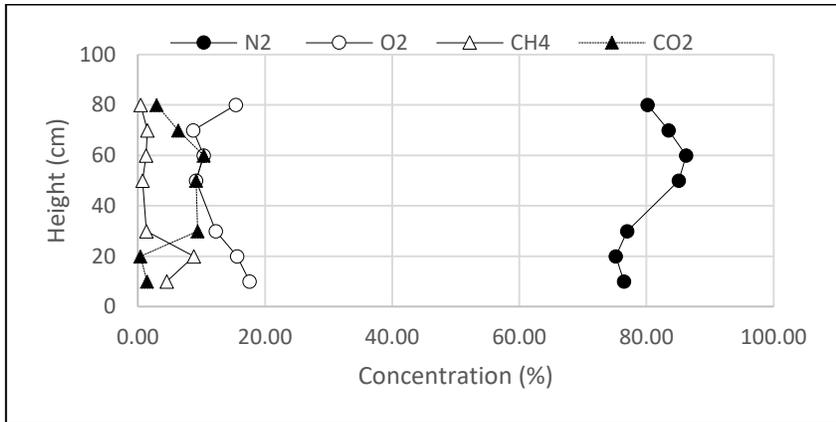


c) Concentration Profiles-Column 1- November 18th

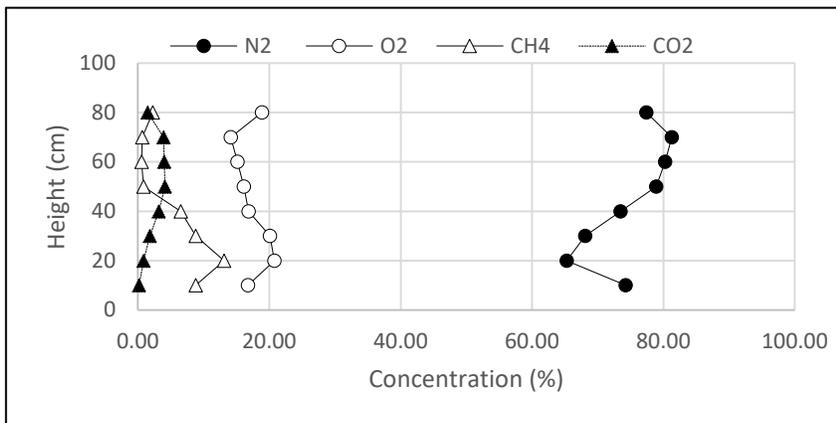
Figure A-11 Gas profiles for November 18th (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- November 24th

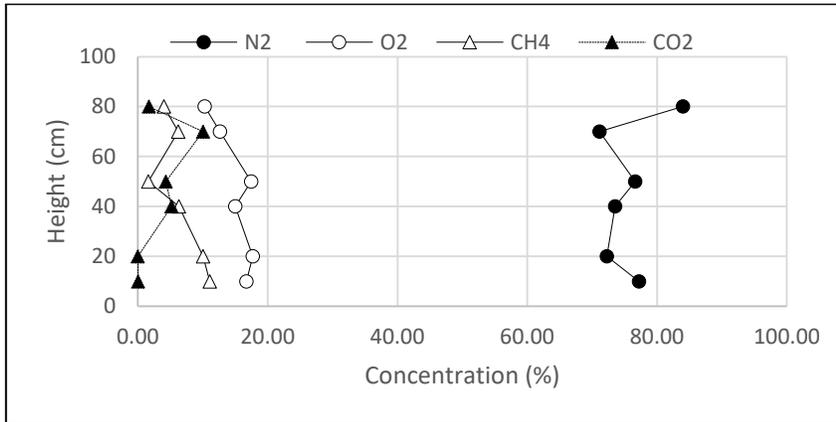


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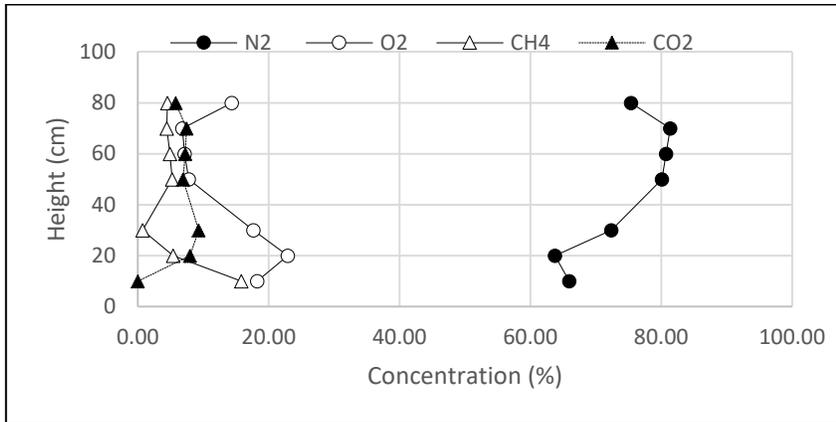


c) Concentration Profiles-Column 1- November 24th

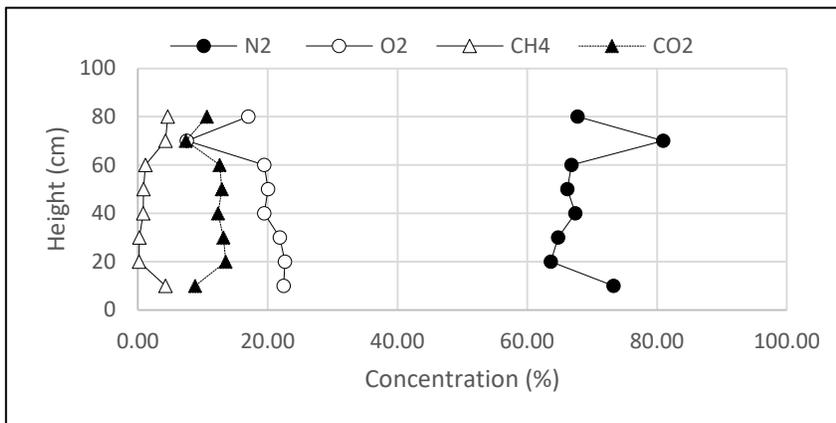
Figure A-12 Gas profiles for November 24th (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- November 30th

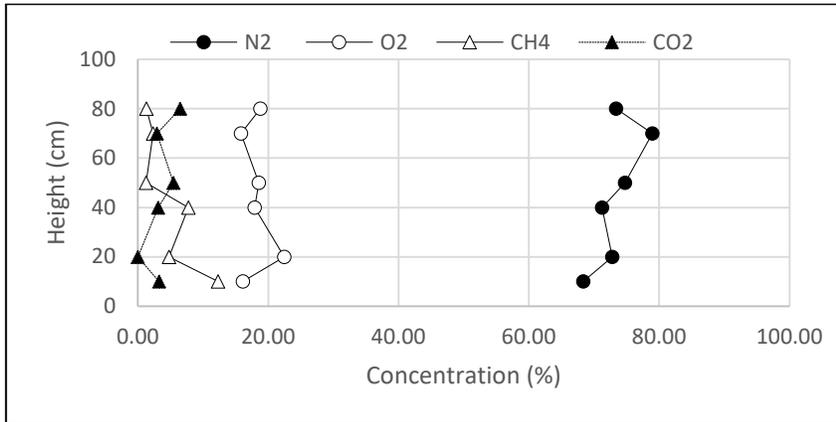


b) Concentration Profiles-Column 2- November 30th

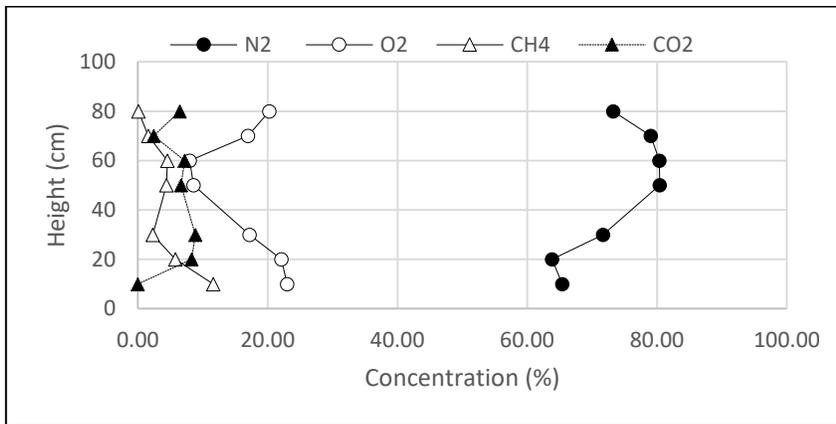


c) Concentration Profiles-Column 1- November 30th

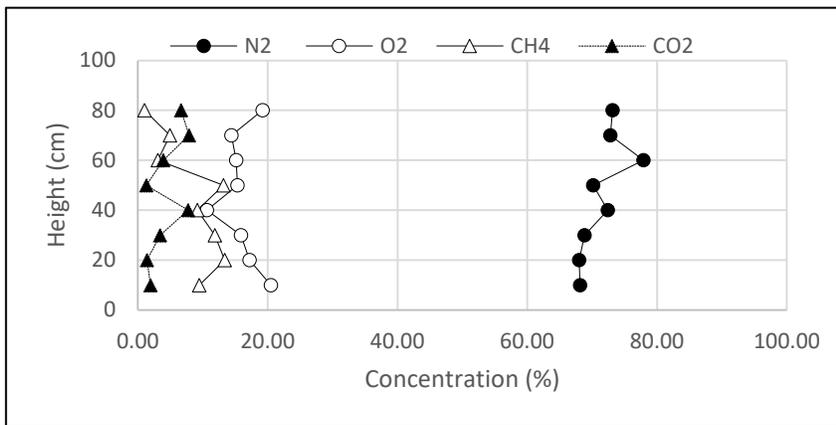
Figure A-13 Gas profiles for November 30th (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- December 4th

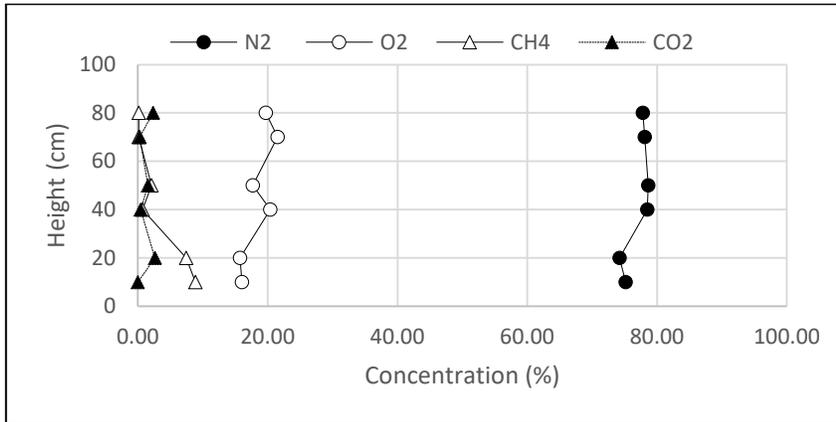


b) Concentration Profiles-Column 2- December 4th

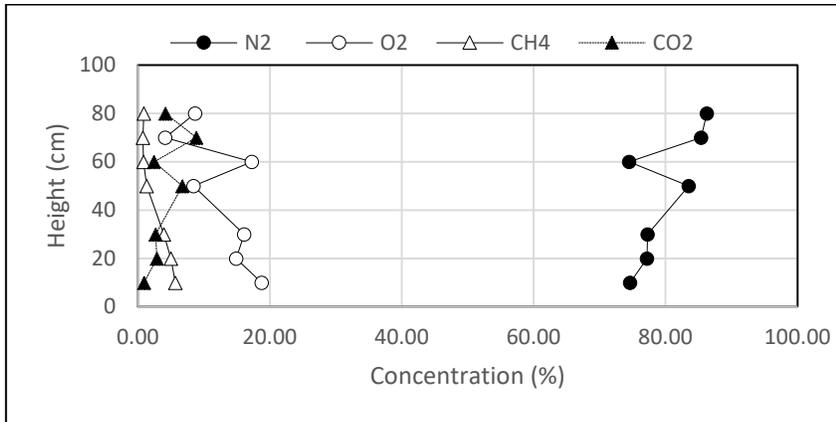


c) Concentration Profiles-Column 1- December 4th

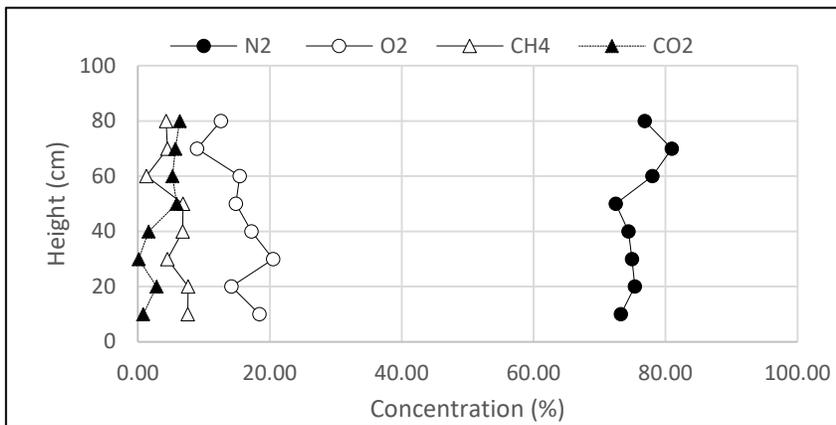
Figure A-14 Gas profiles for December 4th (third stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- December 21st

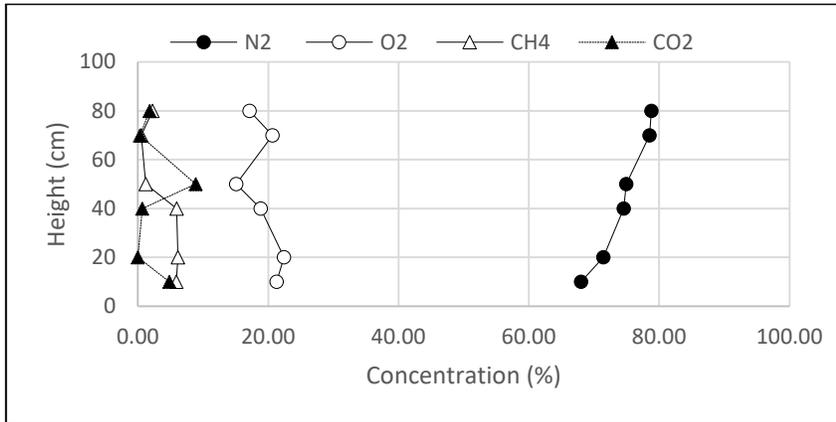


b) Concentration Profiles-Column 2- December 21st

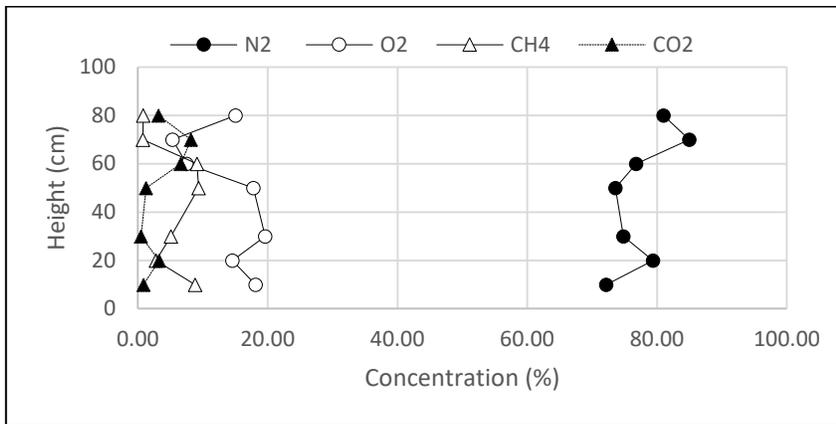


c) Concentration Profiles-Column 1- December 21st

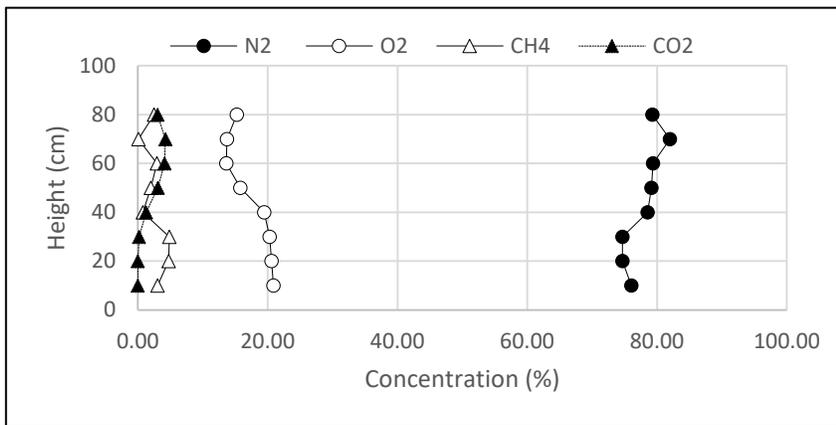
Figure A-15 Gas profiles for December 21st (fourth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- December 27th

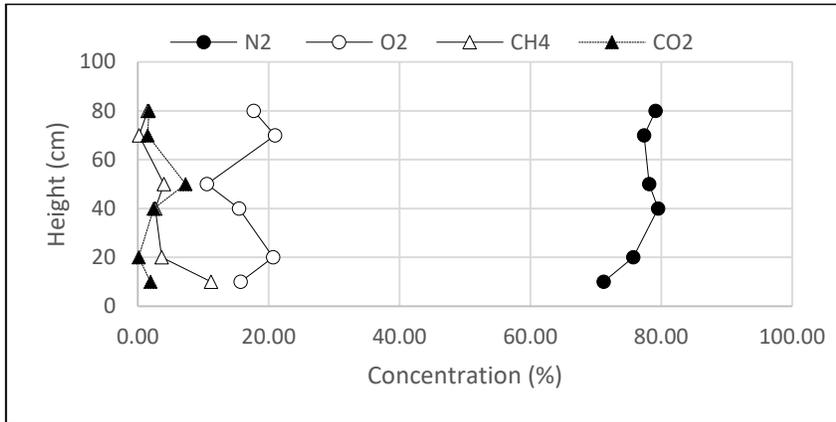


b) Concentration Profiles-Column 2- December 27th

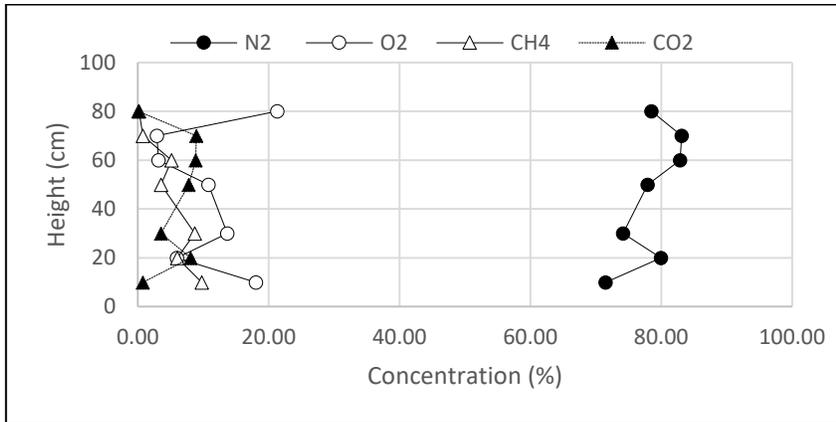


c) Concentration Profiles-Column 1- December 27th

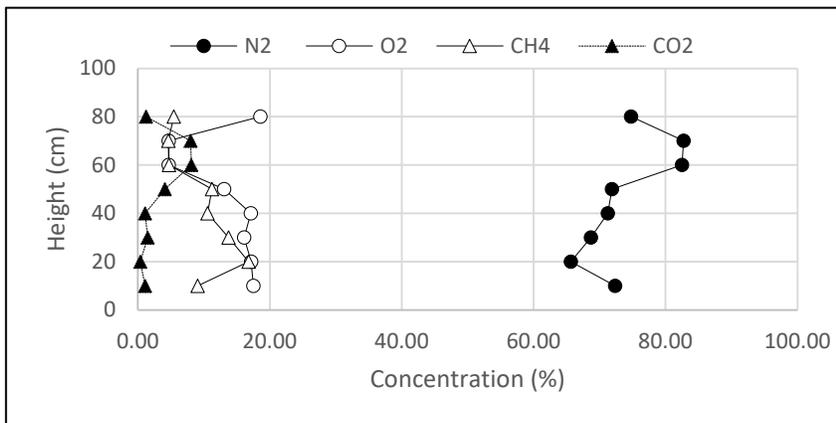
Figure A-16 Gas profiles for December 27th (fourth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- January 18th

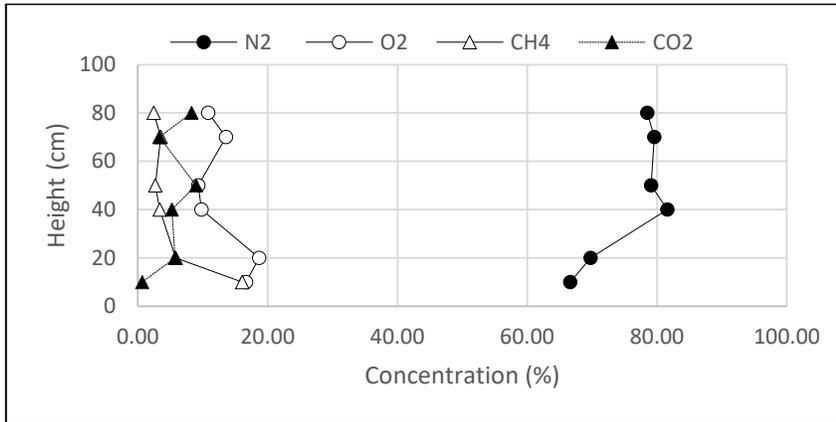


b) Concentration Profiles-Column 2- January 18th

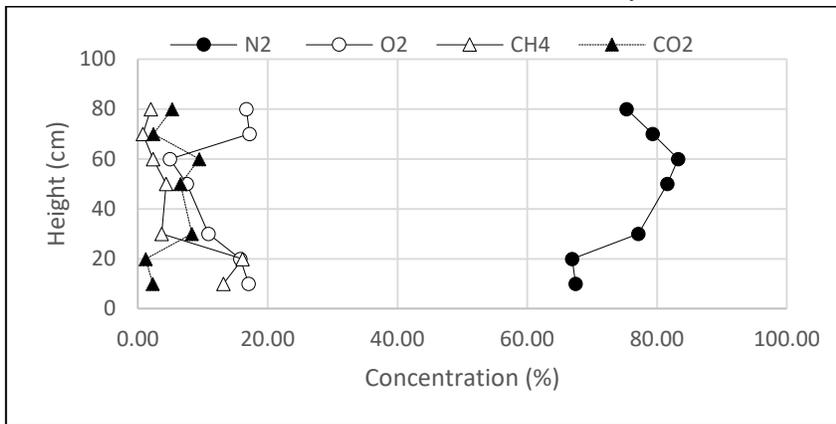


c) Concentration Profiles-Column 1- January 18th

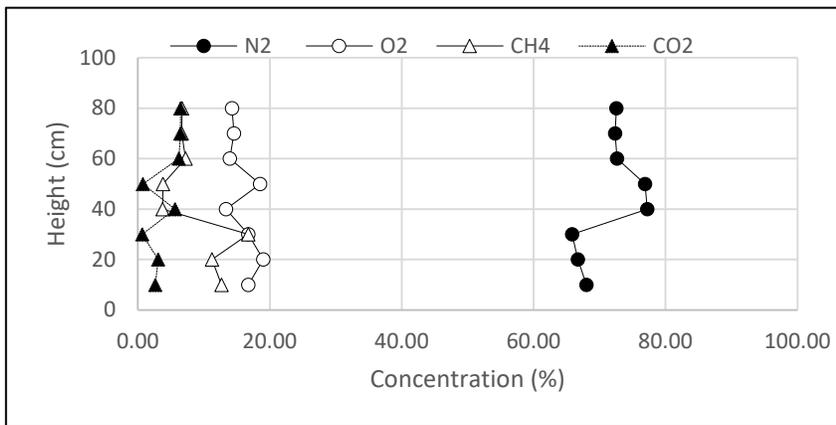
Figure A-17 Gas profiles for January 18th (fourth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- January 21st

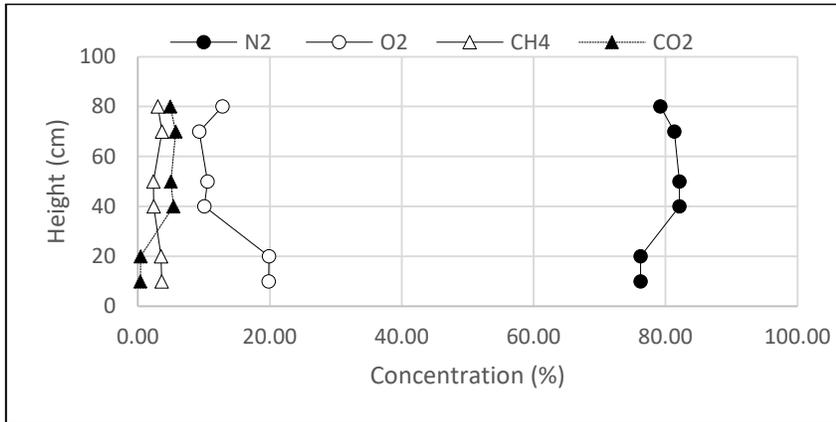


b) Concentration Profiles-Column 2- January 21st

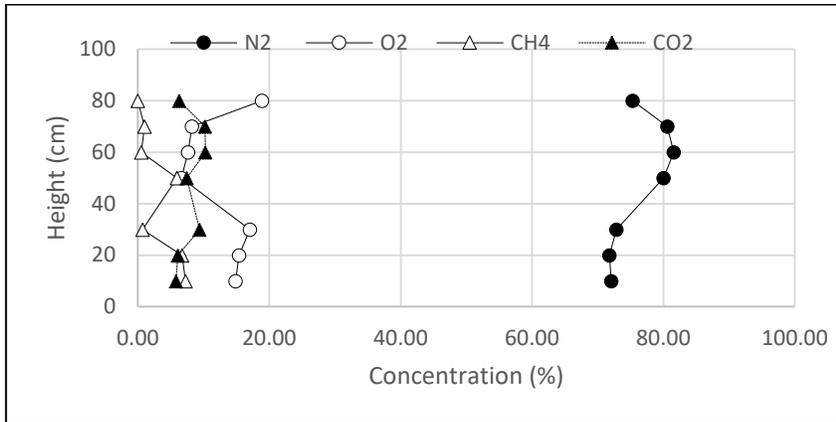


c) Concentration Profiles-Column 1- January 21st

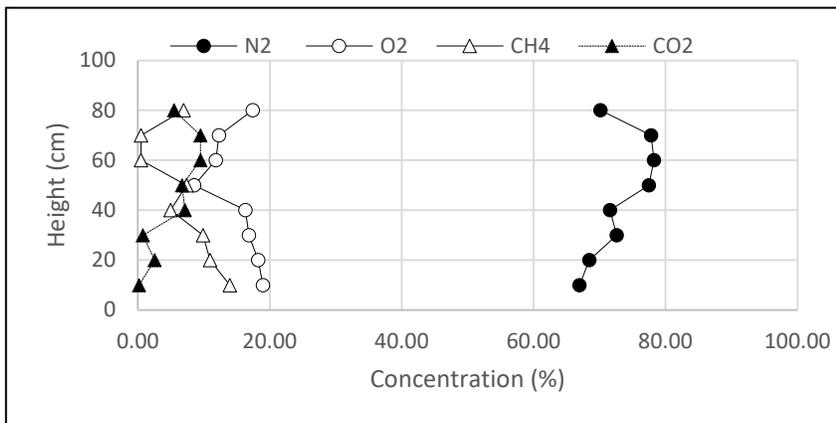
Figure A-18 Gas profiles for January 21st (fourth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- January 26th

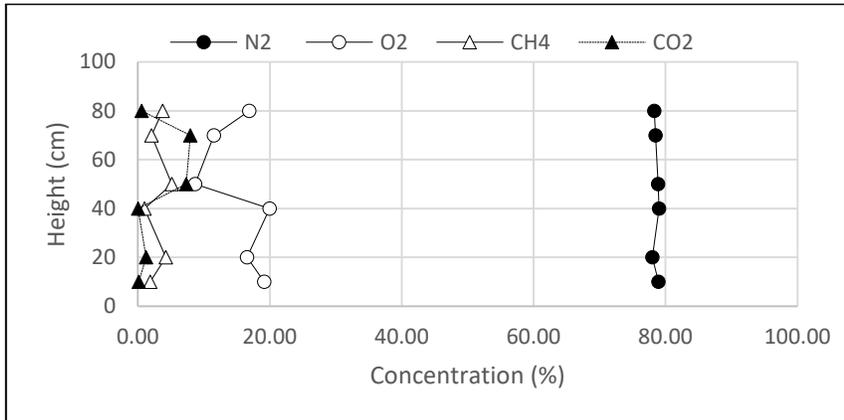


b) Concentration Profiles-Column 2- January 26th

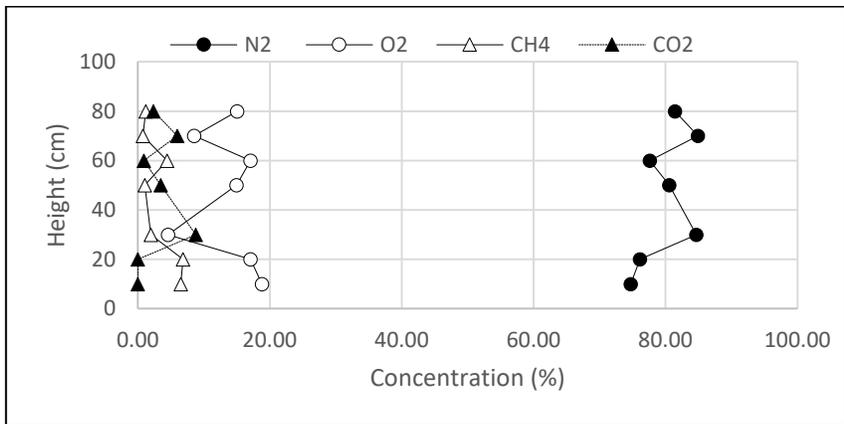


c) Concentration Profiles-Column 1- January 26th

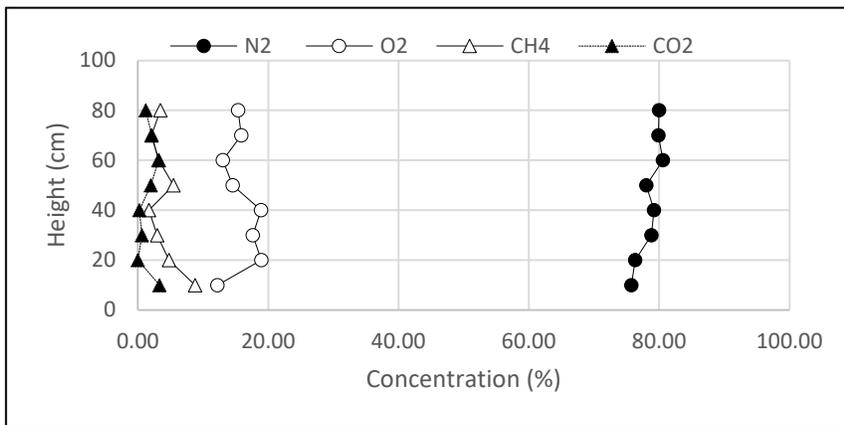
Figure A-19 Gas profiles for January 26th (fifth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- March 26th

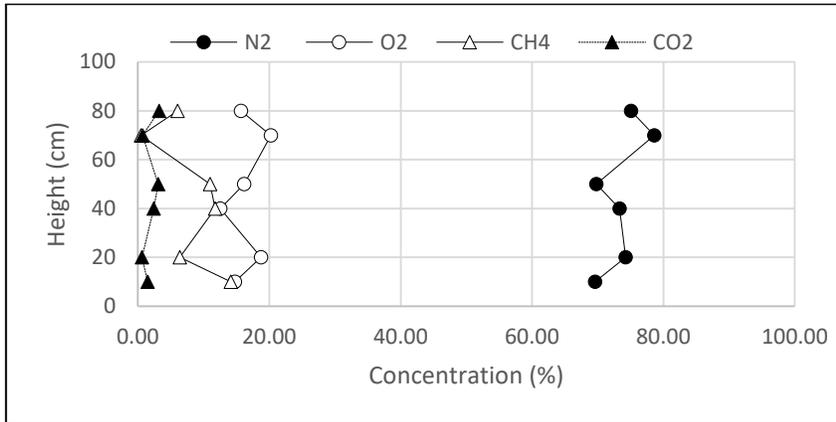


b) Concentration Profiles-Column 2- March 26th

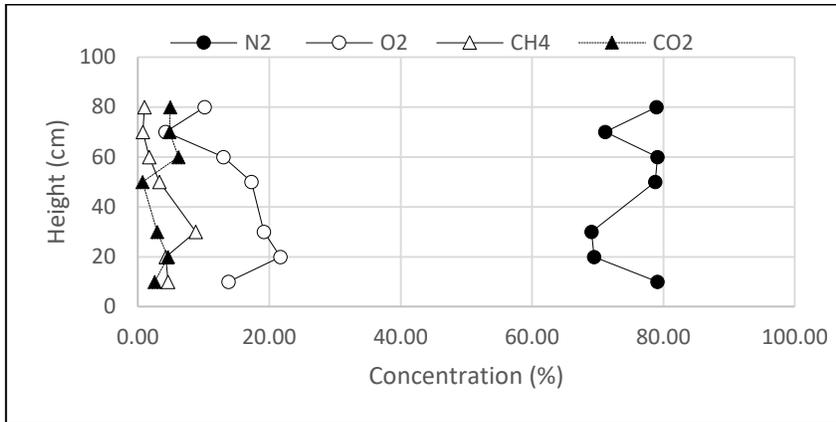


c) Concentration Profiles-Column 1- March 26th

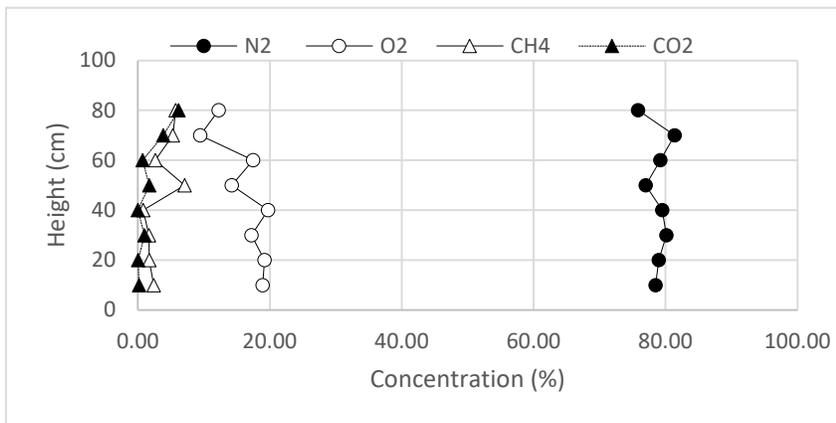
Figure A-20 Gas profiles for March 26th (fifth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- March 28th

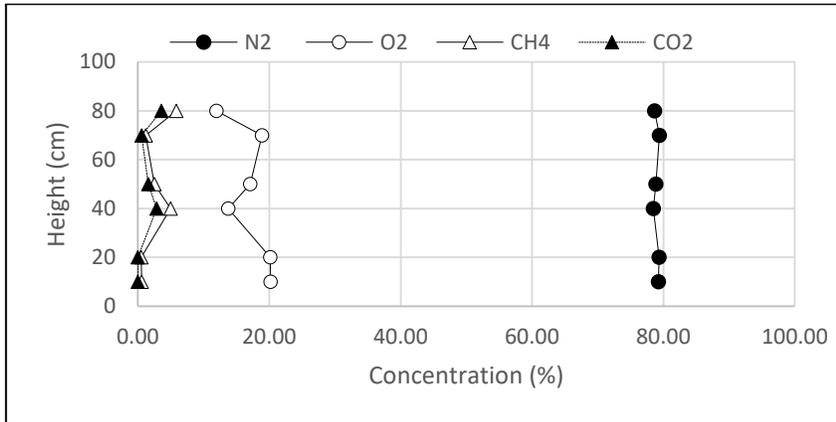


b) Concentration Profiles-Column 2- March 28th

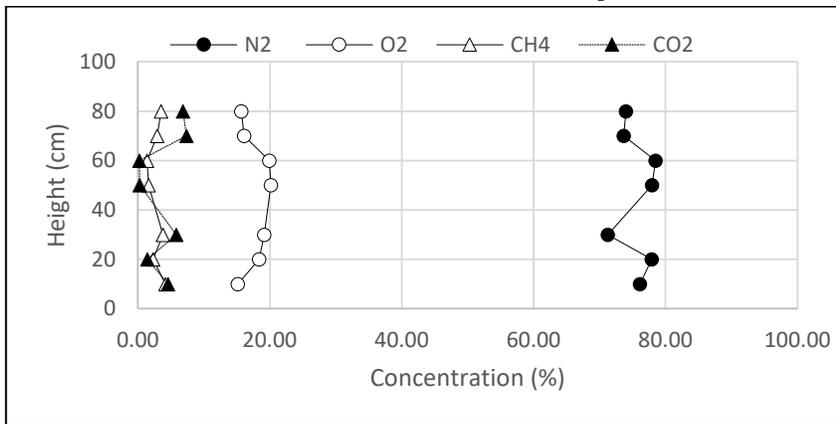


c) Concentration Profiles-Column 1- March 28th

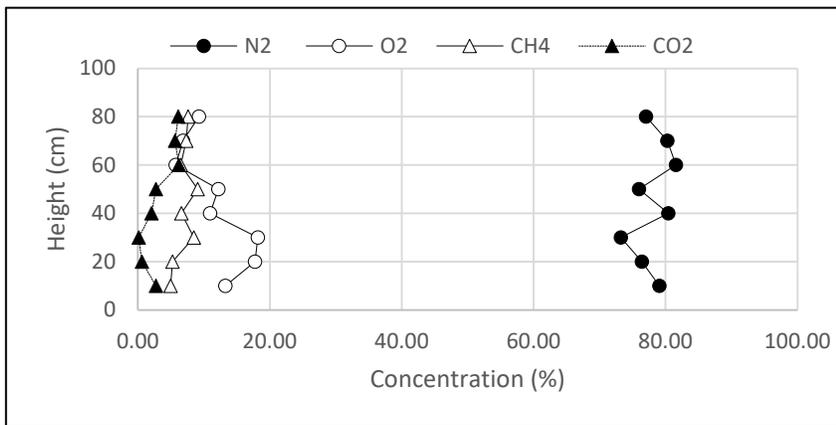
Figure A-21 Gas profiles for March 28th (fifth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level



a) Concentration Profiles-Column 3- April 1st



b) Concentration Profiles-Column 2- April 1st



c) Concentration Profiles-Column 1- April 1st

Figure A-22 Gas profiles for April 1st (fifth stage) for a) Column aerated at three levels b) Column aerated at two levels c) Column aerated at one level

9.4Thesis: Development of Alternative Medium to Sustain Methanotrophs in Methane Biofilters

UNIVERSITY OF CALGARY

Development of Alternative Medium to Sustain Methanotrophs in Methane Biofilters

by

Jesica Goya Sanchez

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
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CALGARY, ALBERTA

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Abstract

Methane biofiltration is a cost-effective technology that reduces the effect of greenhouse gases in the atmosphere through bacterial conversion of methane into carbon dioxide without producing harmful by-products. It is very important that media in which the bacteria live possesses certain characteristics that allows them to grow properly. Compost has been widely used, but it has disadvantages such as instability and compaction issues. The aim of this work was to investigate the performance of flax straw, wood shavings, and lava rock (with and without nutrient addition) used in a mixture along with compost looking to provide better conditions for methanotrophs to thrive and achieve high methane oxidation rates with little degradation from the media. Through laboratory column experiments we found the best material to be the compost:wood shavings mixture in a 30:70 ratio at 70% of FC, with 89% removal efficiency.

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Special thanks to Daniel Larson and Hasti Farrokhzadeh for their technical help during the experimentation stage of this work and for their invaluable friendship.

Finally, I wish to thank all my friends who supported me in overcoming this difficult stage.

Dedication

I dedicate this thesis to two of my greatest supporters who stood by me through thick and thin, my mom, Yolanda and my life partner, Yaneri. This is also for my dad, Daniel, who in the background has always kept an eye on me.

Table of Contents

Abstract	i
Acknowledgements	ii
Dedication	iii
Table of Contents	iv
List of Tables	vi
List of Figures and Illustrations	vii
List of Symbols, Abbreviations and Nomenclature	ix
CHAPTER ONE: INTRODUCTION	1
1.1 Background	1
1.2 Problem definition and research questions	4
1.3 Goal, objectives and scope	5
CHAPTER TWO: LITERATURE REVIEW	6
2.1 CH ₄ in the atmosphere	6
2.2 The effect of methane as a greenhouse gas	7
2.3 Natural and anthropogenic sources of CH ₄	9
2.3.1 The CH ₄ cycle	9
2.3.2 Anthropogenic methane generation in Canada	11
2.3.2.1 Methane generation in the oil and gas sector	12
2.3.2.2 Methane generation in mining	13
2.3.2.3 Methane generation in agriculture	13
2.3.2.4 Methane generation in landfills	14
2.4 Methane emission control	15
2.5 Methane oxidation and biofiltration	17
2.6 Factors affecting methane oxidation	21
2.6.1 Moisture content	21
2.6.2 Temperature	22
2.6.3 Nutrients	22
2.6.4 Oxygen	24
2.6.5 Biofilter Design	25
2.7 Batch experiments	25
2.8 Column experiments	25
2.9 Effect of the materials used in biofilters on methane oxidation	26
2.9.1 Compost	27
2.10 Bulking materials	27
2.10.1 Flax straw	28
2.10.2 Wood shavings	29
2.10.3 Lava rock	30
CHAPTER THREE: MATERIALS AND METHODS	31
3.1 Materials	31
3.1.1 Particle sizes	32

3.1.2 Nutrients	32
3.2 Methods	33
3.2.1 Measurement of Moisture and Water Holding Capacity.....	33
3.2.2 Bulk density and particle density determination	36
3.2.3 Experimental protocol for batch experiments	37
3.2.3.1 Screening batch experiments	39
3.2.3.2 Statistical design of batch experiments.....	40
3.2.3.3 Compost-flax and Compost-wood shavings mixtures	41
3.2.3.4 Compost-lava rock mixtures.....	43
3.2.3.5 Nutrient addition for batch experiments	45
3.2.3.6 Statistical tools for analysis	45
3.2.4 Column experiments.....	46
3.2.4.1 Porosity in column experiments.	48
3.2.4.2 Empty Bed Residence Time (EBRT) and true residence time (τ)	48
3.2.5 Experimental design for column experiments.....	49
3.2.5.1 CH ₄ removal efficiency	52
3.2.5.2 Nutrient addition for column experiments.....	52
CHAPTER FOUR: EXPERIMENTAL RESULTS AND DISCUSSION	54
4.1 Batch experiments.....	54
4.1.1 Screening experiments.....	54
4.1.2 Methane oxidation in Compost-flax and Compost-wood shavings mixtures	55
4.1.3 Methane oxidation efficiencies in Compost-lava rock mixtures	56
4.2 Effect of Nutrient addition on methane oxidation	57
4.3 Effect of moisture content on methane oxidation in batch experiments.....	59
4.4 Statistical analysis for batch experiments	62
4.5 Column experiments.....	63
4.5.1 Methane oxidation efficiencies in flax and wood shavings mixtures.....	65
4.5.2 Methane oxidation efficiencies in Compost-lava rock mixtures	69
4.5.3 Comparison between column experiments.....	72
4.5.4 Comparison with values reported in literature	74
CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS	77
5.1 Conclusions.....	77
5.2 Recommendations.....	79
REFERENCES	80
APPENDIX A: COLUMNS GAS CONCENTRATION PROFILES.....	88
APPENDIX B: DATA FOR ANOVA ANALYSIS.....	94

List of Tables

Table 2-1: GWP of the most common GHGs	9
Table 2-2: Different types of materials used in biofilters	28
Table 3-1: Water holding capacities for the different materials	35
Table 3-2: Dry particle density of the materials	37
Table 3-3: GC operating conditions.....	38
Table 3-4: Material combinations used in the three component experiments	40
Table 3-5: MC and material ratios for batch experiments	40
Table 3-6: Compost – flax and compost – wood shavings mixtures weight ratios	42
Table 3-7: Compost – lava rock mixtures weight ratios	44
Table 3-8: Experimental factors and levels for flax straw, wood shavings and lava rock	45
Table 3-9: Experimental factors and levels for lava rock with and without nutrients	46
Table 3-10: EBRT and Residence time for column experiments	49
Table 3-11: Compost-flax and Compost-wood shavings column experiment characteristics.....	50
Table 3-12: Lava rock mixtures weight ratios	51
Table 4-1: ANOVA for flax straw, wood shavings and lava rock.....	62
Table 4-2: ANOVA for lava rock with and without nutrients	63
Table 4-3: Batch experiments highest efficiencies	64
Table 4-4: Flax and wood shavings column oxidation	66
Table 4-5: Lava rock column experiments oxidation	70
Table 4-6: Column experiments highest CH ₄ removal efficiencies.....	73

List of Figures and Illustrations

Figure 2-1: Averaged GHGs global concentrations.....	6
Figure 2-2: The CH ₄ cycle	10
Figure 2-3 Canada's GHGs Emissions Breakdown by Sector for 2013	11
Figure 2-4: Canada's Emissions Breakdown by GHG for 2013.....	12
Figure 2-5: Composition of Gas from Anaerobic degradation in Landfills.....	15
Figure 2-6: Pathways for oxidation of methane.....	19
Figure 2-7: Photomicrograph of <i>Methylocella</i> , Bar 0±5 µm	20
Figure 3-1: Flax and wood shavings batch experiments.....	43
Figure 3-2: Lava rock batch experiments	44
Figure 3-3: Column design for biofiltration experiments	47
Figure 3-4: Photograph of flax and wood shavings column experiments	50
Figure 3-5: Lava rock column experiments	51
Figure 4-1: Cumulative oxidation for different compost -flax - wood shavings mixtures at FC .	54
Figure 4-2: Cumulative oxidation for compost - flax mixtures at different MC	55
Figure 4-3: Cumulative oxidation for compost - wood shavings mixtures at different MC.....	56
Figure 4-4: Cumulative oxidation for compost - lava rock mixtures at different MC.....	57
Figure 4-5: Cumulative oxidation for compost - lava rock mixtures with nutrients at different MC	58
Figure 4-6: Comparison between lava rock experiments with and without nutrients	59
Figure 4-7: Flax and compost batch experiments at different MC	60
Figure 4-8: Wood shavings and compost batch experiments at different MC	60
Figure 4-9: Lava rock and compost batch experiments at different MC	61
Figure 4-10: Lava rock with nutrients and compost batch experiments at different MC	61
Figure 4-11: Oxidation from flax and wood shavings column experiments	66

Figure 4-12: Gas concentration profile at maximum CH ₄ removal; day 69 (30:70 compost:flax)	67
Figure 4-13: Gas concentration profile at maximum CH ₄ removal; day 69 (30:70 compost:wood shavings at FC)	68
Figure 4-14: Gas concentration profile at maximum CH ₄ removal; day 27 (30:70 compost:wood shavings at 70% of FC)	68
Figure 4-15: Oxidation from lava rock column experiments.....	69
Figure 4-16: Gas concentration profile at maximum CH ₄ removal; day 70 (30:70 compost:lava rock at FC)	71
Figure 4-17: Gas concentration profile at maximum CH ₄ removal; day 91 (30:70 compost:lava rock at 40% of FC)	71
Figure 4-18: Gas concentration profile at maximum CH ₄ removal; day 91 (30:70 compost:lava rock at 40% of FC with nutrients)	72
Figure 4-19: Column experiments oxidation comparison	73
Figure 4-20: Comparison with experiments by Pokhrel (2006)	74

List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
a_i	Radiative efficiency
AGWP	Absolute Global Warming Potential
BM	Bulking Material
$C_i(t)$	Time-dependent abundance of i
$C_{CO_2}(t)$	Time-dependent abundance of CO_2 as reference gas in the denominator
CO_2	Carbon dioxide
CH_4	Methane
$C_{m,in}$	CH_4 concentration in column inlet point
$C_{m,out}$	CH_4 concentration in column outlet point
$C_{n,in}$	Nitrogen concentration in column inlet point
$C_{n,out}$	Nitrogen concentration in column outlet point
DW	Dry Weight ($kg\ kg^{-1}$)
DS	Dry Solids
EBRT	Empty Bed Residence Time
EPS	Exopolymeric substances
GC	Gas Chromatograph
GHG	Greenhouse Gases
GISS	Goddard Institute for Space Studies
GWP	Global Warming Potential
HFC	Hydrofluorocarbons
H_2	Hydrogen
IPCC	Intergovernmental Panel on Climate Change
η	Efficiency
LFG	Landfill Gas
Mo	Initial moisture content (%)
MBF	Methane Biofilter

MC	Moisture Content ($w w^{-1}$)
MC _A	Moisture Content of material A (%)
MC _B	Moisture Content of material B (%)
MC _C	Moisture Content of compost (%)
m _{wet}	Mass of the sample before drying
m _{dry}	Mass of the sample after drying
MMO	Methane Monooxygenase
MSW	Municipal Solid Waste
N ₂ O	Nitrous oxide
NO ₃	Nitrate
NPT	National Pipe Thread
θ	Porosity ($m^3 m^{-3}$)
θ_g	Air filled porosity ($m^3 m^{-3}$)
O(¹ D)	Excited oxygen atom
OH	Hydroxyl radical
pMMO	Particulate Methane Monooxygenase
ppb	Parts per billion
Q	Flow rate ($m^3 s^{-1}$ or $ml min^{-1}$)
RF _{<i>i</i>}	Global mean RF of component <i>i</i>
RF _{CO₂}	Global mean RF of CO ₂
RuMP	Ribulose monophosphate pathway
ρ_{bulk}	Bulk density ($g cm^{-3}$)
$\rho_{particle}$	Particle density ($g g^{-1}$)
sMMO	Soluble Methane monooxygenase
τ	Residence Time
TH	Time Horizon
V	Volume of the mixture (cm^3)
V _f	Total bed volume (ml)
V _t	Total volume (cm^3)
V _w	Volume of water (cm^3)

W	Material weight (g)
W _A	Weight of material A in the mixture (g)
W _B	Weight of material B in the mixture (g)
W _C	Weight of compost in the mixture (g)
FC	Water Holding Capacity (%)
FC _A	Water Holding Capacity of material A (%)
FC _B	Water Holding Capacity of material B (%)
FC _C	Water Holding Capacity of compost (%)
W _s	Weight of solids (g)

Chapter One: **Introduction**

1.1 Background

The concentration of greenhouse gases (GHGs) in the atmosphere has become a major problem in the last few decades. The concentration of methane (CH₄) has increased considerably since the industrial time when concentration was 715 parts per billion (ppb) to 1774 ppb in 2005 (Scheutz et al., 2009).

Even though CH₄ has the second largest radiating force (CO₂ has the first) it has a Global Warming Potential (GWP) 28 times that of CO₂ in a period of 100 years (IPCC, 2013). Since CH₄ has a bigger impact in a short time, it is more effective to reduce the amount of this gas in the atmosphere with more visible results in a shorter time span.

Current mitigation techniques to reduce CH₄ concentrations in the atmosphere include flaring and gas combustion for energy production. Flaring is the main option, but only if CH₄ concentrations are higher than 20% (v v⁻¹); the downside of this technology are the by-products, which are very toxic and released directly into the atmosphere. On the other hand, combustion is best used for producing electricity or hot water but can only be used when the concentrations of CH₄ are higher than 30% (v v⁻¹). Unfortunately, if these concentrations are not present, these technologies cannot be used and an alternative method has to be applied (Nikiema, 2007).

A cost-effective way of minimizing the impact of GHGs is with methane biofilters (MBF), which consist of a medium that can sustain methanotrophic bacteria and through which a continuous flow

of CH₄ rich gas is conducted (Mancebo, 2012). Methanotrophic bacteria convert CH₄ into CO₂, thus generating carbon offsets. By-products generated through biofiltration are less harmful than CH₄ such as water, biomass and CO₂ (Nikiema, 2007).

Some of the sectors that produce GHGs present this issue of having low concentrations of CH₄ in their emissions where conventional GHG mitigation technologies cannot be applied. These sectors are fugitive sources (8%), agriculture (8%) and waste (3%). Fugitive sources include operations from the oil and gas sector and coal mining (Environment Canada, 2015).

In the oil and gas sector, CH₄ comes primarily from fugitive emissions in which this gas is found in such low concentrations and variable flowrates that conventional methods cannot be used to dispose of it. CH₄ sources can be of two types: concentrated emissions consisting mainly of natural gas and dilute emissions where natural gas is less than 1% (v v⁻¹) (Hayes, 2004). Whether or not GHGs from this sector can be reduced through MBF depends on CH₄ flow rates and concentrations, which in turn influence the desing of the MBF.

In the mining sector, most CH₄ emissions (70%) come from mine ventilation air (MVA). To create a safe working environment in the mine, CH₄ is diluted to concentrations under 2% (v v⁻¹), this diluted gas is then released into the atmosphere without further treatment. MBF can be used to treat this gas since it has already been collected and is ready to be sent through the filter (Limbri et al., 2013).

In Canada, the piggery industry is an essential part of the agricultural sector and GHGs can be produced at different stages of slurry management, mostly during storage (65-70%). Slurry is stored in a pit, from which it is relatively easy to collect the gas and dispose of it. Conventional methods have proven to be difficult to use for this source of CH₄ since its concentration are usually under the 20% (v v⁻¹) required for flaring (Girard et al., 2009).

In 2008, 34 million tons of waste were produced , in Canada; 75% of this was stored in landfills or incinerated (Ménard, 2012). Landfills produce landfill gas (LFG) composed of about 55-60% (v v⁻¹) CH₄ and 40-45% (v v⁻¹) CO₂. This gas can only be used if the concentration of CH₄ in the biogas is more than 30% and the produced amount more than 10 to 15 m³ h⁻¹. MBF have potential applications in older or small landfills where common technologies are not very effective because of the amount of LFG that is produced (Nikiema, 2007)

Conversion of CH₄ into CO₂ happens at different rates inside of the biofilter depending on several factors: moisture, temperature, depth, oxygen availability, pH and the media in which the methanotrophs live. This media has to be able to provide the bacteria with the nutrients they need to survive along with the ideal physical and chemical conditions so they can thrive and achieve the best CH₄ to CO₂ conversion rate.

Various compost based media have been tried; however, the problem with these media is that performance decreases with time, the availability of nutrients and oxygen seems to be the main cause for this (Mancebo, 2012). A new and efficient medium has to be identified in order to

enhance carbon offsets. This new medium has to be able to perform at both laboratory and full scale and be cost-effective so it can be used as a new technology for biofiltration.

Materials proposed for this study comprise less degradable and compactable ones, such as wood shavings, flax straw and lava rock. These were used in a mixture along with compost, since this material provides nutrients, water holding capacity and most importantly bacteria. These materials have been tested to try and achieve conditions provided by a 100% compost biofilter bed.

1.2 Problem definition and research questions

Currently used biofilter media shows a decrease in the conversion performance over time most likely due to a reduction in the availability of nutrients and oxygen. A new efficient medium with a material other than compost as its main component has to be identified in order to enhance biofilters' life span. This new medium has to be able to perform in a laboratory and at full scale and be cost-effective so it can be used as a GHG reduction technology.

To address this problem we need to answer the following research questions:

1. What is the minimum amount of compost that can be used for methane oxidation?
2. Will a more porous material provide a higher oxygen level while maintaining methane oxidation?
3. How important is the role of moisture content in methane oxidation and which is the best level?
4. Will inert materials like lava rock provide favourable conditions for methane oxidation?

1.3 Goal, objectives and scope

The goal of this research is to identify a new low-compost material mixture that will allow methane oxidation while having less degradation than compost.

There are some objectives that need to be accomplished to obtain enough information on how these materials work in order to achieve this goal:

1. Determine the combination of flax, wood shavings, lava rock and compost that provides the highest methane oxidation using batch experiments.
2. Determine the best moisture content of mixtures to achieve the highest methane oxidation.
3. Evaluate the performance of methane biofilters with media selected from batch experiments. Columns experiments were conducted to achieve more realistic conditions where porosity could be properly assessed.
4. Identify the effect of nutrient addition on methane oxidation for lava rock based medium.

Chapter Two: LITERATURE REVIEW

2.1 CH₄ in the atmosphere

The concentration of greenhouse gases (GHGs) in the atmosphere has become a major problem in the last few decades. The most important of these are CO₂, CH₄ and nitrous oxide (N₂O). The concentrations of these gases has increased since the pre-industrial time by 40%, 150% and 20% respectively (Figure 2-1). CH₄ concentrations have increased from 715 parts per billion (ppb) to 1774 ppb in 2005 and to 1803 ppb in 2011, this gas comes primarily from anthropogenic emissions which represent 50-65% of global CH₄ emissions in the 2000s (IPCC, 2013).

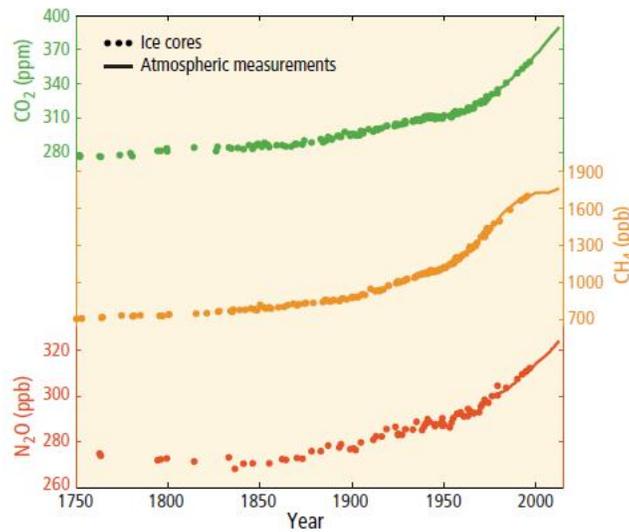


Figure 2-1: Averaged GHGs global concentrations

Source: IPCC, 2013

2.2 The effect of methane as a greenhouse gas

Even though CH₄ in the atmosphere was first discovered by Migeotte in 1948 from infrared absorption features in the solar spectrum (Wahlen, 1993), its importance was revealed later on through three key discoveries. First of all, in 1976 Wei-Chyung Wang and colleagues at NASA Goddard Institute for Space Studies (GISS) realized that CH₄ was a significant GHG since it absorbs the long wave radiation of 4 – 100 μm emitted by Earth's surface; otherwise, this radiation would just escape into space. It is because of this absorption that we have an increase in the surface temperature (Giss.nasa.gov, 2015).

Secondly, CH₄ concentrations in the atmosphere prior to modern records have been constructed from data collected from gas bubbles in ice cores from Greenland and Antarctica. In these cores, annual layers can be identified to create a very accurate timescale. Through the comparison of climate change data we know that CH₄ concentrations have more than doubled over the last 150 years (Giss.nasa.gov, 2015).

Thirdly, the research by Jeff Severinghaus of Scripps Institution of Oceanography and Ed Brook of Washington State University showed that in a warming climate we have a rapid increase in CH₄ concentrations, with a small lag behind temperature. This leads us to the conclusion that CH₄ does affect the climate but its abundance is affected in return by the same climate (Giss.nasa.gov, 2015).

Radiative forcing (RF) is one of the most widely used metrics by itself but also serves as a basis for others that provide for a fast means of comparing the climate effect of emissions. RF quantifies

the influence of a factor on the changes in the energy of the earth-troposphere system, and is usually expressed in watts per square meter over a certain period of time (IPCC, 2013)

Even though CH₄ has the second largest radiating force it has a GWP 28 times that of CO₂ in a period of 100 years (IPCC, 2013). Of all the GHGs that contribute to global climate change, CH₄ represents 16% of the total (IPCC, 2014). The combined effect of GHGs has contributed to an increase in the global mean surface temperature by a range of 0.6°C to 0.7°C in the period from 1951 to 2010 (IPCC, 2013).

Different emissions metrics and indicators have been proposed to evaluate the impact of GHGs, one of these is GWP which is a purely physical indicator and is the most common one. This index is based on the RF over a specific time of an emission of 1 kg of a specific compound (*i*) relative to that of 1 kg of the reference gas CO₂ and is defined as follows in Equation 2.1 (IPCC, 2007):

$$GWP_i = \frac{\int_0^{TH} RF_i}{\int_0^{TH} RF_{CO_2}} = \frac{\int_0^{TH} a_i [C_i(t)] dt}{\int_0^{TH} a_{CO_2} [C_{CO_2}(t)] dt} \quad \text{Equation 2.1}$$

Where:

TH = time horizon

RF_i = global mean RF of component *i*

RF_{CO_2} = global mean RF of CO₂

a_i = radiative efficiency. RF per unit mass increase in atmospheric abundance of *i*

$C_i(t)$ = is the time-dependent abundance of *i*

$C_{CO_2}(t)$ = is the time-dependent abundance of CO₂ as reference gas in the denominator

The numerator and denominator are called the absolute global warming potential (AGWP) of *i* and CO₂ respectively. Table 2-1 shows the different GWP of the most important GHGs.

Table 2-1: GWP of the most common GHGs

	Lifetime (years)	GWP over 20 years	GWP over 100 years
CO ₂	^a	1	1
CH ₄	12.4	84	28
N ₂ O	121	264	265
HFC	1.5	506	138

^a No single lifetime can be given for CO₂.

Source: IPCC 2014a.

Different GWP values have been used throughout the history of IPCC reports due to the fact that as knowledge is improved, so are calculations and estimations. Since CO₂ is the reference gas, any change in its values affects the rest that are related to it (IPCC, 2013).

Since CH₄ has a GWP of 84 in a 20 year span, which translates in a bigger impact in a short time, it is more effective to reduce the amount of this gas in the atmosphere with faster stabilization of its concentrations and its warming effect (Barlaz, 2005).

2.3 Natural and anthropogenic sources of CH₄

2.3.1 The CH₄ cycle

When low levels of ozone (O₃) are present in the troposphere we have the formation of excited oxygen (O(¹D)) through the photolysis process. It is this O(¹D) atom which later reacts with water

vapour to create OH radicals (Atkinson, 2000). CH₄ is mainly destroyed in the troposphere by an oxidation reaction that starts with the OH radical (Wahlen, 1993). In fact, all hydrocarbon chemical species can be broken down into CO₂ and H₂O by this radical (Giss.nasa.gov, 2015). Breakdown of CH₄ in turn, generates water vapour which is also a GHG. This process has been identified as an indirect GHG effect (IPCC, 2007). This is the main process that serves as a CH₄ sink. We must also address the natural and anthropogenic sources of CH₄ to get the whole picture, also known as the CH₄ cycle (Figure 2-2). The main natural sources for CH₄ are wetlands, tundra and ruminants (Whalen, 1993).

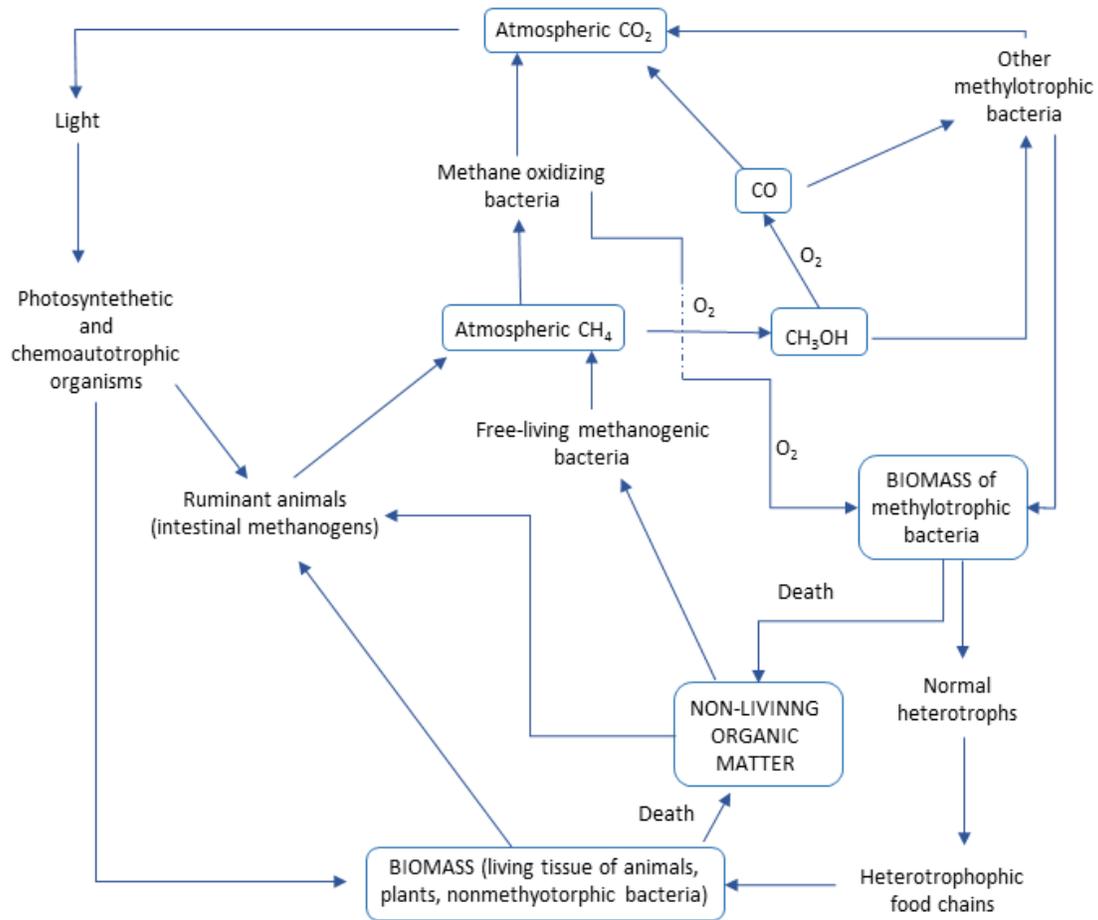


Figure 2-2: The CH₄ cycle

Adapted from Large, 1983

2.3.2 Anthropogenic methane generation in Canada

Canada's total GHG emissions for 2013 were estimated in a total of 726 Megatonnes of CO₂ equivalent. The highest contributing sectors for which MBF is a viable source to reduce GHG emissions are fugitive sources (8%), which includes oil and natural gas and coal mining, agriculture (8%) and the waste sector, which represents 3% of this total (Figure 2-3). For this year, CO₂ was the main GHG contributing with 78% of the total GHGs production; the second most abundant gas was CH₄, which accounted for 15% (Figure 2-4) (Environment Canada, 2015).

CO₂ equivalent emissions is a way of comparing the amount of CO₂ emissions that could cause the same effect, in terms of their warming influence, as an emitted amount of a specific GHG or a mixture of them. This equivalent is obtained by multiplying the GWP of the emitted gas by its emissions (IPCC, 2007)

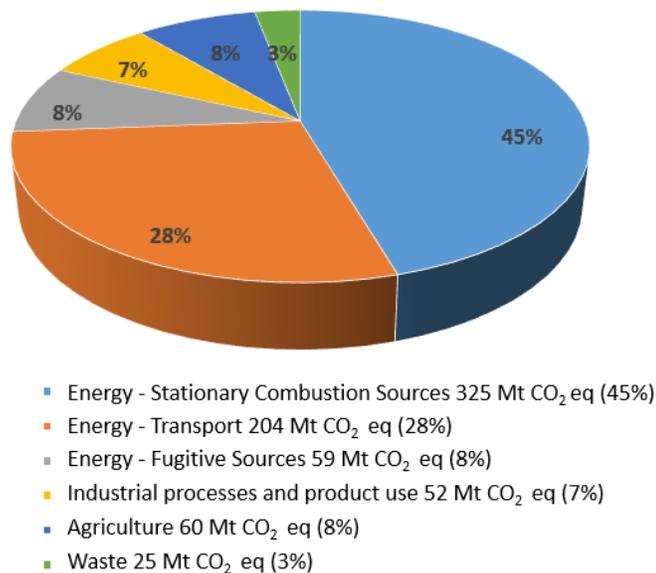


Figure 2-3 Canada's GHGs Emissions Breakdown by Sector for 2013

Source: Environment Canada, 2015

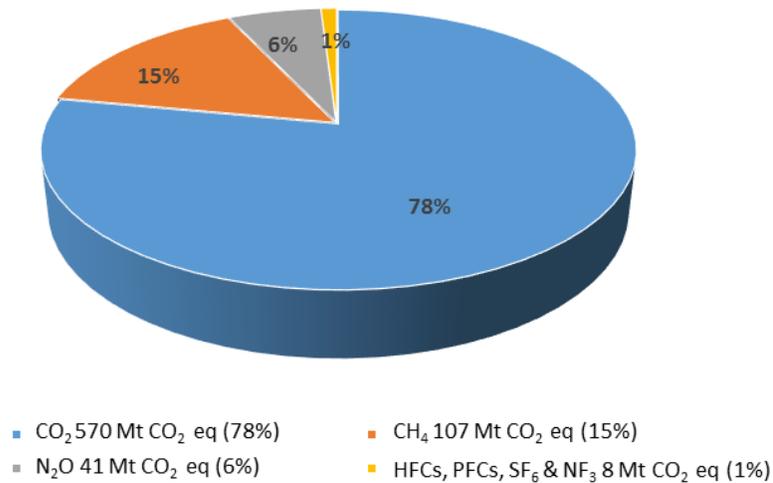


Figure 2-4: Canada's Emissions Breakdown by GHG for 2013

Source: Environment Canada, 2015

2.3.2.1 Methane generation in the oil and gas sector

Most CH₄ produced by the oil and gas sector, considering conventional and non-conventional operations, comes from fugitive emissions (40%) (Hayes, 2004). The main sources for fugitive emissions come from production activities, natural gas processing, transportation and distribution (Picard, 1999).

The main problem with these fugitive emissions is their low CH₄ concentrations, which restricts the possible number of technologies to control them. The main reduction source (flaring) is left out from the choices since CH₄ concentrations are under the 20% (v v⁻¹) required for this process. Another problem arises with the variations in composition and flowrates since gas mixtures can include SO₂ and emissions can occur in ranges from hours to months (Hayes, 2004).

2.3.2.2 Methane generation in mining

The main source of CH₄ in the mining industry comes from mine ventilation air (MVA), where it accounts for approximately 70% of all coal mine CH₄ emissions. CH₄ is formed during the coal creation process and is stored within the coal seam, therefore, it is inevitably released when coal mining activities take place (Limbri, 2014).

Due to safety measures, CH₄ in mines has to be ventilated to be kept under the explosive limit (5-15% v v⁻¹). For gassy mines it is kept at around 0.2 to 1% (v v⁻¹) and for non-gassy around 0.1%. In gassy mines, CH₄ comes from the coal seam where it is found at concentrations of 60 - 95% (v v⁻¹), in this kind of operations it is possible to crack the coal seam before proceeding with the mining operations and to capture the gas for electricity generation. In non-gassy mines, CH₄ can be recovered at concentrations of 30 – 95% (v v⁻¹) and also used for electricity generation. The difficulty in reducing CH₄ emissions into the atmosphere is the very low concentration of CH₄ in the ventilation air along with the high flow rates (50 - 500 m³ s⁻¹) (Limbri, 2014).

2.3.2.3 Methane generation in agriculture

The piggery industry in Canada is a crucial part of the agriculture sector, and is also an important source for GHGs especially CH₄ (49%) and N₂O (51%). Swine slurry is the main source for this GHG, especially during the storage phase (65-70%) (Girard et al., 2009).

Even though the highest production of GHGs comes from the slurry storage pit, it is a relatively easy problem to address since it can be covered in order to collect produced gases. After collection,

these gases could be easily flared but concentrations ($5 - 100 \text{ mg m}^{-3}$) are usually under the flaring requirement and, therefore, another technology needs to be used (Nikiema et al., 2007).

2.3.2.4 Methane generation in landfills

In recently closed landfills, the production of biogas can range between 0 to 11 m^3 per 1000 kg of waste per year. This production decreases overtime and eventually ceases after 30 to 50 years of operation (Nikiema et al., 2007). The contribution of CH_4 from landfills to the atmosphere has increased from 55 Tg yr^{-1} in 1980-1989 to 75 Tg yr^{-1} in 2000-2009 (IPCC, 2013).

Landfills in Canada generate 3% of the total GHG emissions (Ménard et al., 2012). Of the total amount of GHGs produced by the waste sector, CH_4 accounts for 94% of these emissions. The main contributors to this emissions, with 82%, are Municipal Solid Waste landfills (MSW); the rest is produced by wood residues landfills (Environment Canada, 2015).

According to Abichou et al., (2006), an average of $71.3 \text{ g m}^{-2} \text{ day}^{-1}$ up to $1755 \text{ g m}^{-2} \text{ day}^{-1}$, of CH_4 is produced in landfills, while Bogner et al., (1997) found that emissions vary from 0.0004 to $4000 \text{ g m}^{-2} \text{ day}^{-1}$. This great gap between measured emissions comes from the fact that some cover soils are already oxidizing all the CH_4 coming through and even become a sink by oxidizing CH_4 from the atmosphere.

Waste decomposition in landfills has been studied by Farquahar and Rover (1973) where four phases for gas production were identified: I. Aerobic; II. Anaerobic Non-Methanogenic; III. Anaerobic Methanogenic Unsteady; and IV/ Anaerobic Methanogenic Steady. In the first stage

organic compounds are degraded aerobically. In the second stage, organic substances are degraded further into CO₂, acidic compounds and H₂. In stages 3 and 4, CH₄ is produced when bacteria break down acids or reduce CO₂ with H₂. After Christensen and Kjeldsen (1989), a fifth stage was included called Anaerobic Methanogenic Declining, where CH₄ production reaches maturity and starts to diminish. These phases can be seen in Figure 2-5.

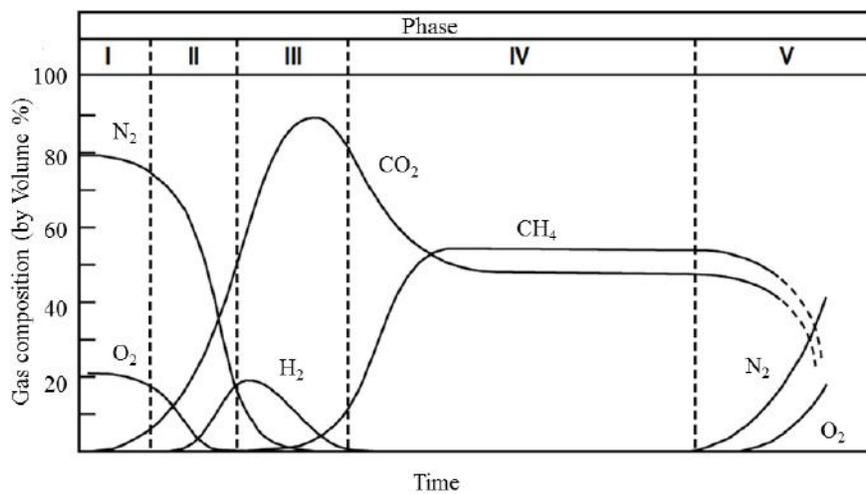


Figure 2-5: Composition of Gas from Anaerobic degradation in Landfills

Source: Christensen and Kjeldsen, (1989)

2.4 Methane emission control

Landfills produce LFG with about 55-60% (v v⁻¹) CH₄ and 40-45% (v v⁻¹) CO₂. LFG emission mitigation technologies range from flaring and gas recovery, natural gas production and generation of electricity to improved waste management policies (Bogner et al., 2008). When the amount of gas that is being produced is not sufficient to be used as an energy source, flaring becomes an option but only if the concentration of CH₄ in the biogas is more than 20% and the amount of biogas produced by the operation is more than 10 - 15 m³ h⁻¹. The problem with this method is

that it produces by-products such as dioxins, which are toxic, and are directly released into the atmosphere (Nikiema et al., 2007).

LFG gas emissions have decreased their growth rate over the last 20 years rate thanks to a wider implementation of gas recovery. CH₄ from LFG was recovered and used for energy purposes for the first time in 1975. More than 1150 plants in the world have now implemented this technology which results in the reduction of more than 105 Mt CO₂-eq year⁻¹ (Willumsen, 2003; Bogner & Matthews, 2003). As of 2013 there are 81 LFG collection systems operating in Canadian landfills (Environment Canada 2015). The use of this technology is expected to spread to developing countries in the next 20 years when waste management in the form of controlled landfilling will be a more common practice (Bogner et al., 2008).

This technology can be used with either vertical or horizontal gas recovery systems and complemented with geomembrane covers, constant monitoring and a perimeter extraction system to collect gas that has migrated (Spokas et al., 2006). Recycling, reducing and reusing waste is being implemented in industrialized countries to help diminish the amount of waste that goes into landfills and that eventually contributes to generate GHGs. (Bogner et al., 2008).

There have been various efforts to minimize the impact and production of GHGs, and a very cost-effective way of doing it is with biofilters. This technology has been defined by Scheutz et al., (2009) as a packed bed reactor filled with a packing material provided with a gas collection and drainage systems. They can be used in landfills with an active or passive inlet gas flow and by way of methanotrophic bacteria, CH₄ in the LFG is converted to CO₂, generating carbon offsets. The

by-products generated from biofiltration are less harmful than CH₄, such as water, biomass and CO₂ (Nikiema et al., 2007).

Bogner et al., (2008) has stated that CH₄ gas recovery from landfills has its maximum efficiency at 75% in developed countries and 50% in developing countries; this provides a wide range of action for biofilters to help reduce net GHG emissions.

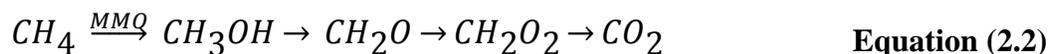
When the previously addressed technologies cannot be used or are not enough to control GHG emissions, biofilters become an inexpensive and easy to install option. This becomes useful particularly for older or small landfills where common technologies are not very effective because of the amount of gases that are produced (Nikiema et al., 2007).

2.5 Methane oxidation and biofiltration

The conversion of CH₄ into CO₂ is done by bacteria called methanotrophs. These bacteria work at different rates inside the biofilter depending on several factors. Some of these factors are: moisture, temperature, depth, oxygen rate, pH and the media in which they live. This media has to be able to provide the bacteria with the nutrients they need to survive along with the ideal physical and chemical conditions so they can thrive and achieve the best CH₄ to CO₂ conversion rate.

Methanotrophs can be found in a great variety of ecosystems, mostly where oxygen and CH₄ are present. The conversion of CH₄ into CO₂ involves a series of intermediate compounds (Equation 2.2) starting with the conversion of CH₄ into methanol (CH₃OH) with the help of Methane

Monoxygenase (MMO) (Figure 2-6). Methanol is then transformed into Formaldehyde (CH₂O) which in turn is converted to Formic acid (CH₂O₂) and finally into CO₂ (Hanson & Hanson, 1996).



Since these bacteria need both oxygen and CH₄ to survive, their activity is confined to a narrow strip where activity reaches its maximum activity. This zone is usually found 15 to 20 cm below the surface (Scheutz et al., 2009).

Methanotrophic bacteria are part of the methylotrophs, which are known for their particular ability to degrade one-carbon compounds in the presence of oxygen (Hanson & Hanson, 1996). One carbon compounds include methane, methanol, methylated amines and halogenated methanes (Chistoserdova et al., 2005).

Methanotrophic bacteria can be divided into three groups based on their carbon assimilation pathways, membrane arrangements and their ability to fix molecular nitrogen and form long-chain unsaturated fatty acids (Whittenbury et al., 1970; Bowman et al., 1993). Type I use ribulose monophosphate (RuMP), Type II uses the serine pathway and type X uses both pathways. MMO is an enzyme common to all three groups and is responsible for starting biological oxidation of CH₄. There are two presentations of this iron-copper enzyme: soluble (sMMO) and particulate (pMMO), the former can be found only in Type II and Type X while the latter is found in all methanotrophs (Hanson & Hanson, 1996).

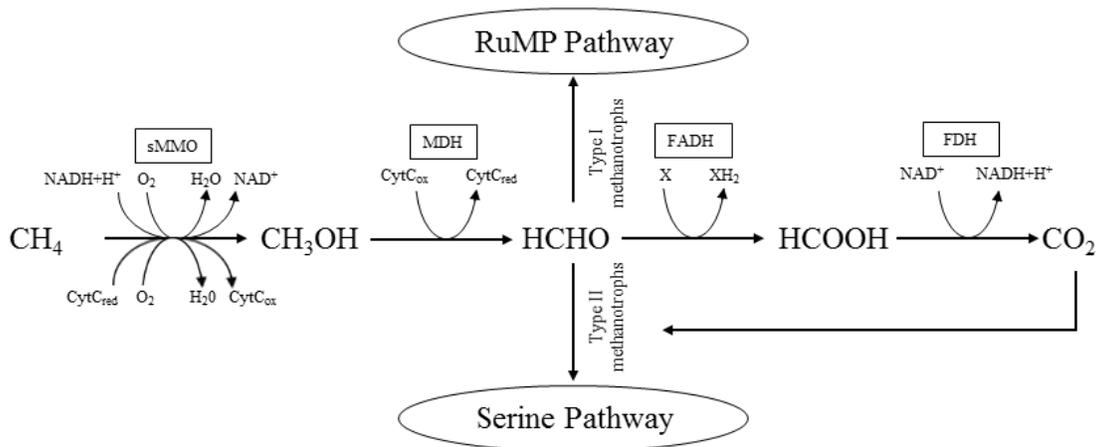


Figure 2-6: Pathways for oxidation of methane

Adapted from Hanson & Hanson, 1996

The categorization of these bacteria into three groups was done taking into consideration factors including cell morphology, growth at 45°C and nitrogen fixation. For example, Type I and II are not able to grow at 45°C , whereas Type X can. Cell morphology in Type I occurs as short rods, usually singly, as cocci or ellipsoids. Type II can be found as crescent-shaped rods, pear-shaped cells or sometimes as rosettes; Type X are found as cocci and often in pairs. Another important factor is the affinity they show for CH_4 , for example, Type II has a low affinity for CH_4 so they grow under high CH_4 concentrations. On the other hand Type I has a high affinity and therefore can grow under low CH_4 concentrations. Growth rate is higher for Type I but so is the death rate while Type II grow slowly but are able to endure extreme conditions for a longer time. Also Type II can out-compete Type I under oxygen and nitrogen limiting conditions (Hanson & Hanson, 1996).

Recently there have been new findings and a new enzyme has been discovered in *Methylocella silvestris* (Figure 2-7), the propane monooxygenase (PrMO), along with the fact that this is a facultative methanotroph which means it is capable of obtaining energy not only from CH₄ but also from propane (Dunfield & Dedysh, 2014). This type of findings suggest that the old conventional classification of three groups is no longer valid but has prevailed due to its ease of use particularly for non-specialized individuals.

Even though bacteria and physical factors like MC and temperature play a very important role in the biodegradation of CH₄, there are other factors like the transfer rate from the gas phase to the biofilm and the biodegradation rate of CH₄ itself that also determine the speed of the reaction (Delhoménie & Heitz, 2005).

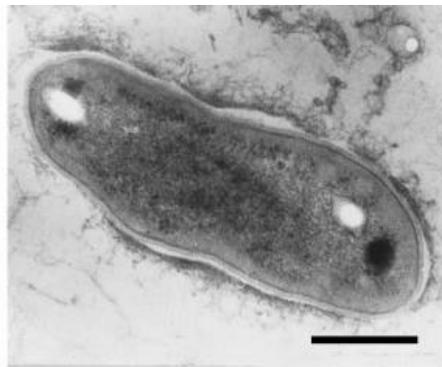


Figure 2-7: Photomicrograph of *Methylocella*, Bar 0±5 µm

Source: Dedysh et al., 2000

CH₄ is a recalcitrant alkane that requires at least a few hours to biodegrade (Delhoménie & Heitz, 2005). Its low water solubility (0.022 g L⁻¹ at 20 °C) is part of what extends the process because

it takes longer to dissolve into the liquid phase for subsequent utilization by methanotrophs (Melse & Van der Werf, 2005).

2.6 Factors affecting methane oxidation

There are several physical factors that affect the conditions of the media in which bacteria live.

Changing these conditions may hinder or enhance bacterial activity. Some of these factors are:

- Moisture content
- Temperature
- Nutrients
- Oxygen concentration
- Porosity
- pH

2.6.1 Moisture content

Water is an essential component to life, thus bacteria require a minimum amount to carry out chemical reactions. Different landfill cover materials studied by Figueroa (1993) showed that the highest conversion efficiency was found in Moisture Contents (MC) of 40 to 80% of Field capacity (FC) and no conversion occurred under 13% of FC; Landfill cover soils studied by Visvanathan et al., (1999) showed maximum oxidation rates at MC between 15 and 20%, whereas at 6%, oxidation was practically inexistent. Mor et al., (2006) found maximum oxidation happening in compost at MC between 45 to 110% (dry weight basis). Huber-Humer et al., (2009) suggests using a MC closer to 50% of the FC of compost materials as an initial value.

2.6.2 Temperature

Bacteria can be divided into three main optimal metabolic temperature ranges. Psychrophiles (live below 20°C), Thermophiles (above 45°C) and Mesophiles (between 20 °C and 45°C) (Gaudy & Gaudy, 1980). Most methanotrophic bacteria are Mesophiles and a temperature range between 20 to 37 °C, according to (Humer & Lechner, 1999), is the one that suits them the best. Some researchers (Kennes & Thalasso, 1998; Delhoméie & Heitz, 2005) have found that this temperature range can go up to 45°C. This has been corroborated by Pokhrel (2006) and by Visvanathan et al., (1999) who identified 30°C and a range between 30 and 36°C, respectively, as the optimum temperature for bacteria to live and have the most activity.

2.6.3 Nutrients

Basic elements like carbon, oxygen, nitrogen, hydrogen, phosphorus, and sulphur are the main components in bacteria, and as such have to be constantly supplied for them to keep existing (Scriban, 1993). Contaminants in the biofilter represent the main food source for bacteria. In this case, carbon is the main contaminant, and since methanotrophs are strict aerobes, oxygen also has to be supplied constantly along with the contaminant. Other macro nutrients (N, P, K, S), if not available in enough quantities in the bed material, have to be supplied through the addition of specific chemical substances like ammonia, nitrate or fertilizer (Delhoméie & Heitz, 2005).

Regarding ammonia nitrogen (NH₃-N), Anthony (1982) found that high concentrations act as an inhibitor towards MMO enzymes and therefore inhibits CH₄ oxidation, these high concentrations were found to be higher than 350 ppm through the work of Humer and Lechner (2001).

This inhibiting effect of ammonia was found by Dalton (1977) and is caused because of the competition between CH_4 and ammonia for an active site in the MMO enzyme. This competition is caused by the fact that this enzyme can oxidize more than just CH_4 .

De Visscher et al., (1999) performed batch experiments with 10% (v v^{-1}) CH_4 and nutrient concentrations where 25 mg of N per kg of soil (DW) was added as a 1.3 g N L^{-1} solution of NH_4Cl and NaNO_3 . This set up was used in different experiments in which activity doubled compared to that of a blank. This experiments proved that ammonium and nitrate can increase bacterial activity instead of inhibiting it.

Wilshusen et al., (2004) reported that concentrations of ammonia and nitrate ranging from 0.5 g N L^{-1} to 1.5 g N L^{-1} cause an inhibitory effect. The concentrations are given per litre of moisture in compost.

Albanna et al., (2007) used a different approach regarding the addition of nutrients since there is not enough information regarding the use of soil fertilizer as the nutrient source. Plant-Prod All Purpose Fertilizer 20-20-20 was used in a concentration of 1.5 grams per kilogram of soil used. Nitrogen is available as nitrate (5.9%), ammoniacal nitrogen 3.9% and urea (10.2%).

This experiment proved that there were no negative effects produced by the addition of this fertilizer, on the contrary, an increase of 43% was seen in the oxidation of CH_4 . The use of a commercially available product as a source of nutrients allows for a more economically feasible component to use in biofilter projects.

Nikiema et al., (2009) performed comprehensive studies on the elimination capacity of CH₄ with the addition of different amounts of nutrients and different inlet loads in biofilters. The highest elimination capacity achieved was 36 g m⁻³ h⁻¹ with an inlet load of CH₄ of 95 g m⁻³ h⁻¹ and a concentration of NO₃ of 0.75 g N L⁻¹; phosphorus was found to increase elimination capacity by 35% with an inlet load of 75 g m⁻³ h⁻¹ and a concentration of 3.1 g L⁻¹. Through this experiment it was found that elimination capacity can be enhanced with a concentration of .75 g N L⁻¹ for an inlet between 55 and 95 g m⁻³ h⁻¹ and 0.5 g N L⁻¹ between 20 and 55 g m⁻³ h⁻¹.

Taking these experiments into consideration we can conclude that nutrients have a positive effect on bacterial efficiency depending on the nutrient concentration and the inlet load of CH₄. These two elements have to be within certain proportions so as not to hinder methanotrophic activity.

2.6.4 Oxygen

Since methanotrophs are obligate aerobes, oxygen has to be constantly supplied and different oxygen concentrations will impact the efficiency of bacteria. Stein and Hettiaratchi (2001) showed that bacterial activity is severely affected (oxidation decreased by 50%) when oxygen concentrations drop from 3% to 1%. On the other hand, efficiency is not increased so much (by 10%) when oxygen varies from 3% to 20%, thus allowing bacteria to work with a small amount of oxygen and to higher depths where this gas is not so abundant. When oxygen is present in high concentrations (10.5%), exopolymeric substances (EPS) are more widely produced and can reduce oxidation (Wilshusen et al., 2004)

2.6.5 Biofilter Design

There are two main designs for biofilter technology. The first consists of big open reactors with ascending gas flow containing CH₄ which are installed outdoors and rely on atmospheric oxygen, and therefore are vulnerable to climate changes and reductions in efficiency. The other main type is a closed reactor design and they are usually installed indoors where temperature can be a controlled factor; they can use both an up flow or down flow configuration for both air and the contaminant gas; this configuration occupies less volume since bed thicknesses can be used more effectively through active aeration.

2.7 Batch experiments

Researchers have used batch experiments, also called incubation experiments to find oxidation capacities from different materials in different conditions. Boeckx (1996) and Christophersen et al., (2000) used this type of experiment under different conditions, such as a varying MC and temperature to assess the change in CH₄ oxidation as well as the maximum obtainable oxidation under optimum conditions.

Some researchers (Wilshusen et al., 2004; Mancebo & Hettiaratchi, 2015; Pokhrel et al., 2011) have worked with compost and compost mixes, the latter in an effort to improve compost's already good conditions for bacteria to live in.

2.8 Column experiments

Landfill conditions created by cover liners are usually replicated using column experiments where depth of the media mirrors original landfill conditions. A few materials and material mixes have

been tried, such as actual landfill cover soils (De Visscher et al., 1999; Hilger et al., 2000; Pokhrel 2006); compost (Figuerola, 1993; Fornés et al., 2003; Wilshusen et al., 2004) compost-soil (Pokhrel, 2006), compost-perlite and compost-sand (Mancebo, 2012). It is also through this type of laboratory experiments that we can study the behaviour of materials for biofilters before trying field scale experiments.

2.9 Effect of the materials used in biofilters on methane oxidation

Various compost based media have been tried with different conversion efficiencies, such as leaf compost with a maximum CH₄ conversion efficiency of 82%, perlite and compost with a maximum conversion efficiency of 100% and the mixture of sand and compost with a maximum conversion efficiency of 62%. The problem with these media is that performance decreases with time, the availability of nutrients and oxygen seems to be the main cause, along with compaction of the material due to degradation (Mancebo, 2012).

Various authors (Scheutz et al., 2009; Janni et al., 2001; Bohn, 1996; Akdeniz et al., 2011) agree that the following characteristics are very desirable for a material that will be used in a biofilter:

1. Large surface area to help bacterial attachment.
2. Low bulk density to reduce the time it takes materials to compact.
3. Good homogeneity so air pockets and clogging do not occur.
4. High porosity to allow gas flow.
5. Slow degradation rate so material does not have to be changed so frequently.
6. Good water retention to avoid continuous water addition.
7. Presence and availability of nutrients.

8. Presence of microflora.

It is expected that by using different materials with different characteristics, these desirable factors for a biofilter's bed will be better than when only using compost.

2.9.1 Compost

Compost has been the most widely used material in biofilters because of its highly desirable properties for bacteria to live in, like the amount of nutrients and its water holding capacity, and because by using it we recycle a material that otherwise has limited use.

The preferred mean particle size should be between 0.5 and 2.5 mm, with the lower specified to prevent excessive bed packing that could prevent proper gas flow and therefore CH₄ oxidation (Bender and Conrad 1995; Kightley et al., 1995; Börjesson et al., 1998).

2.10 Bulking materials

Even though compost is the preferred material for use in biofilters, its porosity eventually decreases over time due to compaction. This has led to the use of other materials by themselves or in mixtures, which includes materials that are difficult to compact and will therefore enhance the biofilter's porosity. Some of the materials that have been used are shown in Table 2-2.

Table 2-2: Different types of materials used in biofilters

Material	Authors
Woodchips or barks	Oh and Choi, 2000; Luo, 2001; Dixit et al., 2012; Mohseni and Allen, 2000; Smet et al., 1996; Smet et al., 1999; Hong and Park, 2004.
Perlite	Weigner et al., 2001; Woertz et al., 2002; Mancebo, 2012.
Vermiculite	Krishnayya et al., 1999; Pineda et al., 2000
Ceramic	Cardenas-Gonzalez et al., 1999
Glass beads	Zilli et al., 2000
Polyurethane foam	Moe and Irvine, 2000; Woertz et al., 2002
Polystyrene	Ottengraf, 1986; Arulneyam and Swaminathan, 2000
Lava rock	Chitwood & Deviny, 2001; Langolf & Kleinheinz, 2006; Akdeniz et al., 2011
Pine nuggets	Akdeniz et al., 2011
Peat	Oh and Choi, 2000
Sand	Mancebo, 2012

2.10.1 Flax straw

Flax is a blue-flowered crop widely distributed in the cooler climate of western Canada, where it is grown across 0.67 million hectares of land (Wood & Layzell, 2003). Oilseed flax is mostly grown for its seeds; however, there is a down turn to its production since this type of crop takes a

long time to degrade and return to the soil. Most farmers find that flax fibres wrap around machinery and make very painful the simple task of remixing it with soil.

Flax is mainly composed of cellulose (~54%), lignin (~23%) and ash (~3.6%). The high contents of lignin are what make it less biodegradable. One of the few uses of flax straw (which is made up of 25% fibre) is in the paper and pulp industry, without any other main uses, farmers have to burn the straw (Buranov & Mazza, 2008).

Disposing of the straw by burning contributes to GHG emissions and this is why it would be a valuable asset to use flax straw in biofilters not only to decrease the amount that is being burnt but also to reduce other sources of GHGs. It is important to mention, that flax straw has not been used in any other study for biofiltration as of this moment.

2.10.2 Wood shavings

Wood chips, bark and bark mulch have been used as a bulking agent in column experiments (Dixit et al., 2012; Saliling et al., 2007; Nicolai and Janni, 2001; Morgan-Sagastume and Noyola, 2006; Chen and Hoff, 2009) because they are commonly available and inexpensive. These materials provide an increase surface area, porosity (Akdeniz et al., 2011) and also appear to enhance bacterial growth in biofilters most likely due to nutrient availability on their surfaces (Saliling et al., 2007).

Wood shavings were used for this study due to their increased surface area compared to wood chips or bark which should provide a better environment for bacteria to grow in.

2.10.3 Lava rock

This is a porous material that comes from volcanic eruptions where magma contains a very high level of dissolved gasses, which result in vesicles when lava cools in the surface. Its chemical composition is predominantly mafic, which means that it is iron and magnesium rich. The abundance of these elements provide this rock with its distinctive black colour that turns red when oxidized due to exposure to weathering.

In a two year column experiment designed for the removal of alpha-pinene conducted by Langolf and Kleinheinz (2006), lava rock was found to have an excellent resistance to degradation with no visible deterioration. The same material performance was found even after five years of use in an industrial system (Langolf & Kleinheinz, 2006).

Akdeniz et al., (2011) also used lava rock in columns reporting respective oxidation efficiencies of 56%, 88%, 87%, 25%, and 0.7% for ammonia, hydrogen sulfide, total reduced sulfur, CH₄ and nitrous oxide. Columns operated at 90% MC for 6 months with almost no compaction which suggests this material can be used for longer periods of time with practically no degradation.

Chapter Three: **MATERIALS AND METHODS**

The goal of this study was to identify a material or mix of materials that would provide methanotrophic bacteria with a suitable environment to convert CH₄ into CO₂ without showing much degradation, thus hindering bacterial activity. To achieve this, laboratory batch experiments were carried out first to evaluate the effect of different MC and material ratios. Later on, column experiments were done taking into consideration results from previous batch experiments to find the best performing material.

3.1 Materials

Different materials were added to the mixture to provide for a specific characteristic of the previously mentioned desired ones. Listed below are some of these characteristics and the materials that provide them:

- Compost: nutrients, high water holding capacity, bacteria.
- Flax: high water holding capacity (when presoaked), low bulk density, little biodegradability.
- Wood shavings: high water holding capacity, low bulk density, little biodegradability.
- Lava rock: high surface area, very little degradation.

Aside from the physical properties that these materials add to the mix, they are being used for economic reasons:

- Flax is abundant in Alberta and sometimes is disposed of by burning; therefore, using it in biofilter helps recycle it and is available at a very low cost, if not free.

- Wood shavings are readily available in most places at low costs.

3.1.1 Particle sizes

According to Williams and Miller (1992), biofilter beds should contain at least 60% particles with a size greater than 4 mm. This has been taken into consideration for the materials used in these experiments, which also coincides with the fact that particle size was kept closer to the mean size so it is easier to replicate experiments and then to use these materials in the field.

Compost was sieved to a size smaller than 2.35 mm to try and preserve homogeneity and avoid other materials like wood and rock particles that could be present. Pine wood shavings were kept between 2.35 and 10 mm. Flax straw was cut to approximately 5 cm so it could be fitted in the jars and to preserve homogeneity in the columns. Lava rock sizes were kept between 12.5 and 14 mm for the batch experiments and between 20 and 28 mm for the columns. This difference in size is due to the fact that bigger size lava rock particles would not be able to fit in the 1 litre jars and in the columns we were trying to use larger size particles to get a better porosity thus allowing better gas flow while still providing a high surface area due to its high intrinsic porosity. Restriction of particle sizes for the different materials was only done to run experiments. In full size biofilters, materials should be used as they are found to avoid increasing operational costs.

3.1.2 Nutrients

Plant-Prod 30-10-10 fertilizer was used for nutrient addition. This material is a mixture of ammonium nitrate and urea (30%), phosphorus as phosphoric acid (P_2O_5) (10%) and potassium as

soluble potash (K₂O) (10%). Micronutrients found in this fertilizer are boron (0.02%), copper (0.05%), iron (0.1%), manganese (0.05%), molybdenum (0.0005%) and zinc (0.05%).

A nutrient ratio of C:N:P 100:5:1 was chosen for the experiments from aerobic waste water treatment processes (Gray, 1999). From these processes we know that this nutrient ratio supplies enough nutrients for the bacteria to perform their enzymatic activities, in this case to oxidize carbon.

3.2 Methods

3.2.1 Measurement of Moisture and Water Holding Capacity

The amount of moisture that a material is able to retain can be determined by the water holding capacity. First, the sample was introduced into an oven at 105°C. The sample was weighted beforehand along with the container, then when the sample is removed from the oven after approximately 12 hours the sample is weighed again. The difference between weights is the amount of water it contained. Then MC was calculated with Equation 3.1. The sample weight remainder is the dry solids (DS).

$$\% \text{ Moisture Content} = \frac{m_{\text{wet}} - m_{\text{dry}}}{m_{\text{wet}}} \times 100 \quad \text{Equation 3.1}$$

Where:

m_{wet} is the mass of the sample before drying

m_{dry} is the mass of the sample after drying = DS

MC of the different mixtures was determined using Equation 3.2 where the different MC and weights of the individual materials are weighted in to find the mixture's final MC.

$$\% \text{ Moisture Content} = \frac{(MC_C W_C) + (MC_A W_A) + (MC_B W_B)}{W_C + W_A + W_B} \quad \text{Equation 3.2}$$

Where:

MC_C = Moisture Content of compost (%)

W_C = Weight of compost in the mixture (g)

MC_A = Moisture Content of material A (%)

W_A = Weight of material A in the mixture (g)

MC_B = Moisture Content of material B (%)

W_B = Weight of material B in the mixture (g)

Since literature reports high efficiencies from a wide range of MC values, for this study we tried to cover this wide range while focusing on the higher values because it is a fact that low MC hinders oxidation.

To measure FCFC, first the materials were to be dried to 104° C for at least two hours and cooled down to room temperature. A filter paper was wetted, drained and then folded to fit into a funnel (volume = 500 cm³). The weight of the funnel and wet filter paper was measured and then the funnel filled with the tested material at a fixed density up to the top.

Then the funnel was clamped to a stand. The bottom tip of the funnel was connected to a tube which is connected at the other end to a burette filled with water and clamped at the same level. Water was released from the burette slowly to drive off the air from the material placed in the funnel. When the test material is saturated, the tube supplying the water was disconnected from the funnel, and a beaker placed at the bottom of the funnel to collect any drained water. The funnel was covered to prevent loss of moisture from the top surface.

The water was allowed to drain for four to five hours or until no water drained into the beaker for at least 30 minutes. The weight of the funnel with the wet sample was measured and the FCFC calculated. With this procedures in mind we determined the MC and FC of each material and mixtures we worked with. Table 3-1 shows FC for the different materials.

Table 3-1: Water holding capacities for the different materials

Material	FC (w w⁻¹)
Compost	61
Flax	73
Wood shavings	67
Lava rock	18

FC of the different mixtures was calculated using Equation 3.3 where the FC and weights of the materials are weighted in to find the mixture's final MC.

$$\% \text{ Water Holding Capacity} = \frac{(WHC_C W_C) + (WHC_A W_A) + (WHC_B W_B)}{W_C + W_A + W_B} \quad \text{Equation 3.3}$$

Where:

FC_C = Water holding capacity of compost (weight %)

W_C = Weight of compost in the mixture (g)

FC_A = Water holding capacity of material A (weight %)

W_A = Weight of material A in the mixture (g)

FC_B = Water holding capacity of material B (weight %)

W_B = Weight of material B in the mixture (g)

3.2.2 Bulk density and particle density determination

Particle density was calculated by introducing a known weight of the material in a graduated cylinder and then adding a known volume of water. The extra volume in the test tube represents the volume of the material. Then particle density was calculated using Equation 3.4. Table 3-2 shows different materials' particle densities.

$$\rho_s = \frac{W}{V_t - V_w}$$

Equation 3.4

Where:

W = material weight (g)

V_t = total volume (cm³)

V_w = volume of water (cm³)

Bulk density was calculated once the material mixture was inside the column apparatus, since the materials by themselves are too porous and difficult to handle and achieve a proper measurement.

The dry weight of the mixture is known along with the volume it occupies in the column, thus bulk density was calculated with Equation 3.5.

$$\rho_b = \frac{W_s}{V} \quad \text{Equation 3.5}$$

Where:

W_s = weight of solids (g)

V = volume of the mixture (cm^3)

Table 3-2: Dry particle density of the materials

Material	Dry particle density (g cm^{-3})
Compost	1.5
Flax	0.52
Wood shavings	0.43
Lava rock	1.44

3.2.3 Experimental protocol for batch experiments

Batch experiments, also called incubation experiments, are used to define how capable is a certain material of oxidizing CH_4 . In this study, the capacity of different medium with different compost proportions was tested to evaluate which is the lowest amount of compost that can convert CH_4 efficiently.

Batch experiments were conducted to study the following:

- How flax, wood and inert materials, like lava rock, work with compost in providing a suitable environment for bacteria to live in.
- The relationship between CH₄ oxidation rates and the amount of compost and MC.
- Determine the impact of nutrient addition on CH₄ oxidation.

Batch experiments were filled with the desired material proportions and MC, then were sealed and injected with CH₄ to achieve a 5% (v v⁻¹) concentration. This concentration is assumed to be enough based on the work of Kightley et al., (1995) and corroborated by Pokhrel (2006). Jars were measured at fixed intervals (3 to 4 days) to record the decline in the concentrations of CH₄ and calculate the amount of oxidized CH₄. Concentration of CH₄ in the head space was determined using a portable micro gas chromatograph (GC) VARIAN CP 4900. The gas composition was determined using the GALAXIE software package in communication with the GC. The method used for the GC is shown in Table 3-3:

Table 3-3: GC operating conditions

	Column 1	Column 2
Length	10 metres	10 metres
Type	MS5	PPU
Injector temperature	100 °C	100 °C
Column temperature	100 °C	80 °C
Column pressure	30 PSI	40 PSI

Analysis length was 1.2 min with a sample line at 110 °C and a TCD detector for both columns.

3.2.3.1 Screening batch experiments

The purpose of these experiments was to determine:

- The behaviour of these materials in two and three material mixtures along a wide range of material ratios.
- The reproducibility of these type of experiments to further continue experimentation.
- The influence of different amounts of compost in the efficiency of CH₄ oxidation.

The first batch experiments were carried out with flax straw and wood shavings along with compost in different combinations including mixtures with three materials. For this design, the amount of compost was restricted to test the mixture's behaviour with reduced amounts of it; also, the amount of wood shavings was restricted (since flax was thought to perform better). The experiment was designed taking into consideration possible material combinations in 10% increments from 10 to 90% to test a wide range of material amounts. The result was 9 combinations which can be seen in Table 3-4 where 15 g were used as 100% material.

Experiments were done with either one, two or three replicates in 1 litre bottles closed with a septa lid. CH₄ was injected in the bottles and kept at a concentration of approximately 5%, and re-injected twice a week when the concentrations went close to 0%. Oxygen was also injected when the concentrations were below 10% to avoid limiting conditions for the bacteria; MC was kept at FC. Experiments were run for 50 days when some of the oxidation rates started to decline.

Table 3-4: Material combinations used in the three component experiments

Material (weight %)	Batch number								
	1	2	3	4	5	6	7	8	9
Compost	10	10	20	40	40	60	60	70	90
Flax	70	90	60	40	60	20	40	10	10
Wood shavings	20	0	20	20	0	20	0	20	0

3.2.3.2 Statistical design of batch experiments

A 3x2 factorial design was used for two material mixtures. MC was used at three levels (40%, 70% and 100% of FC) as well as material ratios (10/90, 20/80 and 30/70). This design was used to thoroughly evaluate the effect of these parameters on CH₄ oxidation (Table 3-5).

Table 3-5: MC and material ratios for batch experiments

FC (%)	100	70	40
Material ratio (weight %)	10:90	10:90	10:90
	20:80	20:80	20:80
	30:70	30:70	30:70

MC levels were chosen to cover a wide range since literature doesn't provide a single value to use, and flax and wood shavings haven't been tested before. While choosing the levels, we considered that bacterial activity usually needs a minimum of 13% of FC (Figueroa, 1993).

For material ratios we chose a maximum of 30% compost in the mixtures. Reasons behind this decision are based in previous research by Pokhrel (2006):

- Increase in CH₄ oxidation is higher when compost fractions are under 70%. Above this level, the increase is less noticeable and higher compost fractions pose operational complications for biofilter operation discussed further below.
- A comparison between the amount of oxygen consumed by 100% soil to 30:70 soil:compost showed that a high amount of compost consumes 19% more oxygen and produces 10% more CO₂ on a mole basis. This is likely due to the presence of a high bacterial population in compost. If this mechanism occurs at higher depths where oxygen is not so abundant, it could pose a limitation in the amount of oxygen needed by methanotrophs to oxidize CH₄.
- Compost has a high degradation rate which is reflected in compaction over long periods of time. This phenomenon can lead to the creation of anaerobic zones and the production of extra CH₄.

After these considerations, it was decided to use a compost ratio under 70% to avoid compaction problems; furthermore, it was reduced to 30% to avoid oxygen consumption from bacterial activity and to provide enough porosity to ensure the presence of oxygen throughout the entire depth of the bed.

3.2.3.3 Compost-flax and Compost-wood shavings mixtures

Experiments were done with two material components since three component mixtures would be hard to replicate on the field and it is easier to assess the effects of each material with only two of

them. From the previous experiments it was determined that mixtures with as little as 10% compost were viable to sustain methanotrophic activity.

This set of experiments (Figure 3-1) was done in duplicate with the same 15 g of material as the previous experiments, with the different proportions shown in Table 3-6. The levels of CH₄ and oxygen were kept as in the previous experiments and run for a total of 119 days.

Table 3-6: Compost – flax and compost – wood shavings mixtures weight ratios

FC (%)	Compost – flax MC (%)	Compost – wood shavings MC (%)	Material ratio (weight %)
100	70	65	10:90
			20:80
			30:70
70	49	46	10:90
			20:80
			30:70
40	28	26	10:90
			20:80
			30:70



Figure 3-1: Flax and wood shavings batch experiments

3.2.3.4 Compost-lava rock mixtures

Batch experiments with lava rock were designed differently than those with wood shavings and flax straw, since lava rock cannot be compacted. Batch weights were a scale down of column experiments where maximum amounts of lava rock represented a constraint if height was to be comparable through the experiments. The maximum amount of lava rock that could be used in a column was 6.47 kg which was used as 90%, with the remaining 10% as compost with a total weight of 718.8 g. This amounts were used as 100% and then scaled down taking into consideration the approximate volume occupied by this mixtures in Batch experiments. The weights used for the batches still had to be reduced to 10% of this weight since they were not comparable to compost amounts used in previous experiments. The final total weight for the batches was 19.5 g, the different material ratios can be seen in Table 3-7. These experiments (Figure 3-2) were also done in duplicate and run for 119 days.

Table 3-7: Compost – lava rock mixtures weight ratios

FC (%)	Compost - lava rock MC (%)	Material ratio (weight %)
100	31	10:90
		20:80
		30:70
70	21.6	10:90
		20:80
		30:70
40	12.5	10:90
		20:80
		30:70



Figure 3-2: Lava rock batch experiments

3.2.3.5 Nutrient addition for batch experiments

Since lava rock is an inert material, the addition of nutrients is important to improve conditions for bacteria to reproduce. A ratio of 100:5:1 (C:N:P) was used through the addition of 30-10-10 Plant Prod Ultimate fertilizer. A solution of 0.14 g L⁻¹ was used for batch experiments with the same material ratios as the experiments without nutrients (Table 3-7).

3.2.3.6 Statistical tools for analysis

A three-way ANOVA analysis was chosen to test the importance and the correlation between the different factors used in batch experiments. The analysis was done in the software IBM SPSS Statistics 22 using data from duplicate experiments in a Univariate General Linear Model with a 95% confidence level.

Two different ANOVA were carried out, the first one used the factors shown in Table 3-8 where we tested the importance of different material ratios, the use of different bulking materials and the variation of MC.

Table 3-8: Experimental factors and levels for flax straw, wood shavings and lava rock

Factor		Level		
		- 1	0	1
A	Compost : bulking material ratio	10:90	20:80	30:70
B	Bulking material	Flax straw	Wood shavings	Lava rock
C	Mixture MC (%)	40	70	100

The second analysis was done for the lava rock batches to compare the effect of nutrient addition on CH₄ oxidation. In Table 3-9 we can see the factors used for the analysis where instead of the bulking material we have whether the sample had nutrients or not.

Table 3-9: Experimental factors and levels for lava rock with and without nutrients

Factor		Level		
		-1	0	1
A	Compost : bulking material ratio	10:90	20:80	30:70
B	Nutrients	No	/	Yes
C	Mixture MC (%)	40	70	100

3.2.4 Column experiments

This type of experiments has been used to simulate landfill cover systems under controlled conditions (Kightley et al., 1995; Hilger et al., 2000; Visvanathan et al., 1999), but for this study it was used to simulate oxidation inside a biofilter under controlled conditions.

The objectives for these column experiments were:

- Up-scale the best materials found from batch experiments.
- Study gas flow through porous material.
- Observe changes in CH₄ oxidation with time.
- Evaluate water retention of different materials.
- Determine the impact of nutrient addition on CH₄ oxidation.

The column apparatus used in the experiments is depicted in Figure 3-3. Columns were constructed from 1 m long clear plexiglass tubes with a 15.2 cm inner diameter and 0.635 cm thickness and their ends were capped with blind flanges. Gas sampling ports were drilled along the column at 5 cm intervals and threaded for 0.635 cm (1/4 inch) National Pipe Thread (NPT) fittings; the sample ports were fitted with 0.635 cm Swagelok – 0.635 cm male NPT adapters. The Swagelok end of the adapters were fitted with 10 mm silicone septa which allowed for gas sampling. A perforated plastic plate was placed above a 10 cm gravel layer at the bottom of the column. The materials were packed in approximately 10 cm layers to preserve a uniform density.

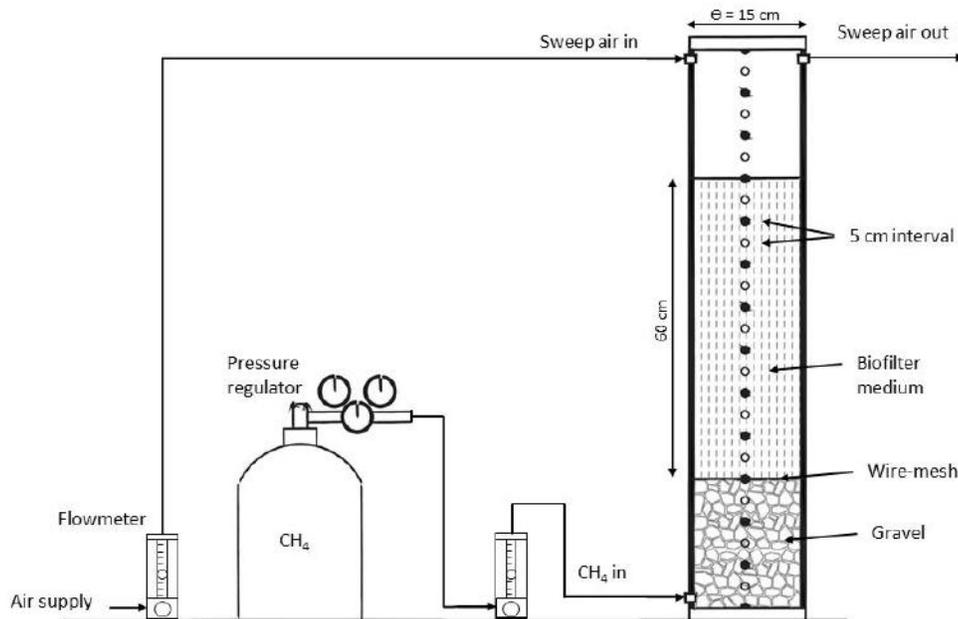


Figure 3-3: Column design for biofiltration experiments

3.2.4.1 Porosity in column experiments.

Determination of the mixture's porosity was done in the columns, since only there can we really see how different porosities affects gas flow through the material. In order to characterize the porosity of material mixtures we used Equation 3.6, shown below.

$$\theta = 1 - \frac{\rho_{bulk}}{\rho_{particle}} \quad \text{Equation 3.6}$$

Where:

ρ_{bulk} = bulk density (g cm^{-3})

$\rho_{particle}$ = particle density (g g^{-1})

3.2.4.2 Empty Bed Residence Time (EBRT) and true residence time (τ)

Other important parameters involved in the column experiment are Empty Bed Residence Time (EBRT) and true residence time (τ). EBRT is the time gas takes to flow through the whole bed volume and is defined by the empty bed filter volume (Equation 3.7). Table 3-10 shows EBRT and residence time for the different materials used in column experiments.

$$EBRT = \frac{V_f}{Q} \quad \text{Equation 3.7}$$

Where:

V_f = the total bed volume (ml)

Q = Flow rate (ml min^{-1})

On the other hand, true residence time (τ) takes into consideration the total granular medium bed volume multiplied by the porosity divided by the gas flow rate (Equation 3.8).

$$\tau = \frac{V_f \theta}{Q} \quad \text{Equation 3.8}$$

Where:

V_f = the total bed volume (ml)

Q = Flow rate (ml min⁻¹)

θ = porosity

Table 3-10: EBRT and Residence time for column experiments

Material ratio (%) (Compost : bulking material)	CH₄ flow rate (ml min⁻¹)	Volume (ml)	EBRT (hr)	Residence time (hr)
30:70 compost : flax straw	190	9500	50	25.5
30:70 compost : wood shavings	190	9700	53.9	26.9
30:70 compost : wood shavings	190	9400	52.2	40.2
30:70 compost : lava rock ^a	190	9100	50.6	22.8
30:70 compost : lava rock ^a	190	8900	49.4	21.8
30:70 compost : lava rock	190	8900	49.4	21.8

^a With nutrient addition

3.2.5 Experimental design for column experiments

In this experiments, CH₄ (99% pure) was supplied from the bottom and air from the top to achieve passive aeration conditions at a rate of 20 ml min⁻¹, gas outflow was collected from the top. Gas

concentration profiles were monitored weekly. Characteristics for these experiments can be found in Table 3-11 and 3-12 and seen in Figure 3-4 and 3-5. Two extra columns were run for the lava rock mixture, further details are given in the results section.

Table 3-11: Compost-flax and Compost-wood shavings column experiment characteristics

Material ratio (%)	Moisture Content (w w ⁻¹)	Porosity	Dry bulk density (g cm ⁻³)
30:70 compost : flax straw	66 ¹	0.80	0.162
30:70 compost : wood shavings	62 ¹	0.73	0.150
30:70 compost : wood shavings	42 ²	0.77	0.150

¹ FC

² 70% of FC

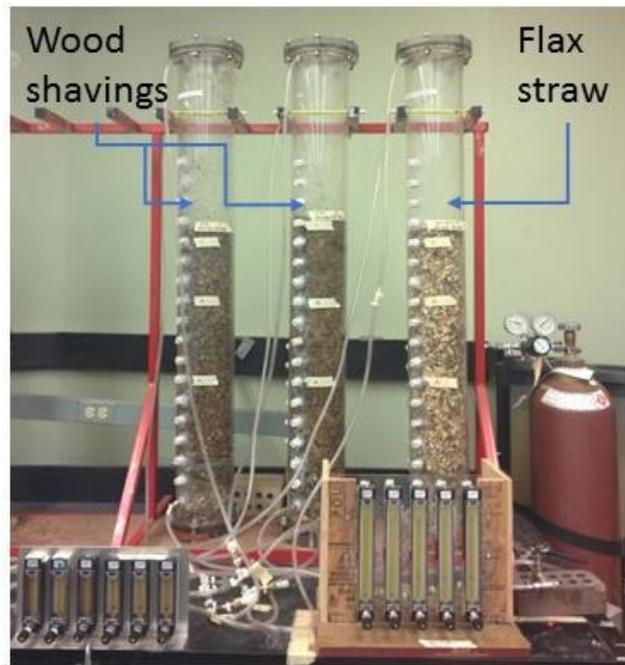


Figure 3-4: Photograph of flax and wood shavings column experiments

Table 3-12: Lava rock mixtures weight ratios

Material ratio (%) (compost : lava rock)	Moisture Content (w w⁻¹)	Porosity	Dry bulk density (g cm⁻³)	Nutrient addition
30:70	12.5 ²	0.45	0.79	No
30:70	12.5 ²	0.44	0.81	Yes
30:70	31 ¹	0.44	0.81	Yes

¹ FC

² 40% of FC



Figure 3-5: Lava rock column experiments

3.2.5.1 CH₄ removal efficiency

The removal efficiency was calculated from Equation 3.9 (Haththotuwa et al., 2012) using the CH₄ and nitrogen concentrations from the inflow and outflow gases at the bottom and top of the filter bed. With this formula we take into consideration the dilution of CH₄ with the sweep air from the top.

$$\text{Removal efficiency} = \left\{ \frac{\left[C_{m,in} - C_{m,out} \left(\frac{C_{n,in}}{C_{n,out}} \right) \right]}{C_{m,in}} \right\} * 100 \quad \text{Equation 3.9}$$

Where:

$C_{m,in}$ is the CH₄ concentration in column inlet point

$C_{m,out}$ is the CH₄ concentration in column outlet point.

$C_{n,in}$ is the nitrogen concentration in column inlet point

$C_{n,out}$ is the nitrogen concentration in column outlet point.

The CH₄ concentration at the outlet point was determined using a portable micro gas chromatograph (GC) VARIAN CP 4900. The gas composition was determined using a GALAXIE software package in communication with the GC.

3.2.5.2 Nutrient addition for column experiments

Since lava rock is an inert material, the addition of nutrients is important to improve conditions for bacteria to reproduce. A ratio of 100:5:1 (C:N:P) was used as optimum nutrient concentrations with the addition of 30-10-10 Plant Prod Ultimate fertilizer. A solution of 60 g L⁻¹ with nitrogen

concentrations of 4050 mg L^{-1} was used for column experiments and supplemented to the material through moisture addition.

Chapter Four: **EXPERIMENTAL RESULTS AND DISCUSSION**

4.1 Batch experiments

In this experimentation stage we tested oxidation efficiencies of different mixtures in batch experiments. Several initial mixtures included three materials, but were discarded for more simple mixes with only two components due to the difficulty it would prove to use them in the field.

4.1.1 Screening experiments

Cumulative CH₄ oxidation after 50 days for three component mixture experiments is presented in Figure 4-1. From the 9 different mixtures we can see the highest oxidation in the mixture with 70:10:20 (compost:flax:wood shavings) followed by 40:60:0 mix with a decrease of 27% and finally the mixture with 10:70:20 with a decrease of 35%. Seeing that the lowest fraction of compost achieved satisfactory oxidations, it was concluded that further experiments could be done within the lower range of compost ratios.

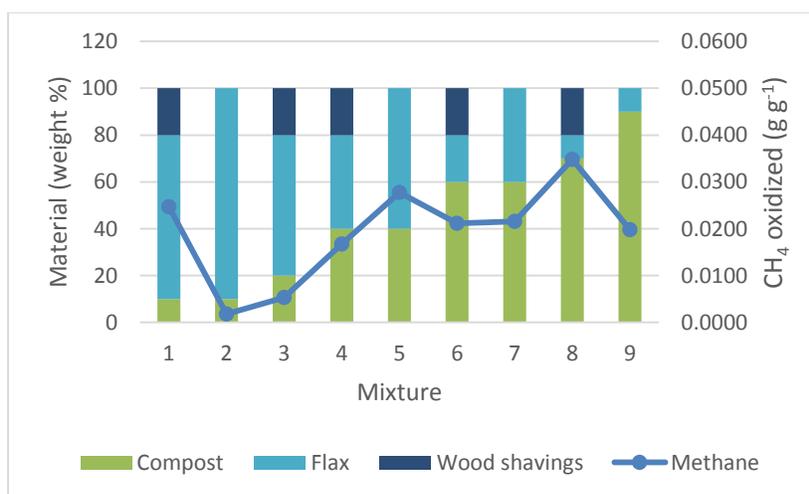


Figure 4-1: Cumulative oxidation for different compost -flax - wood shavings mixtures at FC

4.1.2 Methane oxidation in Compost-flax and Compost-wood shavings mixtures

Figure 4-2 shows CH₄ oxidation rates after 119 days for compost and flax mixtures at different MC. The highest average oxidation can be seen for 30:70 at FC. The second highest oxidation can be seen in the mix 30:70 at 70% of FC with decrease of 8.5% and in third place we have the mix of 20:80 at FC with a decrease of 19%. For this material we can see that the lowest performance was in the mix with 20:80 at 40% of FC.

Figure 4-3 shows the oxidation trends for Compost-wood shavings mixtures after 119 days where we can see that the best performing mix overall was the 30:70 mix at FC closely followed by the same ratio but at 70% of FC with only a decrease of 4.6%. The third best mixture results from the 20:80 ratio at FC whereas the least effective is the same ratio but at 40% of FC.

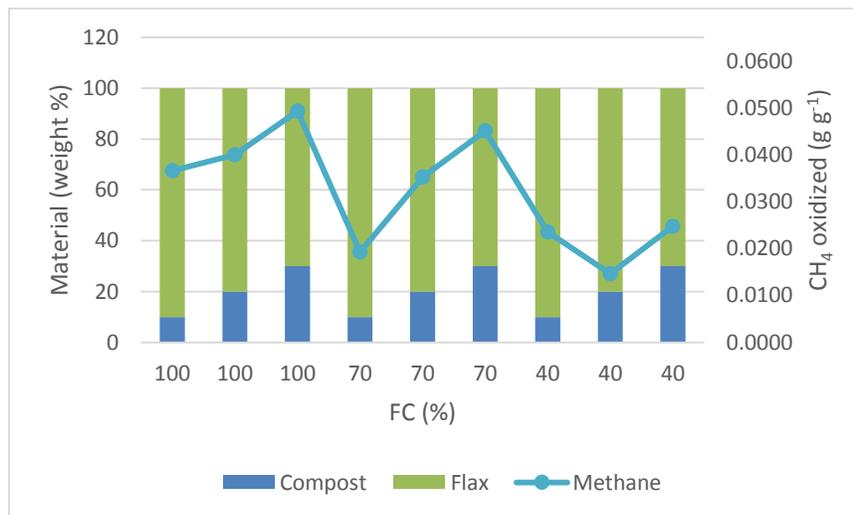


Figure 4-2: Cumulative oxidation for compost - flax mixtures at different MC

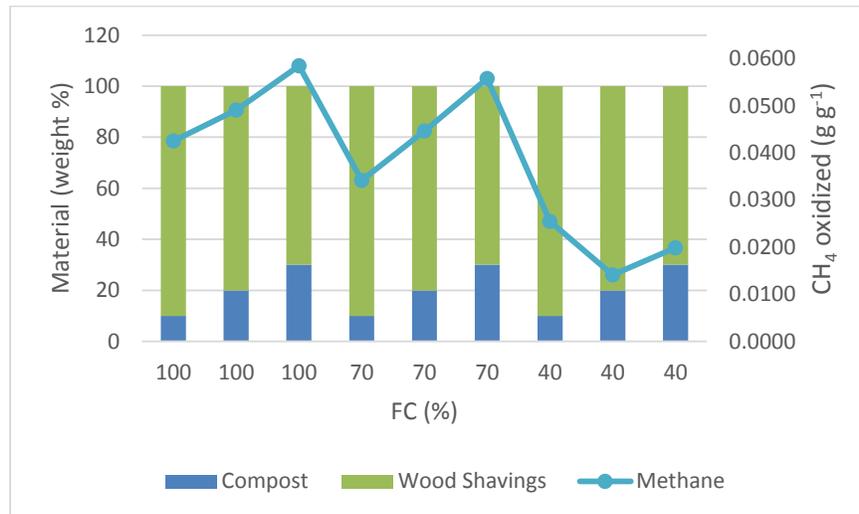


Figure 4-3: Cumulative oxidation for compost - wood shavings mixtures at different MC

4.1.3 Methane oxidation efficiencies in Compost-lava rock mixtures

Where lava rock mixtures are concerned we have a different overall trend from flax and wood shavings mixtures. In this material we can see from Figure 4-4 that the best performing mixtures can be found in the 40% of FC range with the highest efficiency in the mix with a 30:70 ratio. Second to this mix is the 10:90 ratio at the same 40% of FC, and thirdly the 30:70 ratio at FC. The efficiency in these mixtures decreased by 11.4 and 11.8% respectively. The mix with the poorest performance in this case was the 10:90 ratio at FC.

It is very likely that the highest efficiency was found in the mixtures with the lowest MC since the ones at FC appear to have compacted (because of the lack of homogeneity) thus creating zones with little gas exchange (possibly anaerobic), which in turn decreases performance.

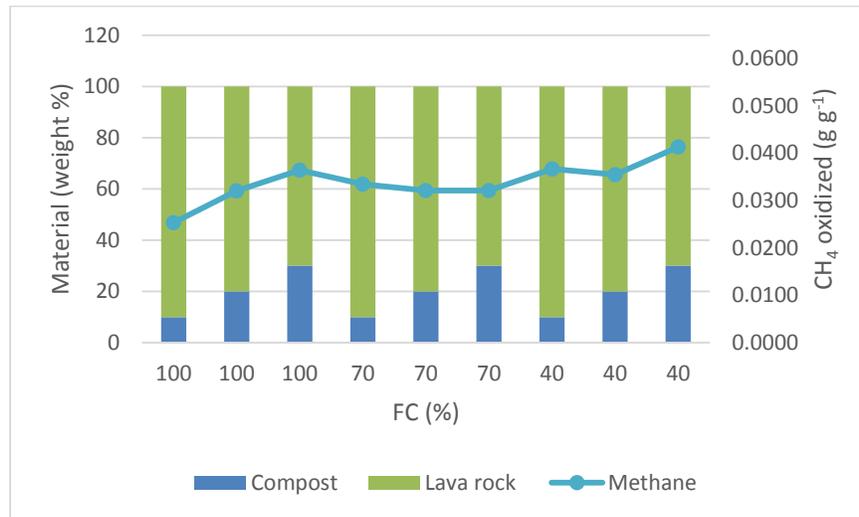


Figure 4-4: Cumulative oxidation for compost - lava rock mixtures at different MC

4.2 Effect of Nutrient addition on methane oxidation

The same mixing ratios of compost and lava rock from the previous experiment were used, only the addition of nutrients was the new variable. From Figure 4-5 we can see the oxidation for compost - lava rock mixtures with nutrients, where the best performing mixture was the 30:70 ratio followed by the 10:90 and the 20:80 all at 40% of FC, the reduction in the oxidation for these two mixtures was 6.8% and 16.7% respectively. The least effective mix was found in the 30:70 ratio at 70% of FC with an efficiency reduction of 46.8%.

The comparison between experiments with and without nutrients can be seen in Figure 4-6. Here we can appreciate that there is an improvement for mixtures at FC and 40% of FC when adding nutrients. This improvement in the oxidation capacity does not apply for the 70% of FC mixes, most likely due to the fact that there was not enough moisture compared to the amount of compost for bacteria to live in.

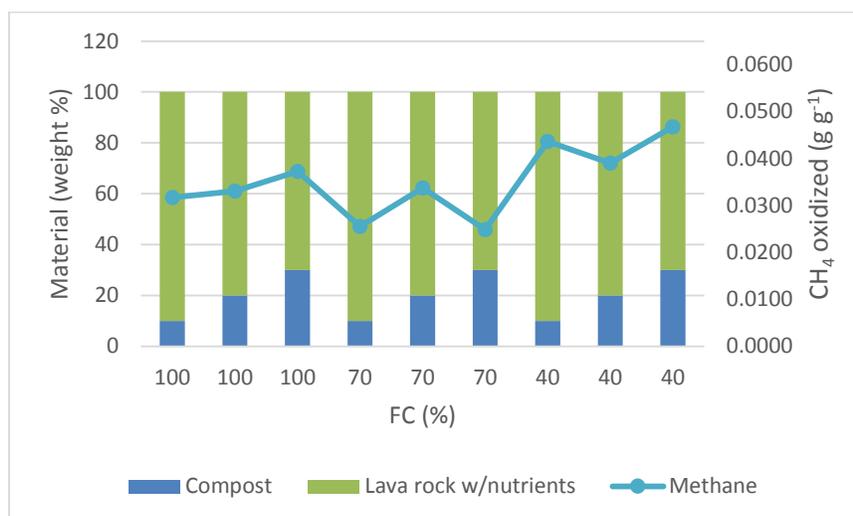


Figure 4-5: Cumulative oxidation for compost - lava rock mixtures with nutrients at different MC

From Figure 4-6 we can see that the highest increase in oxidation is found for the material ratio of 30:70 with an increase of 13% followed by the 10:90 ratio and the 20:80 with 18.8 and 9.9% respectively, all at 40% of FC. In experiments at FC, we can see an increase mostly in the 10:90 mixture, the rest have almost the same performance. In the 70% of FC mixtures we see that there was a negative effect with the addition of nutrients, mostly for the 10:90 and 30:70 mixes; a slight increment was observed on the 20:80 ratio.

Like in the experiments by De Visscher et al., (1999), where an increase in bacterial activity was found with the addition of ammonium and nitrate, for this study it was found that a concentration of 0.14 g L⁻¹ of ammonium and nitrate can increase bacterial activity. Like in the experiments by Albanna et al., (2007) it was proven that urea had a positive effect on CH₄ oxidation as well.

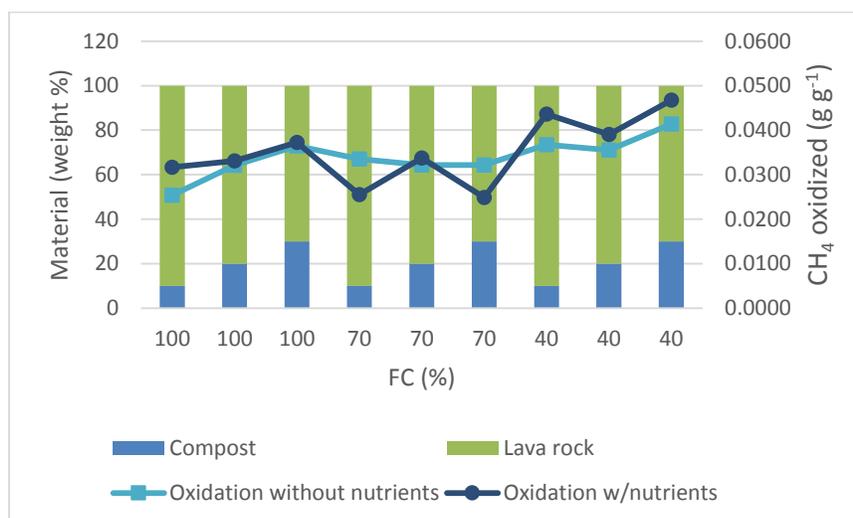


Figure 4-6: Comparison between lava rock experiments with and without nutrients

4.3 Effect of moisture content on methane oxidation in batch experiments

In Figure 4-7 we can see that batch experiments using flax straw have better performance at FC and the lowest at 40% of FC. There is a difference of approximately 20% between experiments with a ratio of 20:80 at 70% and FC with their counter part of 30:70. For the samples with a ratio of 30:70, we see there is only an 8% difference if the material is at FC or at 70%; this is the least difference in between samples at different MC.

Figure 4-8 shows us that approximately the same trend that is found in flax experiments is seen with wood shavings, where the batches at FC show the best performance. For the mixture with the best performance, the 30:70 ratio, we see that there is only a difference of 3.4% between the experiments at FC (highest) and at 70% of FC. An interesting trend can be seen for batches at 40% of FC where the best performing mix was 10:90 ratio even though it has the least amount of compost.

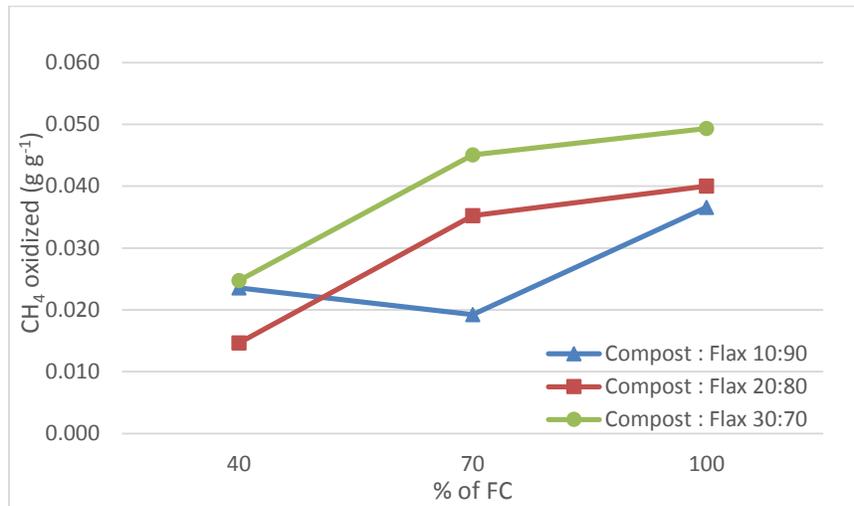


Figure 4-7: Flax and compost batch experiments at different MC

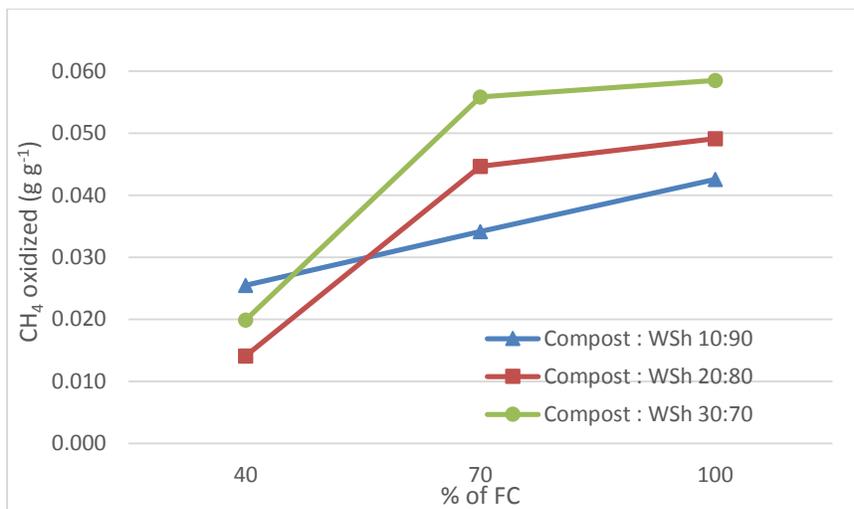


Figure 4-8: Wood shavings and compost batch experiments at different MC

Figures 4-9 and 4-10 show the performance of lava rock mixtures with and without nutrients respectively. This two sets of experiments show a similar pattern between themselves, but a very different one than flax and wood shavings. Here we have the highest performance in the lowest

MC at 40% of FC followed by the mixtures at FC and finally the lowest at 70% of FC. On average, the mixtures with a 30:70 ratio showed the highest oxidation while the 20:80 ratio showed the most constant one with very little difference despite the change in MC.

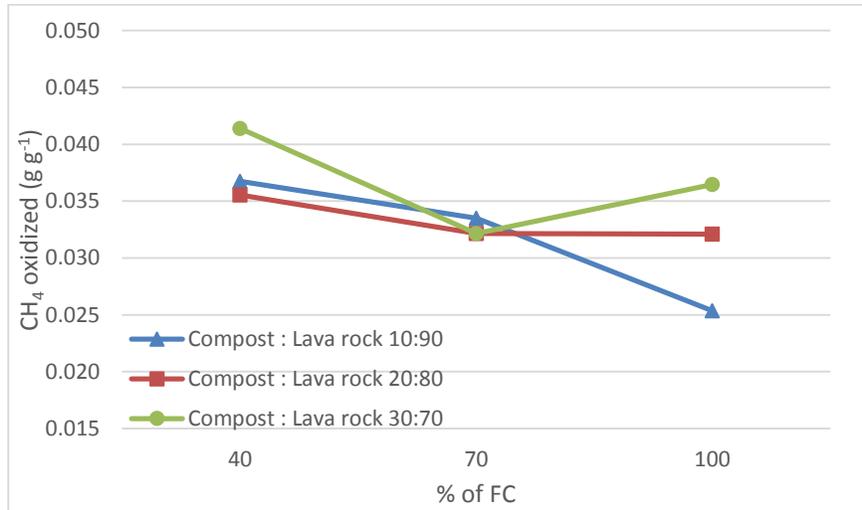


Figure 4-9: Lava rock and compost batch experiments at different MC

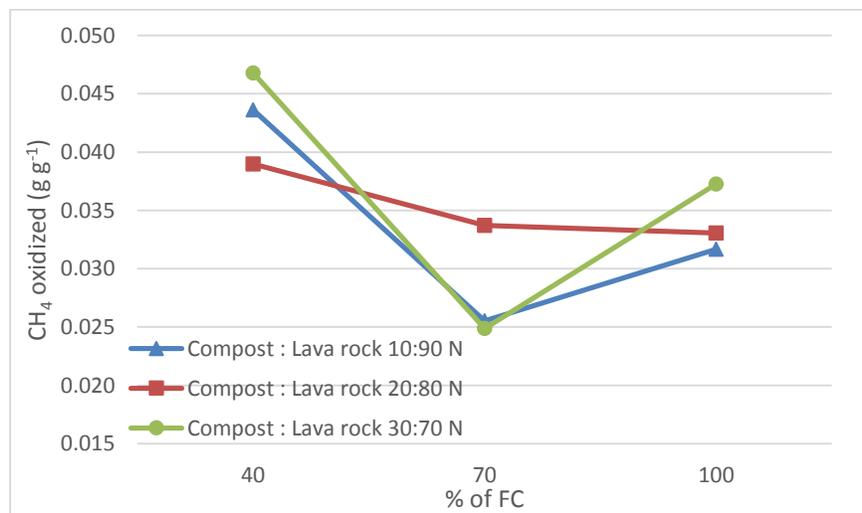


Figure 4-10: Lava rock with nutrients and compost batch experiments at different MC

4.4 Statistical analysis for batch experiments

Interpretation of results from the ANOVA analysis in the SPSS software are done with the significance value. SPSS does the analysis of the F critical value for the specified confidence level and expresses the importance of the parameter in terms of the significance. If this number is lower than the confidence level used for the analysis, in this case 0.05 (95%), we interpret that this factor is highly significant. If it is higher than the confidence level, it is less significant.

Table 4-1: ANOVA for flax straw, wood shavings and lava rock

Source	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Material ratio (A)	5212.942	2	2606.471	14.068	0.000
Bulking material (B)	5474.082	2	2737.041	14.772	0.000
Mixture MC (C)	10423.041	2	5211.521	28.127	0.000
A*B	482.865	4	120.716	0.652	0.631
A*C	2961.254	4	740.313	3.996	0.011
B*C	14619.400	4	3654.850	19.726	0.000
A*B*C	2480.435	8	310.054	1.673	0.151
Error	5002.633	27	185.283		
Total	454300.933	54			
Corrected Total	46656.653	53			

From Table 4-1 we can observe that all the individual factors play a very important role in the oxidation of CH₄. Regarding the interactions, we can see that the most important one was B*C followed by A*C since significances are lower than 0.05. While these interactions are very

significant, we found that A*B and the three way interaction A*B*C are not statistically significant at the 95% confidence level.

From the analysis of the data in Table 4-2 we can conclude that the statistical significance for the interactions between factors is low as well as the individual factors with the exception of MC.

Table 4-2: ANOVA for lava rock with and without nutrients

Source	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Material ratio (A)	2419.704	2	1209.852	3.097	0.070
Nutrients (B)	696.581	1	696.581	1.783	0.198
MC% (C)	3897.976	2	1948.988	4.989	0.019
A*B	203.612	2	101.806	0.261	0.773
A*C	512.362	4	128.090	0.328	0.856
B*C	216.447	2	108.223	0.277	0.761
A*B*C	1356.853	4	339.213	0.868	0.502
Error	7031.274	18	390.626		
Total	397346.445	36			
Corrected Total	16334.808	35			

4.5 Column experiments

Given the oxidation results from the different batch experiments, showing the best three for each mixture in Table 4-3, we considered the best 6 to pursue column experiments. From the ANOVA analysis, we know that MC is the most important factor followed by the interaction of the material

and the MC, therefore, we used the mixtures containing the highest material ratio combined with the most suitable MC for that ratio.

Even though in the lava rock experiment at FC we achieved lower results, we know from the flax and wood shavings experiments, that mixtures at FC usually yield the best results therefore we decided to include that MC for the column experiments.

Table 4-3: Batch experiments highest efficiencies

Bulking material	Material ratio (%)	FC (%)	Oxidation (g g⁻¹)
Wood shavings	30:70	100	<u>0.05849</u>
Wood shavings	30:70	70	<u>0.05581</u>
Flax	30:70	100	<u>0.04930</u>
Wood shavings	20:80	100	0.04908
Lava rock ^a	30:70	40	<u>0.04677</u>
Flax	30:70	70	0.04506
Lava rock ^a	10:90	40	0.04362
Lava rock	30:70	40	<u>0.04138</u>
Flax	20:80	100	0.0400
Lava rock ^a	20:80	40	0.03899
Lava rock	10:90	40	0.03672
Lava rock	30:70	100	<u>0.03646</u>

^a With nutrient addition

4.5.1 Methane oxidation efficiencies in flax and wood shavings mixtures

Materials for column experiments were chosen based on the best performing mixtures in previous batch experiments which can be seen in Table 4-3 . Materials used for the first set of columns were wood shavings in a 30:70 ratio at FC, which had the best performance, followed by the same mixture ratio but at 70% of FC. The third best result was found for compost with flax straw in a 30:70 ratio at FC.

Figure 4-11 shows different oxidations from flax and wood shavings column experiments over a 91 day period where no compaction from the material was found as well as little to no loss of moisture. Formation of EPS was only seen in the flax experiment in the upper 20 cm where oxygen concentrations were higher.

In these experiments, maximum average efficiency was found for the column with a 30:70 ratio of compost and wood shavings at 70% of FC, with an oxidation of $278 \text{ g m}^{-3} \text{ d}^{-1}$; the second highest oxidation was found in the 30:70 compost and wood shavings at FC with an average oxidation of $265 \text{ g m}^{-3} \text{ d}^{-1}$, finally we have the mixture of 30:70 compost and flax with an average oxidation of $231 \text{ g m}^{-3} \text{ d}^{-1}$. In Table 4-4 we can see the maximum oxidation achieved by each experiment as well as the standard deviation

From Table 4-4 and Figure 4-11 we can see that the steadiest oxidations (lowest standard deviation) were achieved by the wood shavings mix at 70% of FC followed by the same material but at FC. We can also see that at the end of the experiment we still had steady oxidation rates that

are expected to continue if these experiments were to be carried out for longer periods of time. Unfortunately, experiments could not be run for a longer period of time to verify this.

Table 4-4: Flax and wood shavings column oxidation

Bulking material	% of FC	Nutrient addition	Average oxidation (g m⁻³ d⁻¹)	Maximum oxidation (g m⁻³ d⁻¹)	Standard deviation
Wood shavings	70	No	278	294	18
Wood shavings	100	No	265	288	19
Flax	100	No	231	236	37

Gas profiles for the column experiments' day of maximum oxidation are shown in Figures 4-12, 4-13 and 4-14. Gas profiles for the whole experimental period can be found in appendix A.

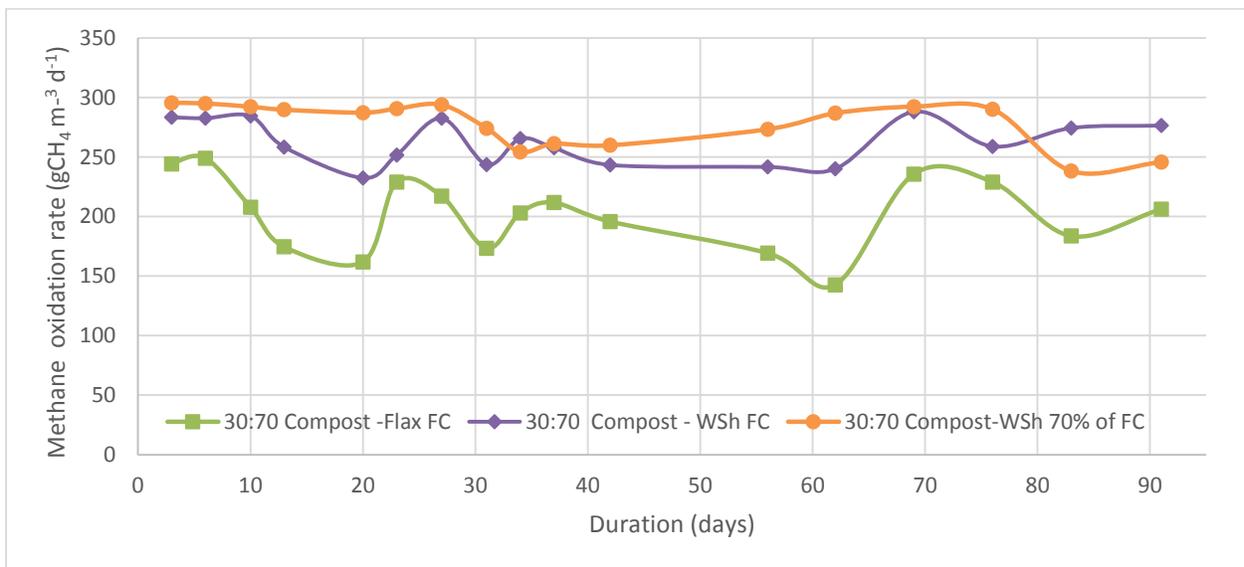


Figure 4-11: Oxidation from flax and wood shavings column experiments

From these profiles we can see that these very porous materials allowed for a very favorable oxygen flow since we have its presence throughout the whole bed. Lower oxygen levels are seen close to the bottom because penetration is difficult due to the aeration regime being passive and because of bacterial activity. Oxygen levels vary from 10% to above 0%, therefore there were no observed anaerobic layers.

In the wood shavings and flax columns at FC we can see that the highest oxidation zone occurred at 40 cm, where CH₄ concentrations drop sharply and there is less available oxygen. From these profiles, we can see that the flax mixture could have provided better conditions for oxidation. This is mostly reflected by the crossing of CH₄ and nitrogen gas profiles further below than in the wood shavings experiments which means CH₄ oxidation started from a lower depth. The formation of EPS in the top 20 cm is most likely the reason why flax did not show a higher oxidation.

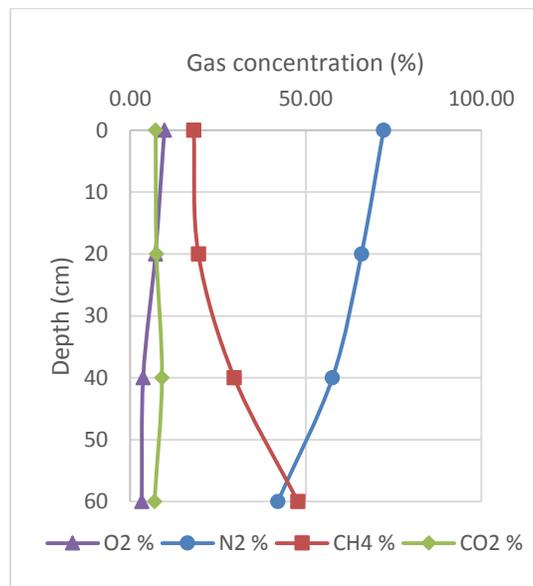


Figure 4-12: Gas concentration profile at maximum CH₄ removal; day 69 (30:70 compost:flax)

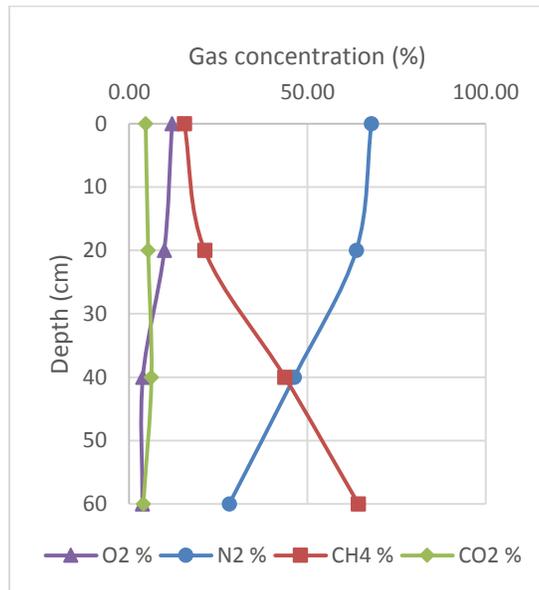


Figure 4-13: Gas concentration profile at maximum CH₄ removal; day 69 (30:70 compost:wood shavings at FC)

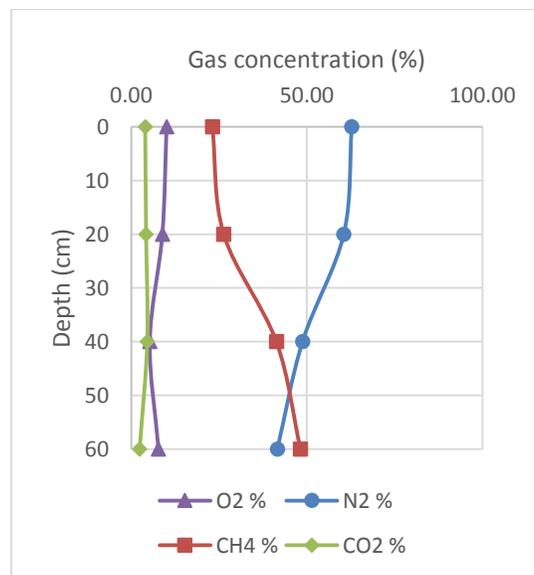


Figure 4-14: Gas concentration profile at maximum CH₄ removal; day 27 (30:70 compost:wood shavings at 70% of FC)

4.5.2 Methane oxidation efficiencies in Compost-lava rock mixtures

As with the flax and wood shavings experiments, we used Table 4-3 as a basis to choose the material for the lava rock column experiments. Where lava rock is concerned, we chose from amongst the best mixtures using the mixtures with a 30:70 ratio; and a MC at FC and at 40% of FC.

Lava rock column experiments are shown in Figure 4-15, where we can compare the effects of different material ratios, MC and addition of nutrients. After running the experiments for 91 days we saw almost no compaction from the material, little loss of moisture and no visible EPS formation.

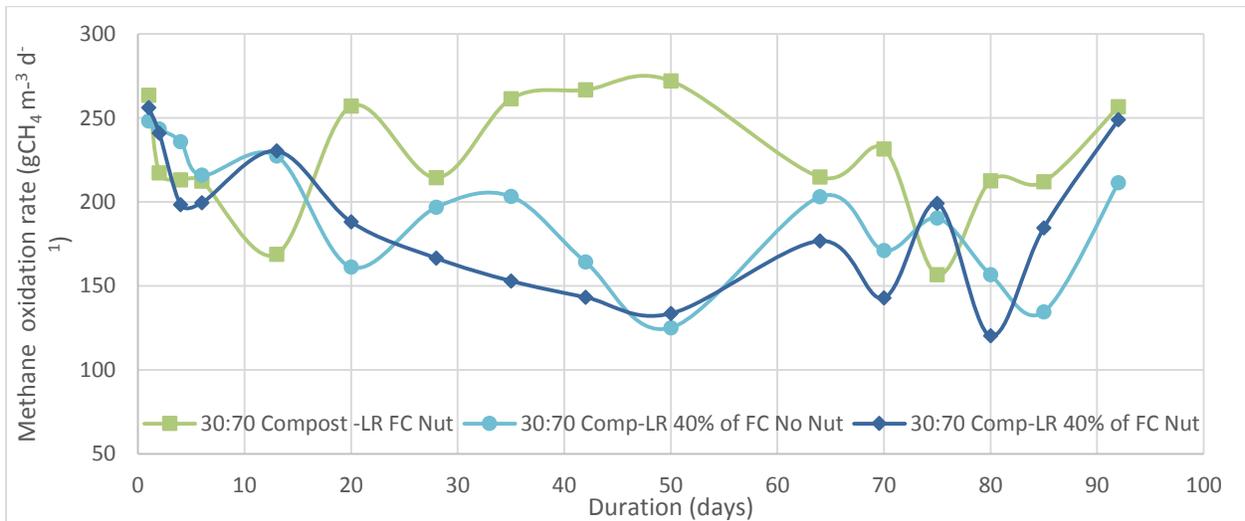


Figure 4-15: Oxidation from lava rock column experiments

In Figure 4-15 and Table 4-5 we can see that the lava rock mixture with a 30:70 material ratio at FC with nutrients showed the highest average oxidation with 231 g m⁻³ d⁻¹ followed by the 30:70

ratio at 40% of FC with no nutrients with an average oxidation of $193 \text{ g m}^{-3} \text{ d}^{-1}$. The third best performance was achieved by the 30:70 ratio at 40% of FC with nutrients with $186 \text{ g m}^{-3} \text{ d}^{-1}$.

Table 4-5: Lava rock column experiments oxidation

Bulking material	% of FC	Nutrient addition	Average oxidation ($\text{g m}^{-3} \text{ d}^{-1}$)	Maximum oxidation ($\text{g m}^{-3} \text{ d}^{-1}$)	Standard deviation
Lava rock	100	Yes	231	272	29
Lava rock	40	No	193	211	38
Lava rock	40	Yes	186	248	42

From nutrient addition in the column experiments we can observe that the addition of 60 g L^{-1} of fertilizer proved to have an inhibitory effect in the 30:70 lava rock column at 40% of FC, however in the 30:70 lava rock at 100% of FC column we saw the highest oxidation. These results lead us to conclude that for this nutrient concentrations, a higher MC is needed to avoid an inhibitory effect on CH_4 oxidation. We can clearly see the negative effect of nutrients at the beginning of the experiment in the lava rock column at 40% of FC followed by an increase in oxidation most likely due to the reduction of nutrient concentrations to those acceptable to bacteria.

Gas profiles from the day with the highest oxidation can be seen in Figures 4-16 to 4-20. The rest of the profiles can be found in Appendix A.

Gas profiles from lava rock allowed us to observe that this material provided enough porosity so that there were no anaerobic zones throughout the bed. Even though oxygen levels were above 10%, there was no visible production of EPS. As well as with flax and wood shavings experiments,

maximum oxidation rates were found at a depth 40 cm deep where CH₄ concentrations dropped considerably.

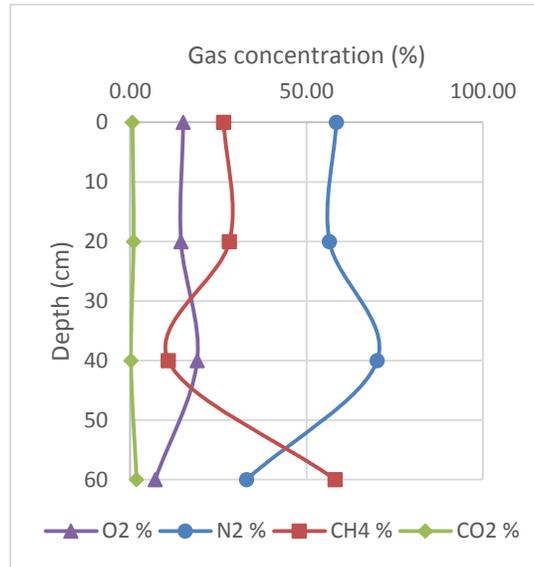


Figure 4-16: Gas concentration profile at maximum CH₄ removal; day 70 (30:70 compost:lava rock at FC)

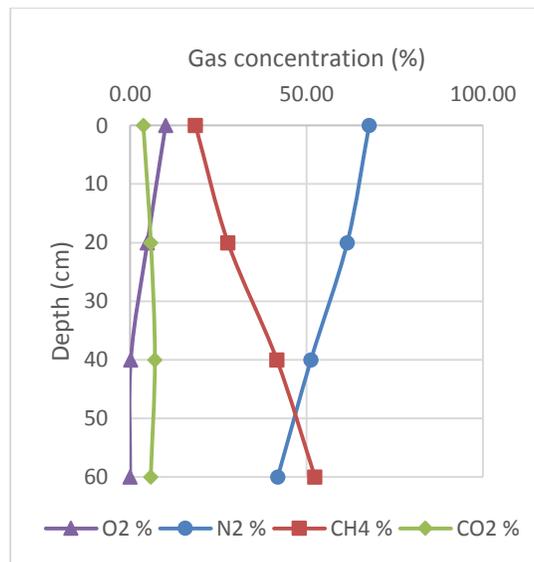


Figure 4-17: Gas concentration profile at maximum CH₄ removal; day 91 (30:70 compost:lava rock at 40% of FC)

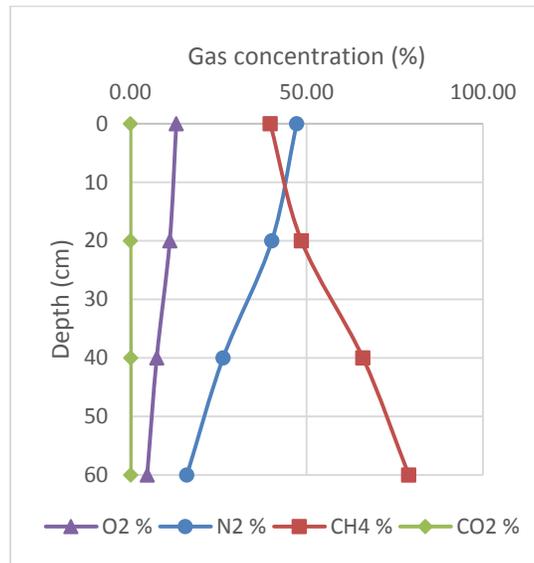


Figure 4-18: Gas concentration profile at maximum CH₄ removal; day 91 (30:70 compost:lava rock at 40% of FC with nutrients)

4.5.3 Comparison between column experiments

From

Table 4-6 and Figure 4-19 we can see the results from column experiments that were run with a 30:70 material ratio with compost and the bulking material mentioned in the table. The best removal efficiencies were provided by wood shavings at 70% of FC with a removal efficiency of 89% and followed by the same mixture at FC (88%) and lava rock at FC (75%) with the addition of nutrients.

Wood shavings columns showed the steadiest and highest oxidations, it is very likely that this is the result of this being a very homogeneous mixture. For lava rock we can see fluctuations in the oxidation, probably due to preferential gas flow since compost was not completely adhered to lava

rock thus creating spaces between these two materials. A similar behaviour can be seen with flax straw which also was less homogeneous than wood shavings.

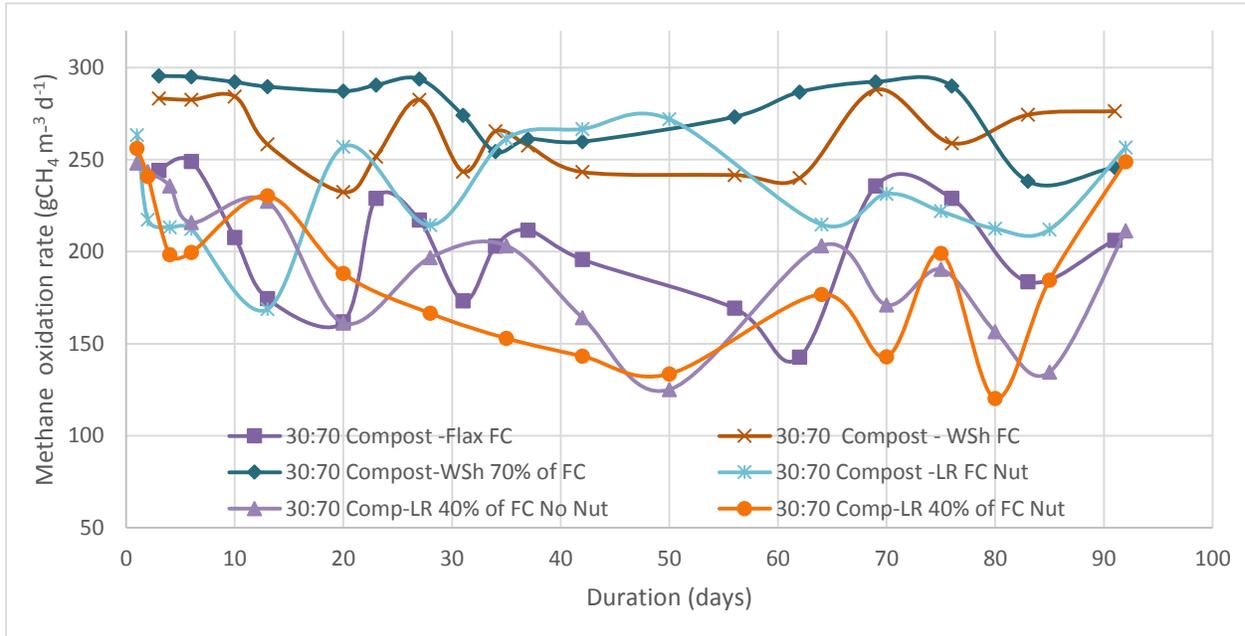


Figure 4-19: Column experiments oxidation comparison

Table 4-6: Column experiments highest CH₄ removal efficiencies

Bulking material	% of FC	Nutrient addition	Average oxidation (g m⁻³d⁻¹)	Maximum oxidation (g m⁻³d⁻¹)	Removal efficiency (%)
Wood shavings	70	No	278	294	89
Wood shavings	100	No	265	288	88
Lava rock	100	Yes	227	272	75
Flax	100	No	231	236	70
Lava rock	40	No	193	211	58
Lava rock	40	Yes	186	248	56

4.5.4 Comparison with values reported in literature

The goal of this study was to develop a mixture that would provide methanotrophic bacteria with conditions similar to, if not better than, compost so that they can convert CH_4 to CO_2 effectively. In Figure 4-20 we can see the comparison between columns with the highest oxidations from this study and the results from experiments conducted by Pokhrel (2006) where columns were filled with 100% compost and 30:70 compost:soil. It is evident that oxidation efficiency from columns with a lower percentage of compost is less than those with 100% compost, but the life time for the new materials is longer while preserving a good porosity. This longer lifetime can be seen especially for the highest oxidizing columns with the lava rock mixtures, when after 91 days oxidation is still occurring at stable rates.

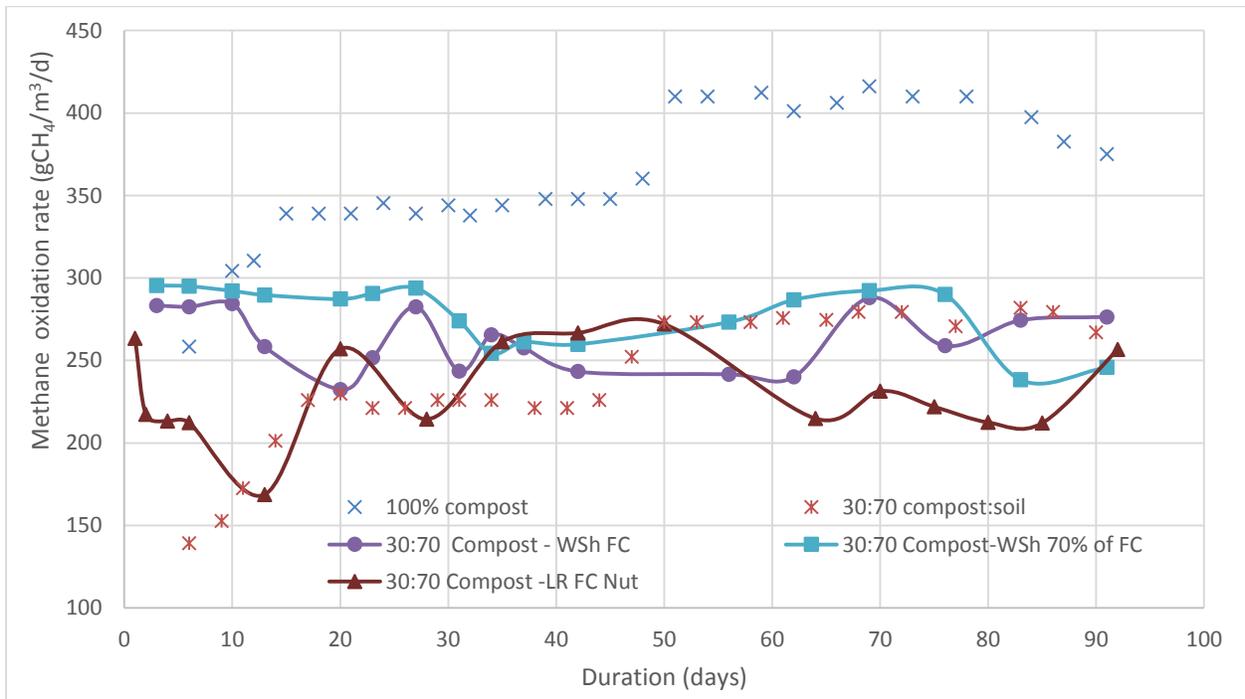


Figure 4-20: Comparison with experiments by Pokhrel (2006)

Compared to the average high oxidation of $410 \text{ g m}^{-3} \text{ d}^{-1}$ by columns with 100% compost, oxidation from columns with 30:70 compost:wood shavings at 70%, wood shavings with a 30:70 ratio at FC and lava rock with a 30:70 ratio at FC with nutrients and yielded lower oxidations by 32%, 35% and 45% respectively. In this study, CH_4 flow rates were not increased as in the study by Pokrel (2006) since no column achieved a 100% elimination capacity.

Compared to the oxidation by the 30:70 compost:soil column ($282 \text{ g m}^{-3} \text{ d}^{-1}$), this study's experiments yielded lower oxidations by 1%, 6% and 20% respectively. From these experiments we can see that bulking materials such as wood shavings and lava rock provide conditions similar to those of soil representing an option to this material.

It is to be noted that even though column experiments were run for a short period of time, wood shavings at FC and lava rock showed no signs of an oxidation decline whereas the 100% compost column did. This results lead us to the conclusion that even though oxidation rates were not very high we can see that they were more stable and should continue exhibiting this trend for a longer period of time. This characteristic of lava rock, and the fact that studies where columns that were run for 5 years showed practically no degradation, provide biofilters with a material that allows for a good oxygen flow and a longer service period thus reducing operational costs.

In the case of wood shavings we have proved that this is a new viable material to be used in Biofilters with low flow rates and CH_4 concentrations. An interesting approach to the use of this material is the doubling even tripling the height of the biofilter thus incrementing CH_4 oxidation

without concerns from compacting material. This would have a good application in projects where surface area is restricted and has to be optimized.

Chapter Five: CONCLUSIONS AND RECOMMENDATIONS

Compost as a material for biofilters has proven to yield high CH₄ oxidation results. The problem with this material is it degrades and its performance decreases. Finding alternate materials to mix compost with is crucial to extend the life of biofilters and to reduce associated costs.

5.1 Conclusions

From the batch experiments we were able to determine:

- Material mixes with as little as 10% compost (by weight) are able to provide the characteristics that bacteria need to live and convert CH₄ to CO₂. It is evident that increasing the compost fraction increases the oxidation capacity but makes the mix more susceptible to performance reduction overtime. The highest performance for all the bulking materials was found with 30% of compost in the mixture.
- Flax and wood shavings supported the highest oxidation at the highest MC. Lava rock was found to perform better at 40% of FC but this is most likely due to the formation of anaerobic zones in mixtures with a high MC.
- Nutrient addition had a positive effect in batch experiments for lava rock mixtures at 40% of FC but the mixtures at 70% and 100% of FC showed either a reduction in the oxidation or almost no increase at all. This result was further explained by the ANOVA analysis where we see that the nutrient factor is not very important in providing higher oxidations.
- From the ANOVA analysis we know that changing the MC provides the most drastic change in results, followed by the interaction of Bulking material * MC and Material ratio

* MC. With this information considerable changes can be done to increase the oxidation efficiency making changes only amongst these factors.

From the column experiments we concluded:

- Column experiments with wood shavings at 70% of FC achieved the highest oxidations since it was a very homogeneous material that allowed a proper gas flow and provided enough moisture for bacteria to live.
- Column experiments that were carried out with a mixture other than 100% compost show lower CH₄ oxidations, but provide a material that does not show signs of a decline in oxidation over the same period of time where compost columns did start to show this.
- Regarding flax and wood shavings, the loss of MC was nearly negligible and it is very likely that in a longer experimental period, wood shavings would have continued to exhibit higher oxidation rates than flax since the material's homogeneity was higher and there was no visible production of EPS.
- Lava rock experiments also showed little loss of MC ($\approx 6\%$) but less stable oxidation rates, most likely due to preferential gas flow since material was not that homogeneous.
- From nutrient addition of 60 g L⁻¹ in column experiments, we conclude that MC should be at 100% of FC to provide the proper concentrations for an increase in oxidation, otherwise at 40% of FC nutrients have an inhibitory effect.
- Considering the results from this study, we conclude that the most suitable material to provide bacteria with a favorable environment to oxidize CH₄ while sustaining little to no degradation or loss of MC, is the compost:wood shavings mixture in a 30:70 ratio at 70% of FC with no nutrient addition showing an average 89% removal efficiency .

- This new media has certain limitations since it cannot oxidize high volumes of gas, but instead should provide longer operational times with steady oxidation rates and reduced maintenance costs.

5.2 Recommendations

- Future experiments using a material mixture with a bulking material and compost should consider using 30% of compost at least to better evaluate the increase in oxidation versus the operational downsides of a higher compost fraction.
- Very different levels of moisture were found to provide high oxidations for the materials used in this study, therefore, it is suggested that a wide range of MC levels be used when screening for new materials for biofiltration.
- Wood shavings and lava rock provided the best conditions for bacterial development and proved to be easy to work when preparing a mixture. Field trials should be conducted with these materials to assess their effectiveness in a larger scale and longer time periods.
- In order to enhance bacterial activity, more comprehensive experiments should be done regarding nutrient addition, with different ratios in different materials.

References

- Abichou, T., Asce, M., Powelson, D., Chanton, J., Escoriza, S., & Stern, J. (2006). Characterization of Methane Flux and Oxidation. *Journal of Environmental Engineering*, 132(2), 220–228.
- Akdeniz, N., Janni, K. a, & Salnikov, I. a. (2011). Biofilter performance of pine nuggets and lava rock as media. *Bioresource Technology*, 102(8), 4974–80. doi:10.1016/j.biortech.2011.01.058
- Albanna, M., Fernandes, L., & Warith, M. (2007). Methane oxidation in landfill cover soil; the combined effects of moisture content, nutrient addition, and cover thickness. *Journal of Environmental Engineering & Science*, 6(2), 191–200. doi:10.1139/s06-047
- Atkinson, R. (2000). Atmospheric chemistry of VOCs and NO(x). *Atmospheric Environment*, 34(12-14), 2063–2101. doi:10.1016/S1352-2310(99)00460-4
- Barlaz, M. a, Green, R. B., Chanton, J. P., Goldsmith, C. D., & Hater, G. R. (2005). Evaluation of a Biologically Active Cover for Mitigation of Landfill Gas Emissions. *Environ. Sci. & Technol.*, 38(18), 4891–4899.
- Bender, M., & Conrad, R. (1995) Effect of CH₄ concentrations and soil conditions on the induction of CH₄ oxidation activity. *Soil Biol Biochem* 27: 1517-1527.
- Boeckx, P., Van Cleemput, O., & Villaralvo, I. (1996). Methane emission from a landfill and the methane oxidising capacity of its covering soil. *Soil Biology and Biochemistry*, 28(10-11), 1397–1405. doi:10.1016/S0038-0717(96)00147-2
- Bogner, J., Meadows, M., & Czepiel, P. (1997). Fluxes of methane between landfills and the atmosphere: natural and engineered controls. *Soil Use and Management*, 13(s4), 268–277. doi:10.1111/j.1475-2743.1997.tb00598.x
- Bogner, J., Matthews, E. (2003). Global methane emissions from landfills: new methodology and annual estimates 1980–1996. *Global Biogeochemical Cycles* 17, 34-1–34-18.
- Bogner, J., Pipatti, R., Hashimoto, S., Diaz, C., Mareckova, K., Diaz, L., Gregory, R. (2008). Mitigation of global greenhouse gas emissions from waste: conclusions and strategies from the Intergovernmental Panel on Climate Change (IPCC) Fourth Assessment Report. Working Group III (Mitigation). *Waste Management & Research*, 26(1), 11–32. doi:10.1177/0734242X07088433

- Bohn, H. L. (1996). Biofilter media. Proceedings of the 89th annual meeting and exhibition of the air & waste management association, June 23–26, 1996, Nashville, TN. Pittsburgh, PA: Air & Waste Management Association.
- Börjesson, G., Sundh, I., Tunlid, A., & Svensson, B.H. (1998) Methane oxidation in landfill cover soils, as revealed by potential oxidation measurements and phospholipid fatty acid analyses. *Soil Biol Biochem* 30: 1423– 1433
- Bowman, J.P., Sly, L.I., Nichols, P.D. & Haywerd, A.C. (1993) Revised taxonomy of the methanotrophs: Description of *Methylobacter* gen. nov., emendation of *Methylococcus*, validation of *Methylosinus* and *Methylocystis* species, and a proposal that the family *Methylococcaceae* includes only the group I methanotrophs. *International Journal of Systematic Bacteriology*, 43, 735–753.
- Buranov, A. U., & Mazza, G. (2008). Lignin in straw of herbaceous crops. *Industrial Crops and Products*, 28(3), 237–259. doi:10.1016/j.indcrop.2008.03.008
- Cardenas-Gonzalez, B., Ergas, S. J., & Switzenbaum, M.S. (1999). Characterization of compost biofiltration media. *Journal of the Air & Waste Management Association*. 49(7): 784–793
- Chen, L., Hoff, S.J., Koziel, J.A., Cai, L., Zelle, B., & Sun, G. (2008). Performance evaluation of a wood chip-based biofilter using solid-phase microextraction and gas chromatography-mass spectrometry-olfactometry. *Bioresour. Technol.* 99, 7767–7780
- Chistoserdova, L., Kalyuzhnaya, M. G., & Lidstrom, M. E. (2005). C1-transfer modules: from genomics to ecology. *ASM News*, 71 (11), 521-528.
- Chitwood, D. E., & Devinny, J. S. (2001). Treatment of mixed hydrogen sulfide and organic vapors in a rock medium biofilter. *Water & Environment Research*. 73(4): 426–435.
- Christensen, T. H., & Kjeldsen, P. (1989) Chapter 2.1 Basic biochemical processes in landfills. In T. H. Christensen, R. Cossu, & R. Stegmann (Eds.), *Sanitary Landfilling: Process, Technology and Environmental Impact* (p. 29). London, UK: Academic Press.
- Christophersen, M., Linderod, L., Jensen, P.E., Kjeldsen, P. (2000). Methane oxidation at low temperatures in soil exposed to landfill gas. *Journal of Environmental Quality* 29, 1989–1997.
- Dedysh, S. N., Liesack, W., Khmelenina, V. N., Suzina, N. E., Trotsenko, Y. a., Semrau, J. D., Tiedje, J. M. (2000). *Methylocella palustris* gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine- pathway methanotrophs. *International Journal of Systematic and Evolutionary Microbiology*, 50(3), 955–969. doi:10.1099/00207713-50-3-955
- Delhoménie, M.-C., & Heitz, M. (2005). Biofiltration of air: a review. *Critical Reviews in*

- Biotechnology*, 25(1-2), 53–72. doi:10.1080/07388550590935814
- Dixit, R., Deshmukh, S., Gadhe, A., Kannade, G., & Lokhande, S. (2012). Treatment of mixtures of toluene and n-propanol vapours in a compost–woodchip-based biofilter. *Environmental Technology*, (February 2014), 37–41. doi:10.1080/09593330.2011.592226
- Dunfield, P. F., & Dedysh, S. N. (2014). Methylocella: A gourmand among methanotrophs. *Trends in Microbiology*, 22(7), 368–369. doi:10.1016/j.tim.2014.05.004
- Environment Canada, (2015). National Inventory Report 1990-2013: Greenhouse Gas Sources and Sinks in Canada - Executive Summary - Climate Change - Environment Canada. [online] Available at: <http://www.ec.gc.ca/ges-ghg/default.asp?lang=En&n=5B59470C-1&offset=3&toc=show> [Accessed 19 Jul. 2015].
- Farquahar, G. J., & Rover, F. A. (1973). Gas production during refuse decomposition. *Water, Air and Soil Pollution*, 2, 483–495.
- Figuerola, R.A. (1993) Methane oxidation in a landfill top soils. In: Christensen, T.H., Cossu, R. & Stegmann, R. (eds.): *Proceedings Sardinia '93. Fourth International Landfill Symposium*, CISA, Environmental Sanitary Engineering Centre, Cagliari, Italy, vol. I, pp. 701–716.
- Fornés, I., C. Ott, and J. Jager (2003). Development of a Landfill Cover with Capillary Barrier for Methane Oxidation-Methane Oxidation in a Compost Layer. *Proceedings of Ninth International Waste Management and Landfill Symposium Cagliari, Italy, October 2003*
- Gaudy, A. F. and E. T. Gaudy (1980). *Microbiology for Environmental Scientists and Engineers*. McGraw-Hill Inc, New York, NY
- Girard, M., Nikiema, J., Brzezinski, R., Buelna, G., & Heitz, M. (2009). A review of the environmental pollution originating from the piggery industry and of the available mitigation technologies: towards the simultaneous biofiltration of swine slurry and methane. *Canadian Journal of Civil Engineering*, 36(12), 1946–1957. doi:10.1139/L09-141
- Giss.nasa.gov,. (2015). NASA GISS: Research Features: *Methane: A Scientific Journey from Obscurity to Climate Super-Stardom*. Retrieved 17 October 2015, from http://www.giss.nasa.gov/research/features/200409_methane/
- Gray, N. (1999). *Water technology*. London: Arnold.
- Hanson, R. S., & Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiological Reviews*, 60(2), 439–471. doi:10.1128/mr.60.2.439-471.1996
- Haththotuwa, C.K., Hettiaratchi, J.P.A., Hunte, C.H., 2012. Actively Aerated Methanobiofilters to

- Control Methane Emissions from Landfills. *International Journal of Environmental Technology and Management* 15 (3/4/5/6), 333 - 345.
- Hayes, R. E. (2004). Catalytic solutions for fugitive methane emissions in the oil and gas sector, *59*, 4073–4080. doi:10.1016/j.ces.2004.04.038
- Hilger, H. A., Cranford, D. F., & Barlaz, M. A. (2000). Methane oxidation and microbial exopolymer production in landfill cover soil, *32*, 457–467.
- Hong, J. H., Park, K. J. (2004). Wood chip biofilter performance of ammonia gas from composting manure. *Compost Science and Utilization*. 12(1): 25–30.
- Huber-Humer, M., Röder, S., & Lechner, P. (2009). Approaches to assess biocover performance on landfills. *Waste Management*, 29(7), 2092–2104. doi:10.1016/j.wasman.2009.02.001
- Humer, M., & Lechner, P. (1999). Alternative approach to the elimination of greenhouse gases from old landfills. *Waste Management & Research*, 17(6), 443–452. doi:10.1177/0734242X9901700607
- IPCC, (2013): *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.
- IPCC, (2014): *Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Edenhofer, O., R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. von Stechow, T. Zwickel and J.C. Minx (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- IPCC, (2014a): *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.
- Janni, K. A., Maier, W. J., Kuehn, T. H., Yang, C.-H., Bridges, B. B., Velsey, D., and Nellis, M. A. (2001). Evaluation of biofiltration of air—An innovative air pollution control technology. *ASHRAE Transactions*. 107(1): 198–214.
- Kennes, C., and Thalasso, F. (1998). Waste gas biotreatment technology. *Journal of Chemical Technology & Biotechnology*. 72(4): 303–319
- Kightley, D., Nedwell, D. B., & Cooper, M. (1995). Capacity for methane oxidation in landfill

- cover soils measured in laboratory-scale soil microcosms . Capacity for Methane Oxidation in Landfill Cover Soils Measured in Laboratory-Scale Soil Microcosms, *61*(2), 592–601.
- Krishnayya, A. V., Agar, J. G., and Wong, T. T. 1999. Design and performance evaluation of vapor-phase biofilters. In: *Proceedings of the Battelle Memorial Institute International In-Situ and On-Site Bioreclamation Symposium*. pp. 111–116. Vol. 5. April 19–22, 1999. San Diego. Battelle Press. Columbus.
- Langolf, B. M., & Kleinheinz, G. T. (2006). A lava rock-based biofilter for the treatment of alpha-pinene. *Bioresource Technology*, *97*(15), 1951–8. doi:10.1016/j.biortech.2005.08.006
- Limbri, H., Gunawan, C., Thomas, T., Smith, A., Scott, J., & Rosche, B. (2014). Coal-Packed Methane Biofilter for Mitigation of Green House Gas Emissions from Coal Mine Ventilation Air. *PLOS ONE*, *9*(4), e94641. doi:10.1371/journal.pone.0094641
- Luo, J. (2001). A pilot-scale study on biofilters for controlling animal rendering process odours. *Water Science & Technology*. *44*(9): 277–285.
- Mancebo, U. (2012). *Compost Physicochemical Characteristics Influencing Methane Biofiltration*.
- Mancebo, U., & Hettiaratchi, J. P. A. (2015). Rapid assessment of methanotrophic capacity of compost-based materials considering the effects of air-filled porosity, water content and dissolved organic carbon. *Bioresource Technology*, *177*, 125–133. doi:10.1016/j.biortech.2014.11.058
- Melse, R.W., and Van der Werf, A. (2005) Biofiltration for mitigation of methane emission from animal husbandry. *Environ Sci Technol* *39*: 5460–5468
- Ménard, C., Ramirez, A., Nikiema, J., & Heitz, M. (2012). Biofiltration of methane and trace gases from landfills: A review. *Environmental Reviews*, *53*, 40–53. doi:10.1139/A11-022
- Moe, W. M., and Irvine, R. L. (2000). Polyurethane foam medium for biofiltration. I-Characterization. *Journal of Environmental Engineering*. *126*(9): 815–825
- Mohseni, M., & Allen, D. G. (2000). Biofiltration of mixtures of hydrophilic and hydrophobic volatile organic compounds. *Chemical Engineering Science*, *55*(9), 1545–1558. doi:10.1016/S0009-2509(99)00420-0
- Mor, S., De Visscher, A., Ravindra, K., Dahiya, R. P., Chandra, a., & Van Cleemput, O. (2006). Induction of enhanced methane oxidation in compost: Temperature and moisture response. *Waste Management*, *26*(4), 381–388. doi:10.1016/j.wasman.2005.11.005

- Morgan-Sagastume, J.M., Noyola, A. (2006). Hydrogen sulfide removal by compost biofiltration: effect of mixing the filter media on operational factors. *Bioresour. Technol.* 97, 1546–1553.
- Nicolai, R.E., Janni, K.A. (2001a). Biofilter media mixture ratio of wood chips and compost treating swine odors. *Water Sci. Technol.* 44, 261–267
- Nikiema, J., Brzezinski, R., & Heitz, M. (2007). Elimination of methane generated from landfills by biofiltration: a review. *Reviews in Environmental Science and Bio/Technology*, 6(4), 261–284. doi:10.1007/s11157-006-9114-z
- Oh, Y. S., and Choi, S. C. (2000). Selection of suitable packing material for biofiltration of toluene, m- and p-xylene vapors. *Journal of Microbiology*. 38(1): 31–35.
- Ottengraf, S. P. P. (1986). Exhaust gas purification. In: *Biotechnology, a Comprehensive Treatise in 8 Volumes*. pp. 426–452. Vol. 8. Rehm, H.-J., and Reed, G., Eds., Verlag Chemie.Weinheim.
- Picard, D. (1999). Fugitive Emissions From Oil and Natural Gas Activities, 103–127.
- Pineda, J., Auria, R., Perez-Guevara, F., and Revah S. (2000). Biofiltration of toluene vapors using a model support. *Bioprocess Engineering*. 23(5): 479–486
- Pokhrel, D. (2006). *Compost based biocap performance*. University of Calgary. Retrieved from <http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Compost+Based+Biocap+Performance#0>
- Pokhrel, D., Hettiaratchi, P., & Kumar, S. (2011). Methane diffusion coefficient in compost and soil–compost mixtures in gas phase biofilter. *Chemical Engineering Journal*, 169(1-3), 200–206. doi:10.1016/j.cej.2011.03.013
- Saliling, W., Westerman, P., & Losordo, T. (2007). Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations. *Aquacultural Engineering*, 37, 222–233. doi:10.1016/j.aquaeng.2007.06.003
- Scheutz, C., Kjeldsen, P., Bogner, J. E., Visscher, A. De, Gebert, J., Hilger, H. A., & Spokas, K. (2009). Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions. *Waste Management & Research : The Journal of the International Solid Wastes and Public Cleansing Association, ISWA*. doi:10.1177/0734242X09339325
- Scriban, R. (1993). *Biotechnologie*. 4th edition. Tec & Doc Lavoisier. Paris.

- Smet, E., Chasaya, G., van Langenhove, H., and Verstraete, W. (1996). The effect of inoculation and the type of carrier material used on the biofiltration of methyl sulphides. *Applied Microbiology & Biotechnology*. 45(1–2): 293–298.
- Smet, E., van Langenhove, H., and Philips, G. (1999). Dolomite limits acidification of a biofilter degrading dimethyl sulphide. *Biodegradation*. 10(6): 399–404
- Spokas, K., Bogner, J., Chanton, J. P., Morcet, M., Aran, C., Graff, C., Hebe, I. (2006). Methane mass balance at three landfill sites: What is the efficiency of capture by gas collection systems? *Waste Management*, 26(5), 516–525. doi:10.1016/j.wasman.2005.07.021
- Van Dijken, J. P., & Harder, W. (1975). Growth yields of microorganisms on methanol and methane: A theoretical study. *Biotechnology and Bioengineering*, 17, 15-30.
- Visvanathan, C., Pokhrel, D., Cheimchaisri, W., Hettiaratchi, J. P. a., & Wu, J. S. (1999). Methanotrophic activities in tropical landfill cover soils: effects of temperature, moisture content and methane concentration. *Waste Management & Research*, 17(4), 313–323. doi:10.1177/0734242X9901700408
- Wahlen, M. (1993). The Global Methane Cycle. *Annual Review Of Earth And Planetary Sciences*, 21(1), 407-426. <http://dx.doi.org/10.1146/annurev.earth.21.1.407>
- Weigner, P., Paca, J., Loskot, P., Koutsky, B., and Sobotka, M. (2001). The start-up period of styrene degrading biofilters. *Folia Microbiologica*. 46(3): 211–216.
- Whittenbury, R., Philipps, K.C., and Wilkinson, J.F. (1970) Enrichment, isolation and some properties of methane- utilizing bacteria. *J Gen Microbiol* 61: 205–218.
- Williams, T. O., & Miller, F. C. (1992). Biofilters and facility operations— Part II. *Biocycle*, 33, 75–79
- Willumsen, H. (2003). Landfill gas plants worldwide: number and types. In: *Proceedings Sardinia '03 Waste Management and Landfill Symposium*. CISA, University of Cagliari, Sardinia, Italy, 2003.
- Wilshusen, J. H., Hettiaratchi, J. P. A., De Visscher, A., & Saint-Fort, R. (2004). Methane oxydation and formation of EPS in compost: effect of oxygen concentration. *Environmental Pollution*, 129(2), 305–14. doi:10.1016/j.envpol.2003.10.015
- Wilshusen, J. H., Hettiaratchi, J. P. A., & Stein, V. B. (2004). Long-term behavior of passively aerated compost methanotrophic biofilter columns. *Waste Management*, 24(7), 643–653. doi:10.1016/j.wasman.2003.12.006

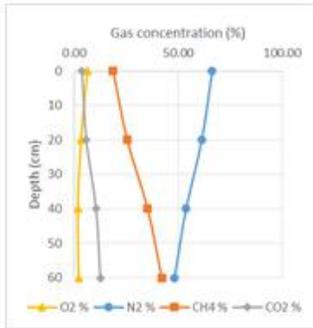
Woertz, J. R., van Heiningen, W. N. M., van Eekert, M. H. A., Kraakman, N. J. R., Kinney, K. A., and van Groenestijn, J. W. (2002). Dynamic bioreactor operation: effects of packing material and mite predation on toluene removal from off-gas. *Applied Microbiology & Biotechnology*. 58(5): 690–694.

Wood, S. and Layzell, D.B. (2003). A Canadian Biomass Inventory: Feedstocks for a Bioased Economy. *BIOCAP Canada Foundation*, pp. 18–24.

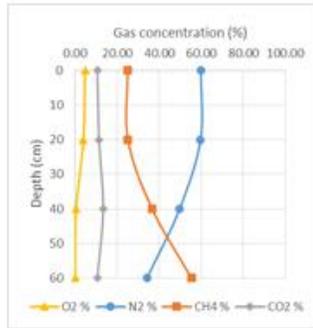
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Appendix A: Columns gas concentration profiles.

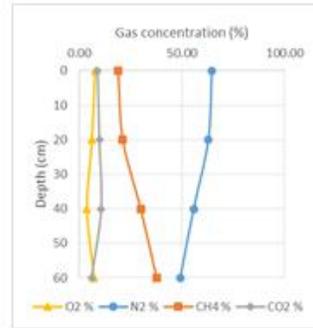
30:70 Compostflax at Field Capacity



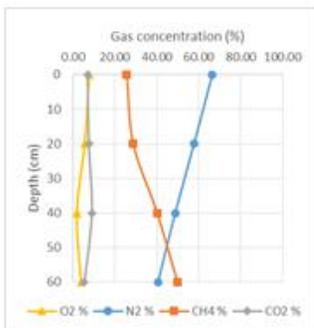
Day 13



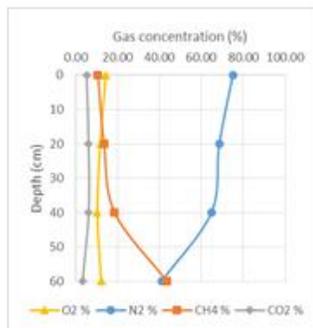
Day 20



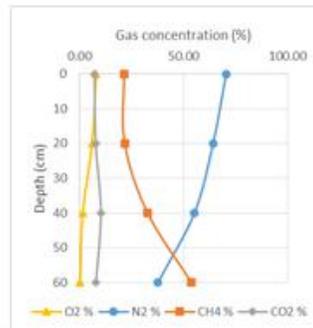
Day 27



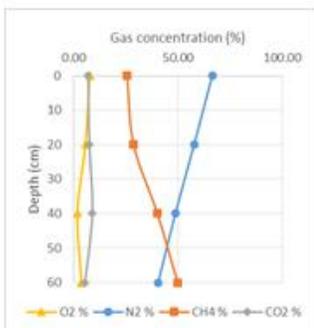
Day 34



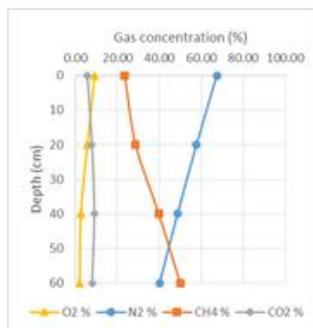
Day 42



Day 62

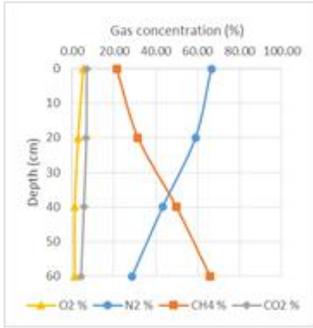


Day 76

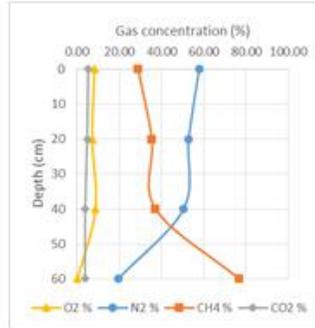


Day 91

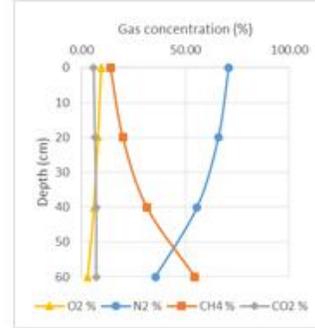
30:70 Compost:Wood shavings at Field Capacity



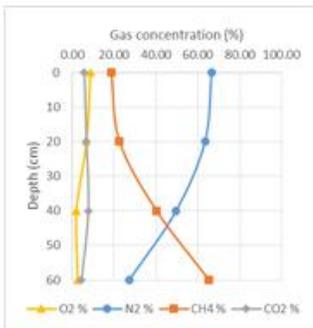
Day 13



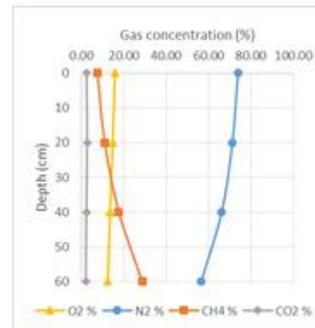
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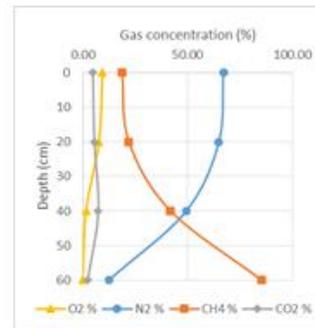
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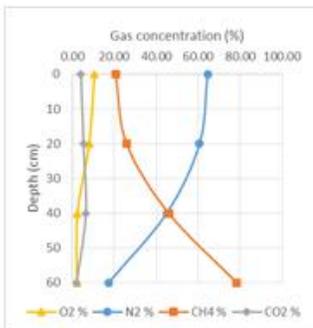
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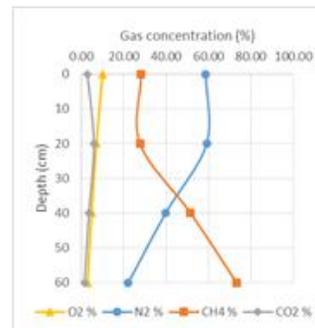
Day 42



Day 62

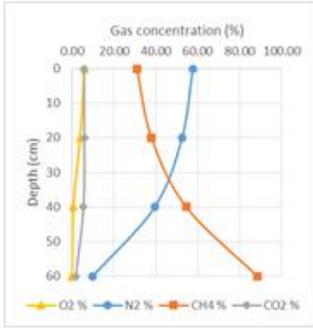


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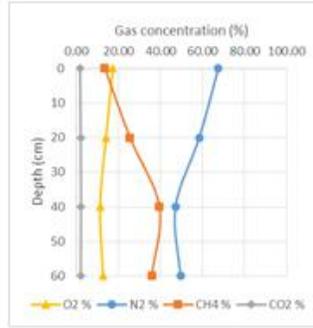


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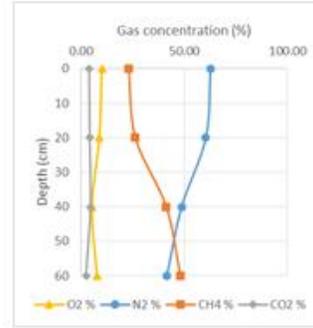
30:70 Compost:wood shavings at 70% of Field Capacity



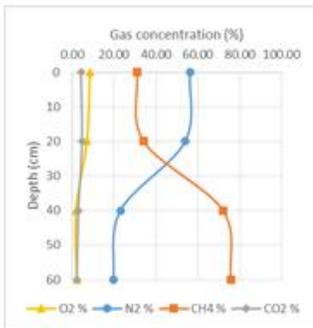
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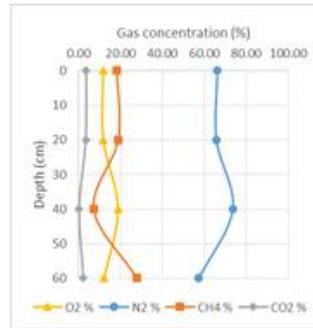
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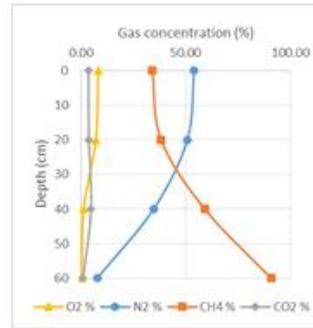
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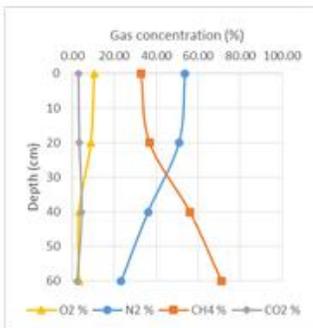
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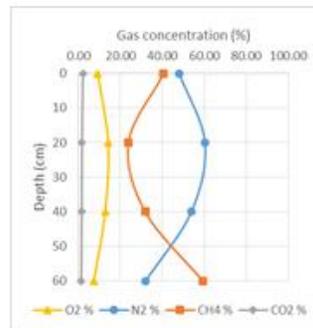
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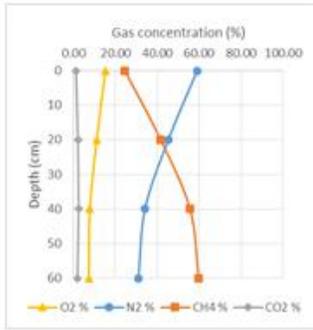


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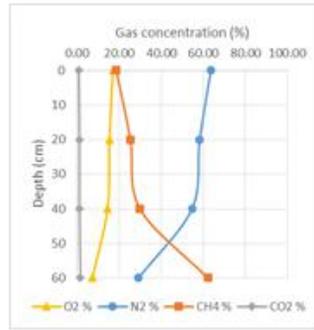


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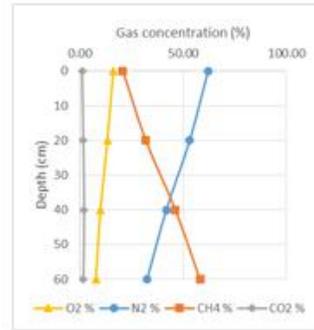
30:70 Compost:lava rock at Field Capacity with nutrients



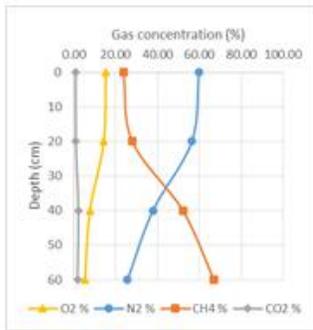
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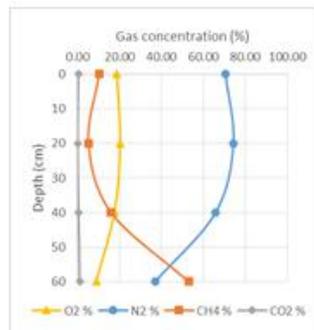
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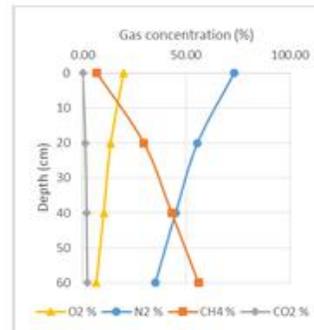
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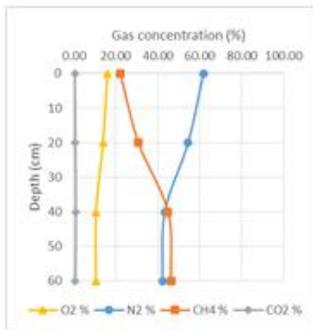
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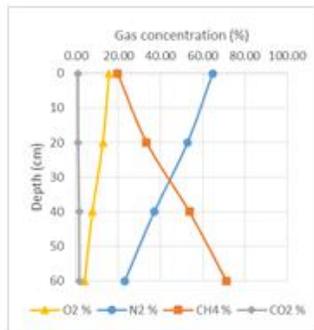
Day 42



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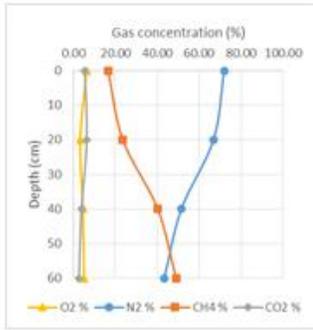


Day 76

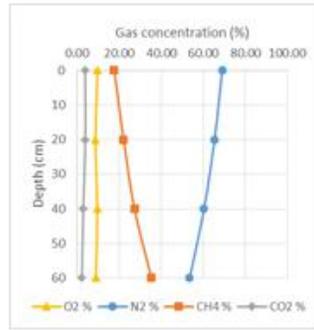


Day 91

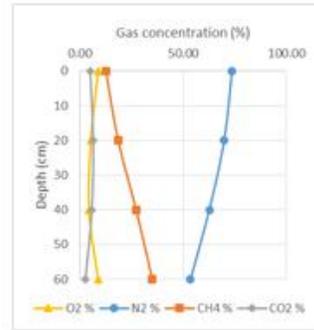
30:70 Compost:lava rock at 40% of Field Capacity



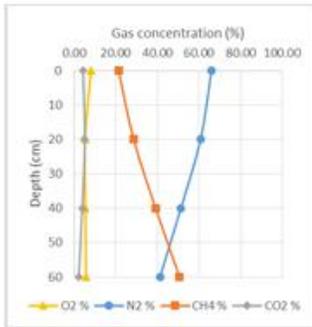
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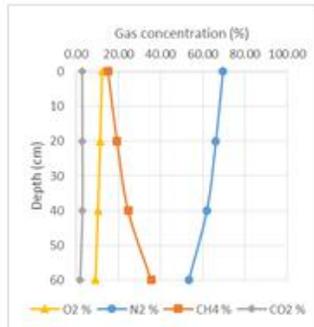
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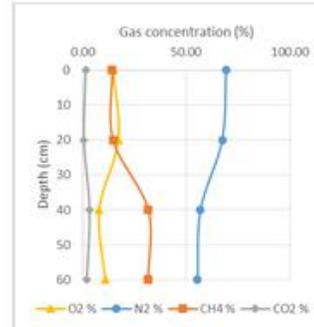
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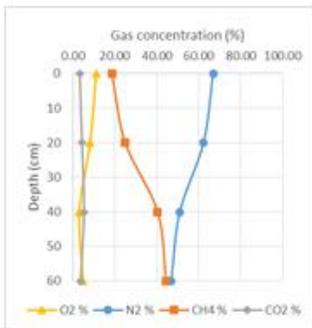
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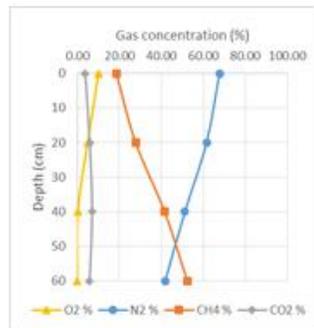
Day 42



Day 62

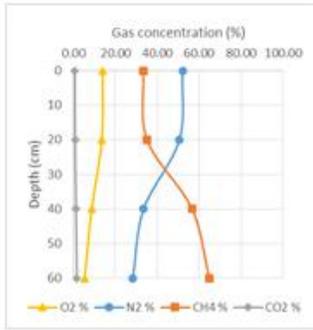


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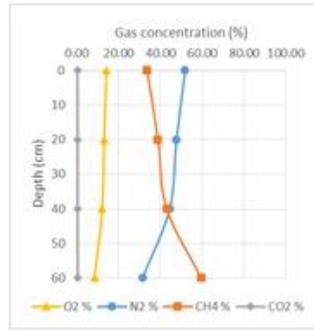


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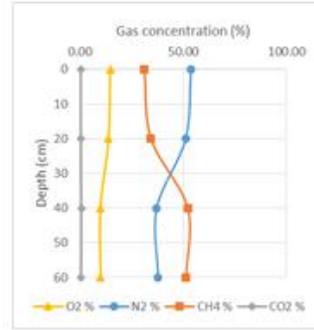
30:70 Compost:lava rock at 40% of Field Capacity with nutrients



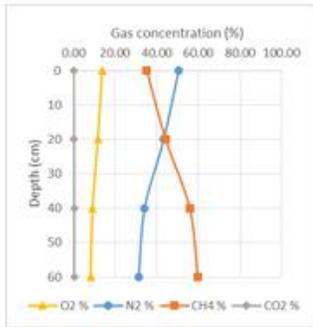
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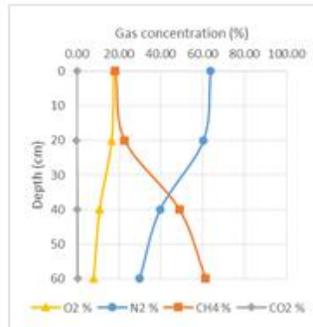
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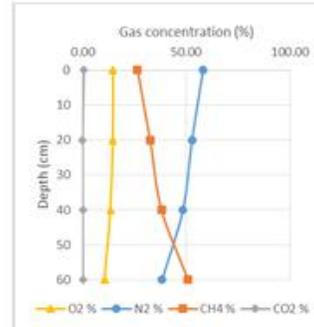
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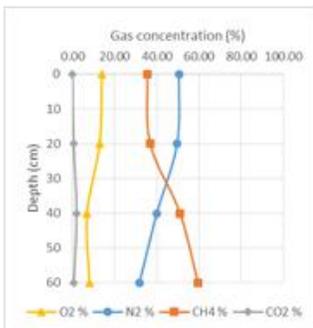
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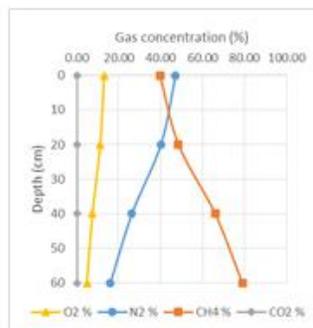
Day 42



Day 62



Day 76



Day 91

Appendix B: Data for ANOVA analysis.

Oxidation data from flax, wood shavings and lava rock batches

Factor	Factor			CH ₄ oxidation (g of CH ₄ per g of material) (119 days)
	Ratio A	Material B	MC % C	
1	1	-1	1	0.389342469
	1	-1	1	0.342300239
2	0	-1	1	0.19693907
	0	-1	1	0.20329903
3	-1	-1	1	0.160733026
	-1	-1	1	0.167979365
4	1	-1	0	0.153533972
	1	-1	0	0.231327634
5	0	-1	0	0.166754019
	0	-1	0	0.185677775
6	-1	-1	0	0.138260343
	-1	-1	0	0.162168797
7	1	-1	-1	0.30454078
	1	-1	-1	0.166065838
8	0	-1	-1	0.086424975
	0	-1	-1	0.060117116
9	-1	-1	-1	0.049262886
	-1	-1	-1	0.115472696
10	1	0	1	0.430800393
	1	0	1	0.420205434
11	0	0	1	0.24055853
	0	0	1	0.250332232
12	-1	0	1	0.194285575
	-1	0	1	0.19565485
13	1	0	0	0.341523491
	1	0	0	0.341752678
14	0	0	0	0.209477632
	0	0	0	0.237103769

15	-1	0	0	0.18721203
	-1	0	0	0.184884275
16	1	0	-1	0.270105567
	1	0	-1	0.239092669
17	0	0	-1	0.061342376
	0	0	-1	0.079622857
18	-1	0	-1	0.042789014
	-1	0	-1	0.089769892
19	1	1	1	0.212847998
	1	1	1	0.281338491
20	0	1	1	0.130271751
	0	1	1	0.190775242
21	-1	1	1	0.117931077
	-1	1	1	0.127264914
22	1	1	0	0.360622786
	1	1	0	0.292795376
23	0	1	0	0.160380754
	0	1	0	0.161051277
24	-1	1	0	0.111318797
	-1	1	0	0.104879015
25	1	1	-1	0.316925398
	1	1	-1	0.399291229
26	0	1	-1	0.209186348
	0	1	-1	0.146070892
27	-1	1	-1	0.151350775
	-1	1	-1	0.126955483

Oxidation data from lava rock batches with and without nutrients.

	Factor			CH ₄ oxidation (g of CH ₄ per g of material) (119 days)
	Ratio A	Nutrients B	MC % C	
1	1	-1	1	0.212848
	1	-1	1	0.281338
2	0	-1	1	0.130272
	0	-1	1	0.19078
3	-1	-1	1	0.11793
	-1	-1	1	0.12726
4	1	-1	0	0.36062
	1	-1	0	0.2928
5	0	-1	0	0.16038
	0	-1	0	0.16105
6	-1	-1	0	0.11132
	-1	-1	0	0.10488
7	1	-1	-1	0.31693
	1	-1	-1	0.39929
8	0	-1	-1	0.20919
	0	-1	-1	0.14607
9	-1	-1	-1	0.15135
	-1	-1	-1	0.12696
10	1	1	1	0.41304
	1	1	1	0.20463
11	0	1	1	0.18754
	0	1	1	0.14312
12	-1	1	1	0.13899
	-1	1	1	0.1115
13	1	1	0	0.24464
	1	1	0	0.25309
14	0	1	0	0.11956
	0	1	0	0.21767
15	-1	1	0	0.02606
	-1	1	0	0.14124

16	1	1	-1	0.48095
	1	1	-1	0.36967
17	0	1	-1	0.19944
	0	1	-1	0.19048
18	-1	1	-1	0.15239
	-1	1	-1	0.16213

9.5Report: Monitoring Protocol Development

Contents

Summary:	2
Background	3
I. Methods optimization for bacterial community analysis and quantitative estimation of methanotroph populations in compost biofilters (2014)	6
II. Monitoring protocol development: determination of reliable indicator species of biofilter activity (2015-2016)	23
III. Development of microbial consortia for seeding biofilters in acidic environments (2016).	31
Future Work	34
Outputs and deliverables	34
References	35

Project Name:

Control of point-source low-volume methane emissions using methane biofiltration technology: Development of the Monitoring Protocol

Project Conducted By:

Roshan Khadka, Joongjae Kim, and Peter Dunfield

Summary:

The primary goal of this portion of the project was to develop a simple and cost-effective strategy for monitoring the methane oxidation efficiency of a field methane biofilter via DNA-based detection and quantification of bacteria. Methods for extraction and analysis of bacterial DNA from the biofilter materials were optimized in 2014. We standardized our bacterial diversity analysis protocol (based on 16S rRNA gene analysis) and quantitative polymerase chain reaction (qPCR) specific to methanotrophic bacteria. In 2015 and 2016, we used microcosm experiments to determine the best candidate bacterial species for monitoring. The hypothesis was that some members of a methanotrophic community will be enduring, long-lived bacteria because they form recalcitrant survival states like spores and cysts. These will be poor targets for monitoring, as their populations may reflect prior methanotrophic activity rather than current methanotrophic activity. However, other methanotrophic species follow a "boom-and-bust" existence where they grow and die rapidly in response to the availability of methane. Populations of these bacteria will be good monitoring targets, as high population levels will indicate high recent activity. We performed growth and starvation experiments using the compost biofilter material. Unfortunately, most the methanotrophs in the biofilter were very recalcitrant, their populations did not decline even under long-term starvation of several months. The genus *Methylomicrobium* was the best candidate, as it did show some decline during starvation. However, the effect was not marked, and a careful alignment and analysis of methanotroph-specific gene sequences revealed that it was nearly impossible to develop a desired qPCR assay specific for this genus, as it is too closely related to other (more recalcitrant) methanotrophs.

However, unexpectedly these experiments suggested that some non-methanotrophic bacteria showed the desired rapid response to biofilter methane-oxidation activity and would be better monitoring candidates. These were bacteria are associated with *Methylophilum* spp. (such as *Methylotenera* spp., and the uncultured PRDO1AO11B group), and *Xanthomonas* spp. These bacteria are themselves incapable of methane oxidation but are often found associated with methanotrophs because they grow on

byproducts of methanotrophy like methanol or formaldehyde. These bacteria grew rapidly with the methanotrophs in our experiments and died rapidly when methane was removed from the systems. Therefore, we have instituted a monitoring system for these "methanotroph-associated methylotrophs". This protocol is now ready for optimization and field testing.

In addition to the primary goal of developing a monitoring protocol development, our secondary goal was to develop methanotrophic microbial consortia that could be added to biofilter materials to improve efficiency under particular conditions, especially acidification caused by H_2S in sour gas streams. Adding pure isolates of acidophilic verrucomicrobial methanotrophs did not show a significant improvement in methane oxidation in acidified biofilters. Therefore, natural acidic peat samples from Alberta and BC were used to enrich methanotrophs for seeding acidified biofilters. Nearly 70% of the methanotrophs enriched belonged to the alphaproteobacterial methanotrophs, particularly *Methylosinus* spp. and *Methylocapsa* spp. Enriched microbial consortia will be added to acidified compost and methane oxidation rates tested.

Background:

One of the key aspects of developing methane biofilter systems as a strategy to decrease greenhouse gas (GHG) emissions to the atmosphere is monitoring the amount of methane destroyed. This requires a tool for accurate measurement of methane oxidation efficiencies in biofilter systems. This is necessary both to accurately assess carbon budgets as well as to intervene when a biofilter ceases to be effective at removing methane from a waste gas stream. Measurement of CH_4 emissions generally involves labor-intensive methods like flux chambers. These are difficult to apply in remote areas, and due to the high spatial heterogeneity can deliver very inaccurate results. Gas profile analyses give more rapid flux estimates, but rely on accurate estimation of diffusion coefficients and gas-filled porosity, which are not trivial (Gebert et al., 2010). Other methods used to estimate CH_4 oxidation rates include gas push-pull tests (Henneberger et al., 2011) and measuring CO_2/CH_4 soil gas ratios to estimate CH_4 oxidation efficiency (Gebert et al., 2011), but both have drawbacks. The first is still labor intensive, the second is only useful under constant subsurface conditions. It is also unclear of the relationship of these measures to CH_4 efflux.

Another option is to monitor population sizes of methanotrophic bacteria. Most of the known methanotrophs belong to the phylum *Proteobacteria*, but nonproteobacterial methanotrophs belonging to the phylum *Verrucomicrobia* (Op den Camp et al., 2009) or candidate phylum NC10 (Ettwig et al., 2009) also exist. Because methanotrophs are specialists, they only survive where CH_4 is present, and therefore large populations are indicative of large CH_4 fluxes. In theory, the populations respond only to CH_4 availability,

and therefore the population size should indicate the CH₄ availability and the amount being oxidized.

Different species of methanotrophs are adapted to diverse environmental conditions of temperature, pH, and salinity (Dunfield, 2009). The first step in aerobic methane oxidation, the oxidation of methane to methanol, is catalyzed by methane monooxygenase, which exists in two known forms: soluble methane monooxygenase (sMMO), or particulate methane monooxygenase (pMMO). The pMMO is universal to all known methanotroph species presently known except *Methylocella* and *Methyloferula* while the soluble form is present in only a few genera. The pMMO enzyme and the genes encoding it (*pmoCAB*) are therefore often used as molecular biomarkers for methanotroph detection and analyses in the environment (McDonald et al., 2008). These genes are well conserved and can be easily aligned across species and groups, demonstrating that they have evolved from a common ancestor. Major taxa based on 16S rRNA gene sequence phylogeny are consistent with taxa defined on *pmoA* gene sequence phylogeny, indicating limited horizontal transfer of these key genes (Stein et al., 2012). This also means that *pmoA* gene can be used effectively to identify individual methanotroph taxa in the environment

At present, the most rapid and reliable method to measure methanotroph populations is to count the methanotroph-specific *pmoA* gene via quantitative PCR (qPCR), and use the number of detected genes as an estimate of the total population size (Kolb et al., 2003). This method requires only about 0.5 g of soil and can be performed entirely in the laboratory. Up to 50 samples can easily be analyzed in 2 days. It is possible to independently assess individual species as well as the total overall population. Henneberger et al. (2011) demonstrated that population levels of methanotrophs assessed via qPCR of *pmoA* genes were correlated with CH₄ oxidation rates and soil CH₄ concentrations in a Swiss landfill. Although their analysis was based on only a few sites, their results do suggest that analysis of *pmoA* genes might be a quick and practical method to predict the location of CH₄ emission hotspots in the field.

In biofilter systems operated by the Hettiaratchi lab in the past, a closed flux chamber method to measure point emissions of gas on the surface of a biofilter has been used. This approach is highly versatile and accurate, since one is able to increase the number of points measured to ensure overall measurement accuracy. However, a drawback is that it only gives a snapshot of biofilter performance at the time of on-site data collection. Therefore, if this method is to be used in calculating GHG reductions, it is necessary to undertake such measurements frequently to account for changes of performance due to environmental conditions. Although, such measurements could be few and far between in an established methane biofilter, some situations may warrant taking measurements every few weeks, increasing the cost of monitoring and of the overall project. A monitoring method that provides information about the growth trends

of methanotrophs and, therefore, the methane biofilter performance trends over a long period could overcome the limitations of the flux chamber method and significantly decrease the cost of methane biofilter monitoring.

A potential advantage of using a microbiological indicator compared to physical and chemical methods to monitor the biofilters is therefore that the latter are instantaneous snapshots, while the methanotroph population size is an integrated effect of longer time periods. Methane concentrations in soil air may vary quickly, but methanotrophs typically have doubling times between 6-24 h. Populations therefore do not respond to short-term methane changes on the order of hours to days, but instead reflect an integrated average of soil CH₄ levels over a period of weeks. Different species also respond over different time scales. Spore- and cyst-forming species of *Alphaproteobacteria* like *Methylocystis* and *Methylosinus* withstand starvation and therefore tend to have constant populations over months, while other species like the *Gammaproteobacteria* *Methylococcus* and *Methylomonas* follow a boom-and-bust strategy, growing and dying quickly (days to weeks) in response to changing CH₄ levels (Henckel et al., 2000). We predict that measuring populations of the former will allow us to see historical hotspots, while monitoring the latter will allow us to predict more recent hotspots. However this method is only an improvement over other methods if a sampling protocol can be developed that overcomes the scale of variability. That is, a sampling protocol will need to be developed that exhaustively samples an average population, so that hotspots are not missed in a similar manner as they can be using flux methods.

Besides methanotrophs, other bacteria in the biofilters may change dramatically across sites and over the effective biofilter lifetimes due to souring and other toxic effects. Monitoring overall microbial communities in the methane biofilters over their lifetime by molecular fingerprinting (next-generation pyrotag sequencing) of the universal 16S rRNA gene, as well as the methanotroph-specific genes *pmoA* and *mmoX* (McDonald et al 2008), may allow the identification of species or species assemblages that are indicative of biofilter failure. Community changes (i.e. changes in species present) will correlate to the monitored CH₄ oxidation efficiencies, and suggest strategies to sustain performance. Particular bacteria such as obligate anaerobes will effectively indicate biofilter failure and suggest strategies to sustain performance. The predominance of spore and cyst-forming as opposed to ephemeral methanotroph species could also suggest problems with the biofilter caused by such issues as restricted aeration.

I. Methods optimization for bacterial community analysis and quantitative estimation of methanotroph populations in compost biofilters (2014)

Initial experiments in 2014 were aimed to develop the molecular protocols needed for detecting and quantifying methanotrophs in biofilter columns and lab microcosms. Biofilters studied used compost as a packing material, so the methods were optimized to this material.

Methods:

Column setup. Four acrylic columns (150 cm height; 14 cm inner diameter) were packed with compost at moisture contents of 123% by dry weight (Figure 1). Methane (99% pure) was fed through the bottom of the columns. Sweep air was fed through the headspace of the columns (passive aeration) or through the methane inlet located at the bottom of the column (active aeration). The compost was sampled at several levels through the column via side-port openings (Figure 1), after 50 and 100 days to determine methanotrophic bacterial population.

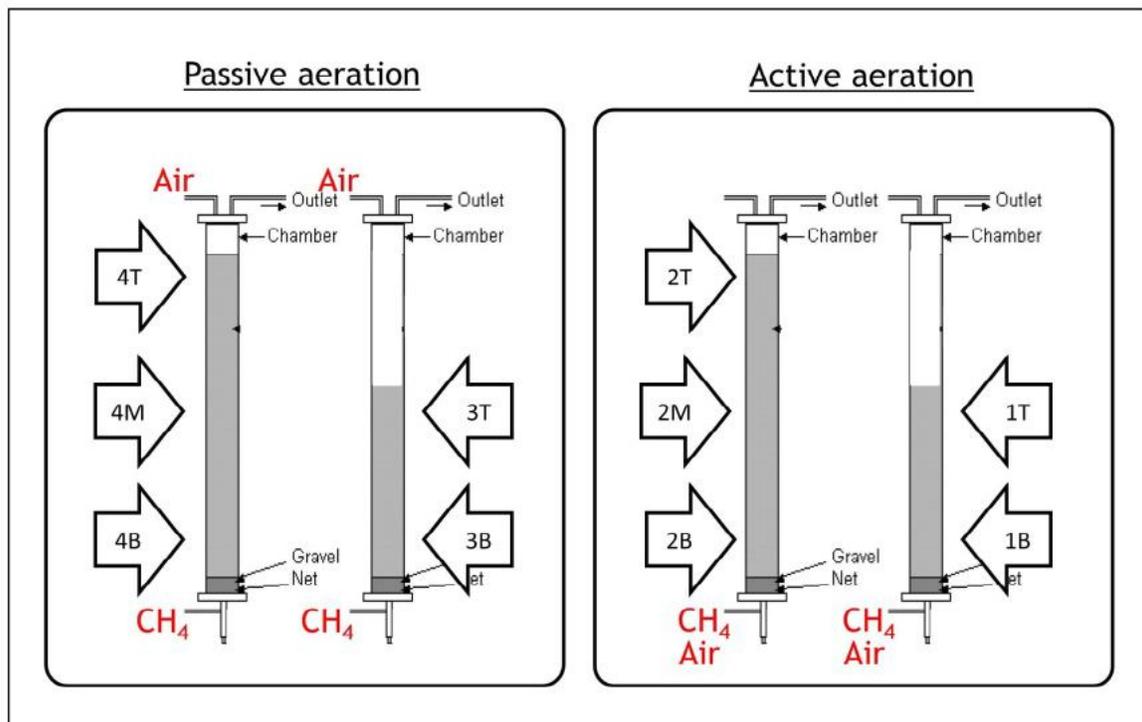


Figure 1. Schematic of laboratory-scale biofilter columns. T, Top; M, Middle; B, Bottom.

DNA extraction and PCR amplification. For the conventional PCR and real-time quantitative PCR, DNA was extracted from 0.5 g of compost samples using the FastDNA SPIN Kit for Soil (MP Biomedicals) following the manufacturer's instruction with the following modification. Since it was difficult to get the high purity DNA from compost, additional purification steps using 5.5 M guanidine thiocyanate (GTC) were introduced in the washing step (Knief et al., 2003). The DNA concentration was determined by using a Qubit Fluorometer with a Quant-iT™ dsDNA HS Assay Kit (Invitrogen) and the purity of DNA was determined by using Nanovalue Plus™ (GE Healthcare). The 16S rRNA gene as a marker gene was amplified in a reaction mixture (50 µl) containing FailSafe PCR 2× Premix F (Epicentre Biotechnologies), 1 U Taq DNA polymerase, 0.5 µM of each of universal 9f and 1492b primers and 1 µl of the DNA extract. The PCR was run for: 94 °C for 5 minutes; then 30 cycles of 94°C for 45 s, 48°C for 1 minute, and 72°C for 2 minutes; with a final elongation of 72°C for 5 minutes.

Sequencing of the 16S rRNA gene. To analyze microbial diversity, the 454 pyrotag DNA sequencing technique targeting the 16S rRNA gene was performed. The 16S rRNA genes were amplified from the DNAs extracted from the compost samples through two rounds of PCR, using amplification with non-barcoded universal 16S primers 926Fw (AAACTYAAAKGAATTGACGG) and 1392R (ACGGGCGGTGTGTRC) followed by amplification using FLX Titanium amplicon primers 454T-RA-X and 454T_F containing the 16S rRNA gene targeted primers 926f and 1392r at their 3'-ends, along with adapters necessary for the Roche Titanium chemistry. The reverse primer contained a unique 10-bp nucleotide identifier barcode sequence that allowed for sequences to be binned according to the particular sample. The first PCR was for 25 cycles with 30 s at 94°C, 45 s at 55°C, and 90 s at 72°C. The PCR products were used as templates for a second PCR of 10 cycles under the same conditions. PCR products were checked on an agarose gel and purified with the EZ-10 Spin Column PCR Purification Kit (Bio Basic Inc.). Purified PCR products (-150 ng total DNA) were sent to the Genome Quebec and McGill University Innovation Centre, Montreal, Quebec, for analysis on the Roche 454 Genome Sequencer FLX Instrument using titanium chemistry. QIIME (Quantitative Insights Into Microbial Ecology, v1.3) was used to analyze sequencing results. OTUs were identified taxonomically using BLAST against the Silva 108 reference database (Caporaso et al., 2010).

Note that for cost reasons the sequencing platform was changed from Roche 454 to Illumina MiSeq in 2015 (see Section 2). For this The hypervariable V4 region of the 16S rRNA gene were amplified according to the Illumina protocol (http://www.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). The Illumina MiSeq system (Illumina, Inc.) was used to sequence 16S rRNA gene amplicons using standard protocols

http://www.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf.

Results and Discussion:

DNA extraction from the compost. Since the compost contains large amounts of humic substances and phenolic compounds, which inhibit the polymerase enzyme in PCR, the DNA bound to silica matrix was washed with 500 μ l of 5.5M guanidine thiocyanate (GTC) for 1-4 times until the binding matrix has returned to its original colour. DNA was successfully extracted from the composts using the modified DNA extraction methods (Figure 2), and the purity of DNA determined was good enough to perform the further molecular experiments (data not shown). To confirm the purity of the extracted DNA, 16S rRNA gene was selected as a marker gene to test a PCR amplification to see if the PCR is properly working. As shown in figure 2, the marker genes were successfully amplified from the composts tested without any interference.

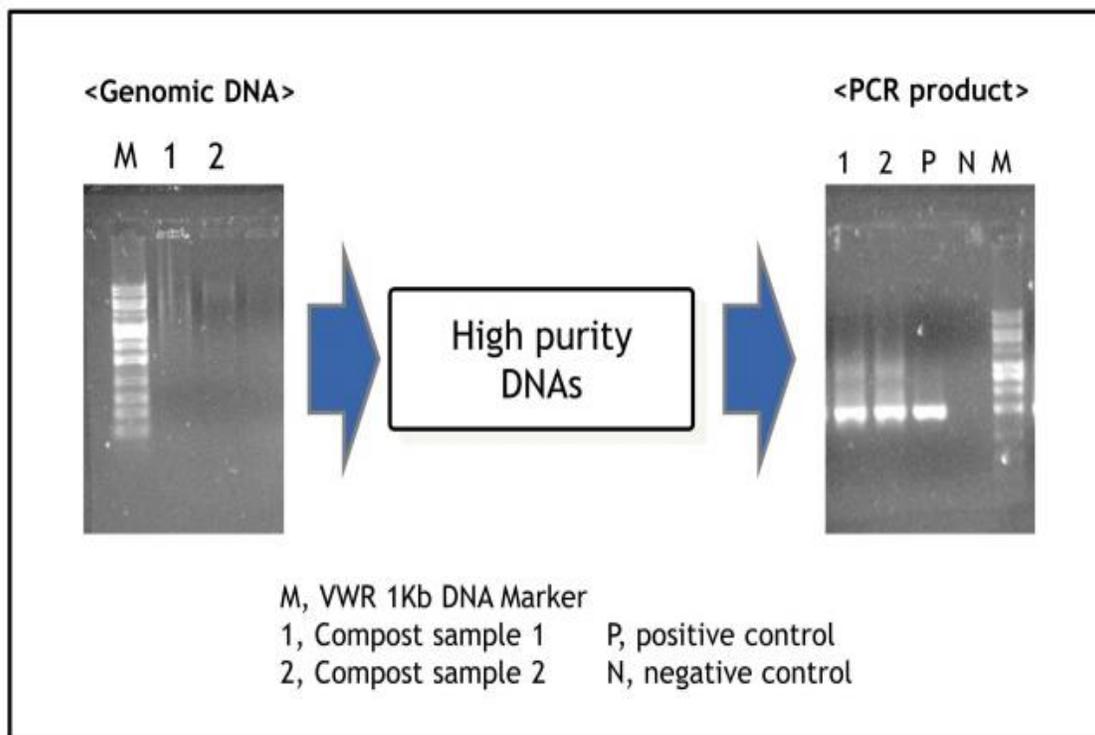


Figure 2. Extraction of DNA from the compost and amplification of 16S rRNA gene from the extracted DNA by Polymerase Chain Reaction.

Microbial community analysis based on 16S rRNA gene The compost used for biofilter experiment was subjected to high throughput 16S rRNA gene amplicon

sequencing to analyze the microbial community. The majority of sequences collected from the compost belonged to the phyla *Proteobacteria* (30.1%), *Bacteroidetes* (12.4%), *Actinobacteria* (9.7%), *Planctomycetes* (7.5%), and *Firmicutes* (5.6%) (Figure 3).

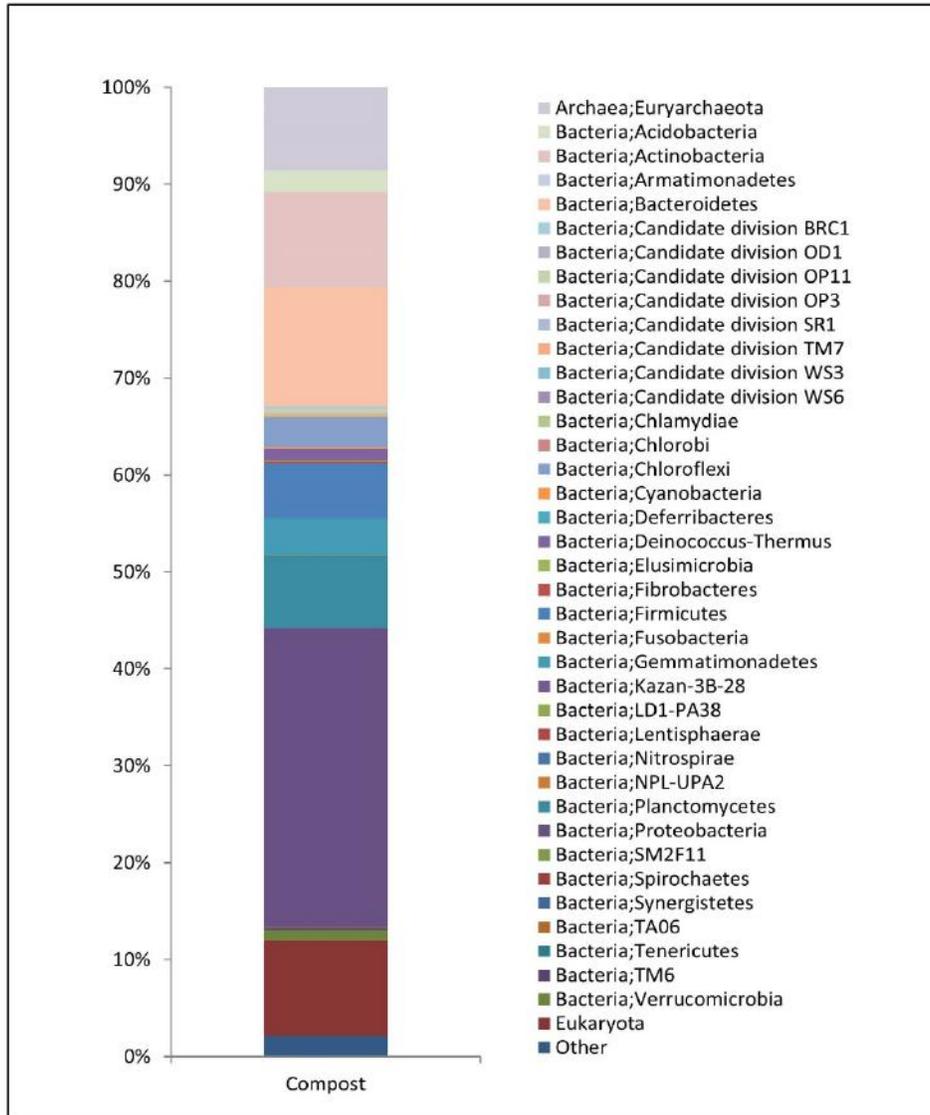


Figure 3. Microbial community analysis based on 16S rRNA of the compost using pyrotag sequencing.

Methanotrophic bacterial communities identified based on 16S rRNA gene pyrotag sequencing are summarized in Table 1. In this compost sample, gammaproteobacterial methanotrophs were dominant. Specifically, the genera *Methylocaldum* and *Methylobacter* in the family *Methylococcaceae* were detected. Some unknown genera in the family *Methylococcaceae* or the order *Methylococcales* may or may not represent methanotrophs.

Table 1. Major methanotrophic groups of compost sample based on % of total reads in 16S rRNA gene pyrotag sequencing analysis.

Rank	% Read	Phylum	Class	Order	Family	Genus
2	0.04	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcales</i>	<i>Methylococcaceae</i>	<i>Methylocaldum</i>
3	0.03	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcales</i>	Hyd24-01	
4	0.03	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcales</i>	<i>Methylococcaceae</i>	
5	0.02	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcales</i>	<i>Methylococcaceae</i>	<i>Methylobacter</i>

Methanotrophic community analysis based on the *pmoA* gene. Methanotrophic species in compost from an active lab-scale biofilter column (Figure 1), were determined by high-throughput sequencing of *pmoA* genes (Table 2). *Methylocaldum* spp. *Methylocystis* spp. and *Methylomicrobium* spp. were detected as the major methanotrophic groups. Although not numerically most abundant, the presence of *Methylomicrobium* spp., which we hypothesized to be a good indicator species of activity based on literature research (see Section II Table 1), is promising and suggested that this species could be targeted.

Table 2. Methanotrophs found in the compost by *pmoA*-targeted sequencing. the number of reads is a relative measure of the abundance of each species detected.

OTU / Reads	Identity	Closest neighbor (%)
OTU_1/20380	<i>Methylocaldum tepidum</i>	99
OTU_2/6478	<i>Methylocaldum szegediense</i>	99
OTU_3/4965	<i>Methylocystis hirsute</i>	97
OTU_4/861	<i>Methylocaldum sp.</i>	80
OTU_5/1071	<i>Methylocaldum gracile</i>	95
OTU_7/119	<i>Methylocystis echinoides</i>	96
OTU_8/64	<i>Methylomicrobium album</i>	94
OTU_9/58	<i>Methylocystis sp.</i>	92
OTU_11/56	<i>Methylomarinovum sp.</i>	85
OTU_19/6	<i>Methylocystis sp.</i>	89
OTU_22/19	<i>Methylosinus trichosporium</i>	94
OTU_23/2	<i>Methylovulum sp.</i>	88

Bacterial communities at different zones of the column. During the operation of the lab-scale methane biofilter system, white/beige layers were found 10 cm above from the bottom of the column (Figure 4). To determine, what kinds of microorganisms formed these layers, the composts from white layer and non-white layer were analyzed by 16S rRNA gene-targeted Illumina sequencing.



Figure 4. White clog formation during the laboratory-scale biofilter column operation.

Methanotrophic bacterial communities in white and non-white samples identified based on 16S rRNA gene targeted sequencing are summarized in Tables 3 and 4. The materials differed dramatically in their community composition (Fig. 5). The white material was predominated by methanotrophs like *Methylobacter* sp. (OTU_1), and *Methylosinus* sp. (OTU_13), *Methylocystis* (OTU_121), and *Methylosinus* (OTU_13) as well as methylotrophic bacteria like *Hyphomicrobium* (OTU_47). On the contrary, non-methanotrophic bacteria comprised the main population in the non-white samples.

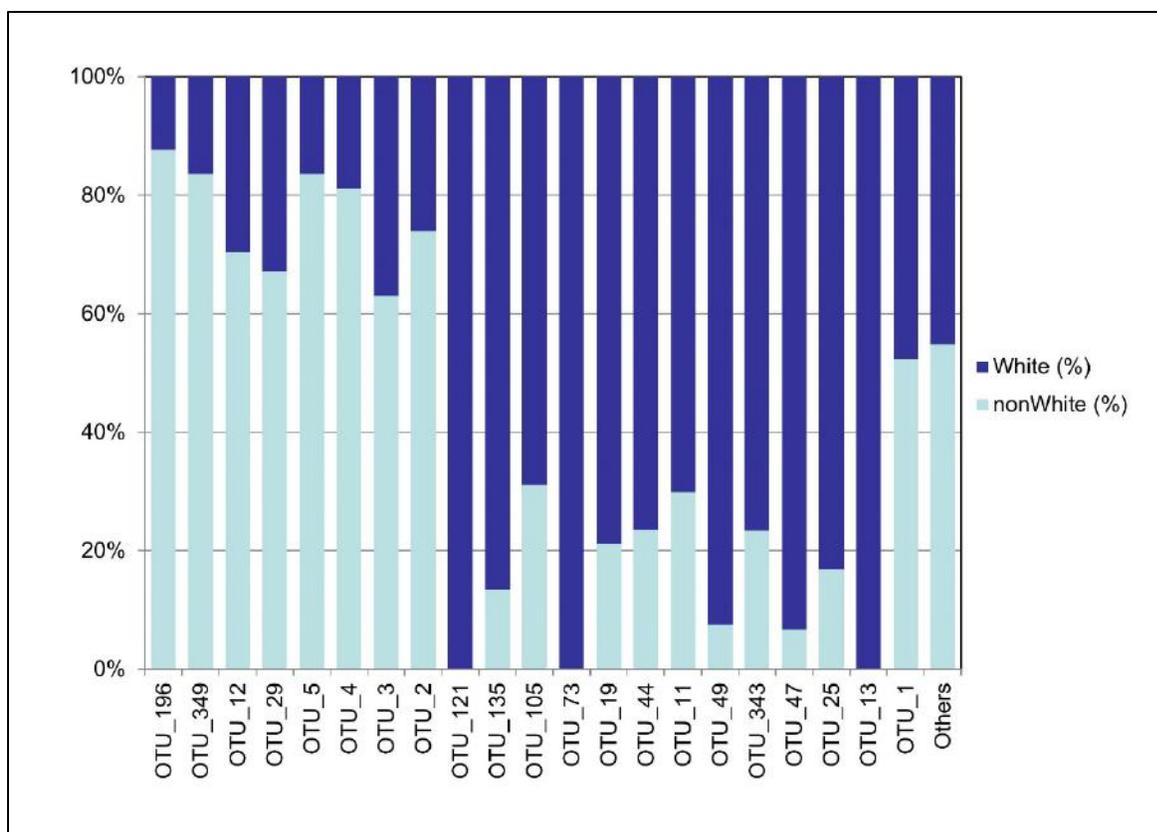


Figure 5. Microorganisms in White/Non-White Samples from methane biofilter column operation. Designation of OTUs are given in Tables 3 and 4.

Table 3. Dominant microbes in white sample detected by 16S rRNA gene-targeted sequencing

OTU ID	White (%)	Non-White (%)	Taxonomy
OTU_1	22.6	24.9	Proteobacteria(100);Gammaproteobacteria(100);Methylococcales(100);Methylococcaceae(80);Methylobacter(74);
OTU_13	6.3	0.0	Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100);Methylocystaceae(93);Methylosinus(83);
OTU_25	3.5	0.7	Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100);Hyphomicrobiaceae(100);Hyphomicrobium(90);
OTU_47	2.5	0.2	Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100);Hyphomicrobiaceae(100);Hyphomicrobium(87);
OTU_343	2.3	0.7	Bacteroidetes(100);Cytophagia(100);Cytophagales(100);Cytophagaceae(99);Ohtaekwangia(99);
OTU_49	2.2	0.2	Proteobacteria(100);Gammaproteobacteria(100);Methylococcales(100);Methylococcaceae(100);Methylodum(100);
OTU_11	2.1	0.9	Bacteroidetes(100);Cytophagia(90);Cytophagales(90);Cytophagaceae(87);unclassified;
OTU_44	1.8	0.5	Bacteroidetes(100);Sphingobacteriia(100);Sphingobacteriales(100);Chitinophagaceae(100);Flavisolibacter(73);
OTU_19	1.3	0.4	Proteobacteria(100);Gammaproteobacteria(100);Xanthomonadales(99);Xanthomonadales_Incertae_Sedis(99);Steroidobacter(99);
OTU_105	1.2	0.5	Bacteroidetes(100);Cytophagia(99);Cytophagales(98);Cytophagaceae(96);Ohtaekwangia(94);
OTU_73	1.2	0.0	Bacteroidetes(100);Sphingobacteriia(100);Sphingobacteriales(100);Chitinophagaceae(100);Niastella(97);
OTU_135	1.2	0.2	Planctomycetes(100);Phycisphaerae(100);WD2101_soil_group(100);unclassified;unclassified;
OTU_121	1.0	0.0	Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100);Methylocystaceae(100);Methylocystis(100);

Table 4. Dominant microbes in non-white sample detected by 16S rRNA gene-targeted sequencing

OTU ID	non-White (%)	White (%)	Taxonomy
OTU_4	2.9	0.7	Firmicutes(100);Bacilli(100);Bacillales(99);Planococcaceae(96);Planococcus(78);
OTU_5	2.9	0.6	Actinobacteria(100);Actinobacteria(100);Micrococcales(100);Micrococcaceae(100);Arthrobacter(100);
OTU_2	2.7	0.9	Chloroflexi(100);Anaerolineae(99);Anaerolineales(99);Anaerolineaceae(99);unclassified;
OTU_3	1.4	0.8	Actinobacteria(100);Acidimicrobiia(100);Acidimicrobiales(100);OM1_clade(99);unclassified;
OTU_12	1.3	0.5	Euryarchaeota(100);Methanomicrobia(100);Methanosarcinales(100);Methanosarcinaceae(100);Methanosarcina(100);
OTU_196	1.3	0.2	Chloroflexi(100);Anaerolineae(100);Anaerolineales(100);Anaerolineaceae(100);Anaerolinea(61);
OTU_29	1.1	0.5	Chloroflexi(100);Anaerolineae(95);Anaerolineales(95);Anaerolineaceae(95);unclassified;
OTU_349	1.1	0.2	Proteobacteria(100);Betaproteobacteria(100);Burkholderiales(100);Comamonadaceae(100);unclassified;

Comparison of other packing materials for the methane biofilter performance. To find the most cost-effective packing material for methane biofilter system, wood shaving and flax seed straw were tested for the methane oxidation activity. Four acrylic columns (150 cm height; 14 cm inner diameter) were packed with compost and wood shaving or flax seed straw at moisture contents of 123% by dry weight (Figure 6). Methane (99% pure) was fed through the bottom of the columns. After operation, compost/packing materials were removed from the three different levels for the microbial community analysis.

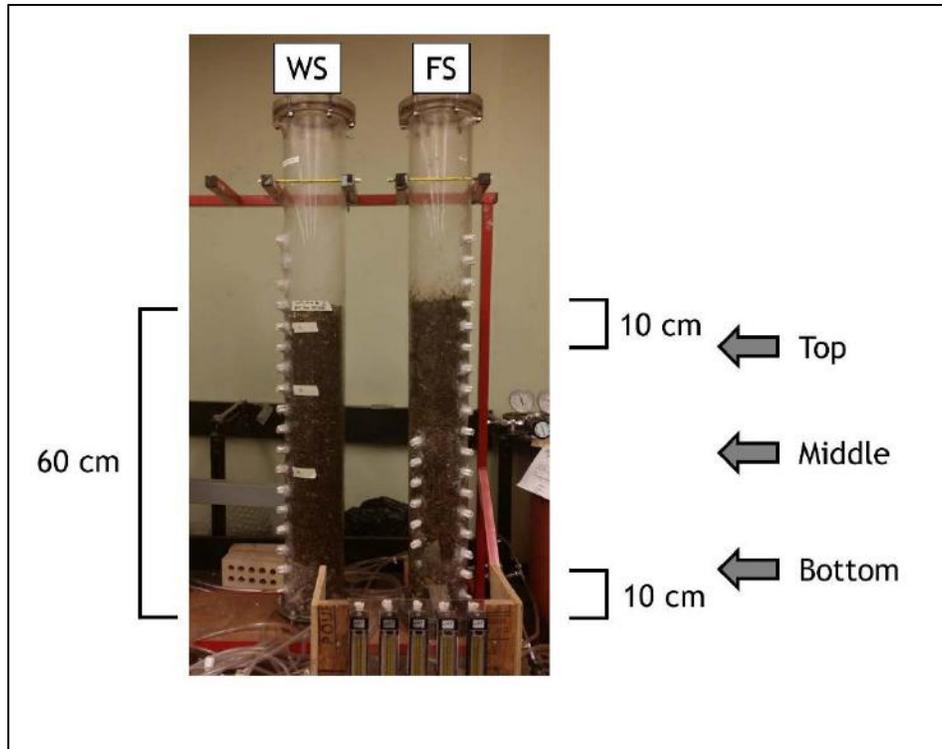


Figure 6. Laboratory-scale biofilter columns for the comparison of wood shaving (WS) and flax seed straw (FS) as a methane biofilter filling material. Grey arrows are the spot the samples were taken (top, middle, and bottom).

Methylosinus, an alphaproteobacterial methanotroph, was the major group in both materials (13.8% of the total community in wood shaving and 39.1% in flax seed straw) at the bottom part of the column. In both case, methylotrophic groups (*Hyphomicrobiaceae*) were also detected as the one of the major groups (16.1% in wood shaving and 21.3% in flax seed straw). This contrasted to the compost biofilters, in which gammaproteobacterial methanotrophs like *Methylocaldum* and *Methylobacter* predominated.

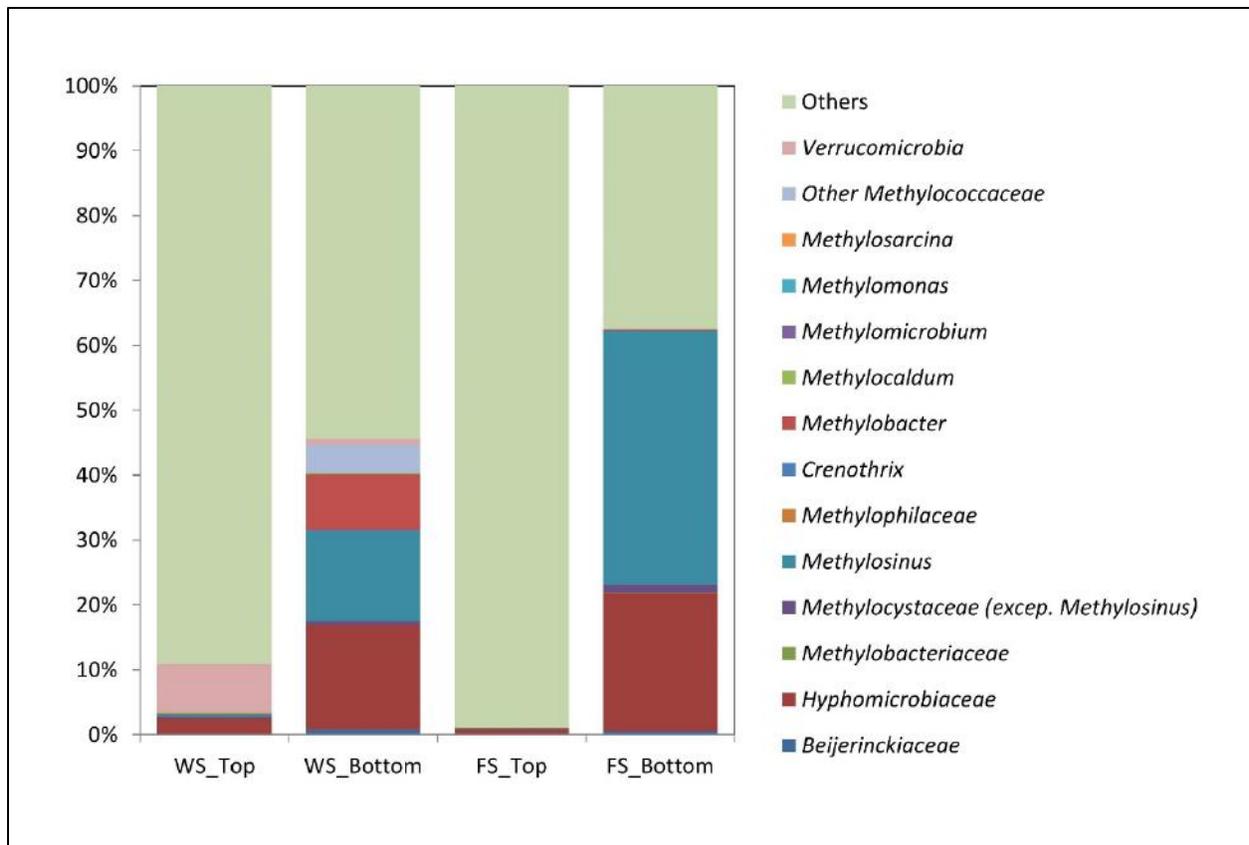


Figure 7. Microbial community analysis of methane biofilter filled with different materials using 16S rRNA gene-targeted Illumina sequencing. WS, Wood shaving; FS, Flax seed straw. Top and Bottom are the sampling spot on the biofilter column.

The major implication of these data is that the methanotrophic communities are dependent on packing material, and the monitoring systems we develop based on microbial communities may therefore need to be adjusted depending on the final packing materials used. However, since this has not yet been finalized, we have concentrated our further efforts on compost as a packing material.

Real-time *pmoA* PCR quantification. Quantitative PCR was performed on the DNA extracted from the compost samples from methane biofilter systems, using *pmoA* gene specific primers, A189f / mb661b for total methanotrophs, A189f / Mc468b for the *Methylococcus* (*Gammaproteobacteria*) group, and II223f / II646b for Type II (*Alphaproteobacteria*) methanotrophs (Kolb et al., 2003). PCR assays were prepared using a QIAgility instrument (v4.13.5, Qiagen). Reaction mixtures contained 1 μ L of DNA, 6.25 μ L of 2X RotorGene SYBR Green PCR Master Mix (Qiagen), 1 μ M of each primers and 4.75 μ L of RNase-free water (Qiagen). qPCR was performed on the RotorGene Q (Qiagen). Cycling conditions were consisted with a three-step

thermoprofile: an initial denaturation of 5 min at 94°C; 40 cycles of 94°C for 60 s, 55°C for 45 s and 72°C for 45 s; and a final elongation step of 72°C for 10 min (Martineau et al., 2010). Fluorescence data were obtained during the last step of each cycle. Each measurement was taken in duplicate. The specificity of each reaction was verified by melt curve analysis. A serial dilution of a *pmoA* gene cloned from *Methylosinus trichosporium* OB3b was used as a standard for the total methanotrophs and the type II group; while a dilution of *pmoA* gene cloned from *Methylococcus capsulatus* Bath was used as a control for the *Methylococcus* group. The measured DNA in a PCR from the cloned fragment amount via Qubit was converted to target molecules per microliter, and a *pmoA* gene standards curve was made by serial dilutions adjusted to 10^8 - 10^1 target molecules per microliter.

qPCR quantification of the methanotrophic population. In order to examine the methanotrophic population in biofilter media, qPCR was applied for the quantitative analysis of the methanotrophic communities. Initially the qPCR system was applied to a simple batch culture experiment involving the incubation of compost in a sealed serum vial with methane added to the headspace (Figure 8). Methane was monitored using gas chromatography and methanotrophic microbial population was analyzed by using qPCR technique. Methane consumption was stopped at 10 and 14 days since the oxygen level was declined below the threshold, therefore, air was fed to keep oxidizing methane. Methane was completely consumed at 18 days. Comparing the methane consumption with *pmoA* gene copy number changes, gene copy number had dramatically increased during the fast methane consumption period as expected, and however the high level of *pmoA* gene copy number was maintained after methane consumption rate decreased (Figure 8).

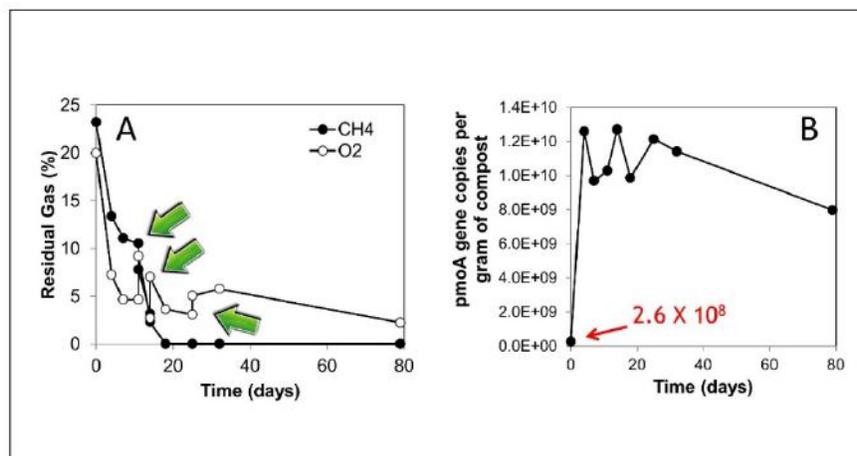


Figure 8. Batch cultivation of the compost. (A) Methane oxidation activity of the compost, (B) Quantification of total methanotrophs. Arrows indicate the air feeding points. Initial *pmoA* gene copy number was 2.6×10^8 copies per gram of compost.

It was surprising that the *pmoA* gene copy number did not quickly decline even after there had been no methane in the system more than 60 days. There appeared to be a slow decline from day 30 to day 80 d. This indicates that methanotrophs in the compost do not die immediately when the substrate methane is depleted, but keep alive or possibly enter a resting state like a spore or cyst. This may be a problem with using the qPCR technique to monitor biofilter activity, since it will accurately predict the number of methanotrophs and therefore the “potential” methane oxidation rate of the system, but may not predict the present and actual methane oxidation rate. It will be critical to know the time window over which the *pmoA* gene counts trace the historical growth of methanotroph populations. In addition, we will conduct qPCR experiments examining specific groups of methanotrophs to see if some are more ephemeral and some are more permanent. Among the methanotrophs detected in the compost, *Methylobacter* are known to form spores that may last some time, while *Methylomicrobium* should follow a boom-and-bust strategy. The latter may better track short term changes in activity, while the former should track longer term changes.

The qPCR results with the meso scale laboratory column systems showed that the methanotrophic population levels significantly increased over the time, from 0 d to 50 d and 100 d after the initiation of methane flow (Figure 9). This again is promising data that the qPCR system works in a broad sense to show the methane oxidation potential and the methane oxidation history of the column.

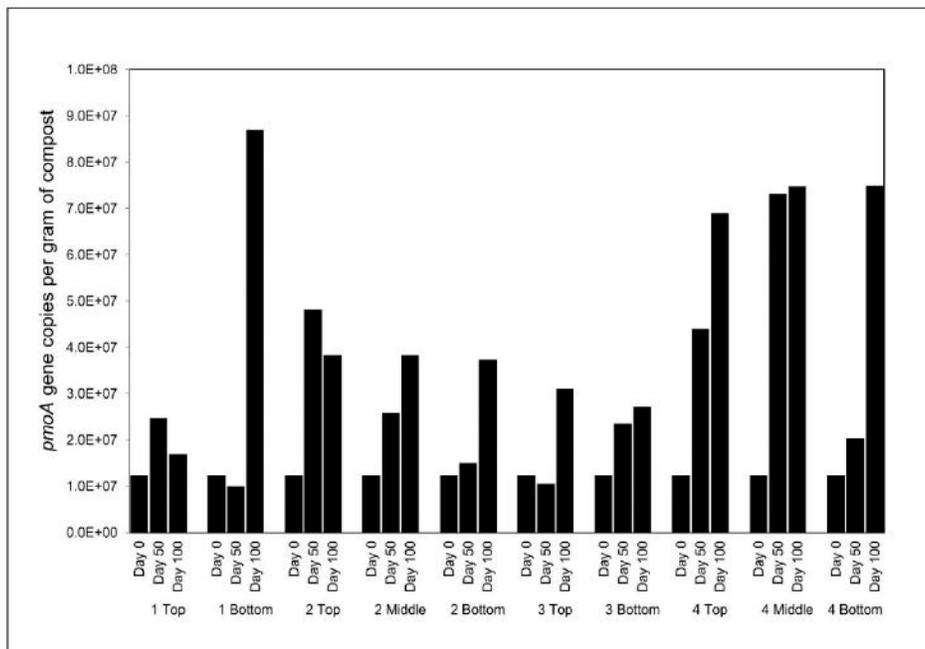


Figure 9. Abundance of *pmoA* gene copies in the biofilter columns with depth over the time. 1 and 2, Active aeration; 3 and 4, Passive aeration; T, Top; M, Middle; B, Bottom.

However, there were no consistent trends with depth and aeration type in this experiment, possibly due to sampling issues. Likely the small samples taken from the side ports of the columns did not integrate enough over space, and microscale differences in methanotroph growth were not properly averaged to determine small population differences. Large scale population growth over time was shown clearly, but smaller differences in treatments were not captured precisely. Therefore, following more extensive standard guidelines for soil sampling will be required to obtain the representative samples.

Group specific primers for *Methylococcus* and Type II (*Alphaproteobacteria*) methanotroph groups were also tested with the same samples. *Methylococcus* showed an overall increasing trend over the time regardless the sampling depth or aeration methods, but type II methanotrophs showed irregular patterns over the time on the sampling depth or aeration methods (Figure 10). These results are interesting because the type II methanotrophs were not detected from the original compost sample. It is believed that type II methanotrophs may be near the detection threshold of the qPCR amplification and/or the group specific primers used for the type II methanotrophs could not cover the whole group of type II methanotrophs. These results also support the need to test new primer sets for specific methanotrophic groups, to discover which is best suited to our goals.

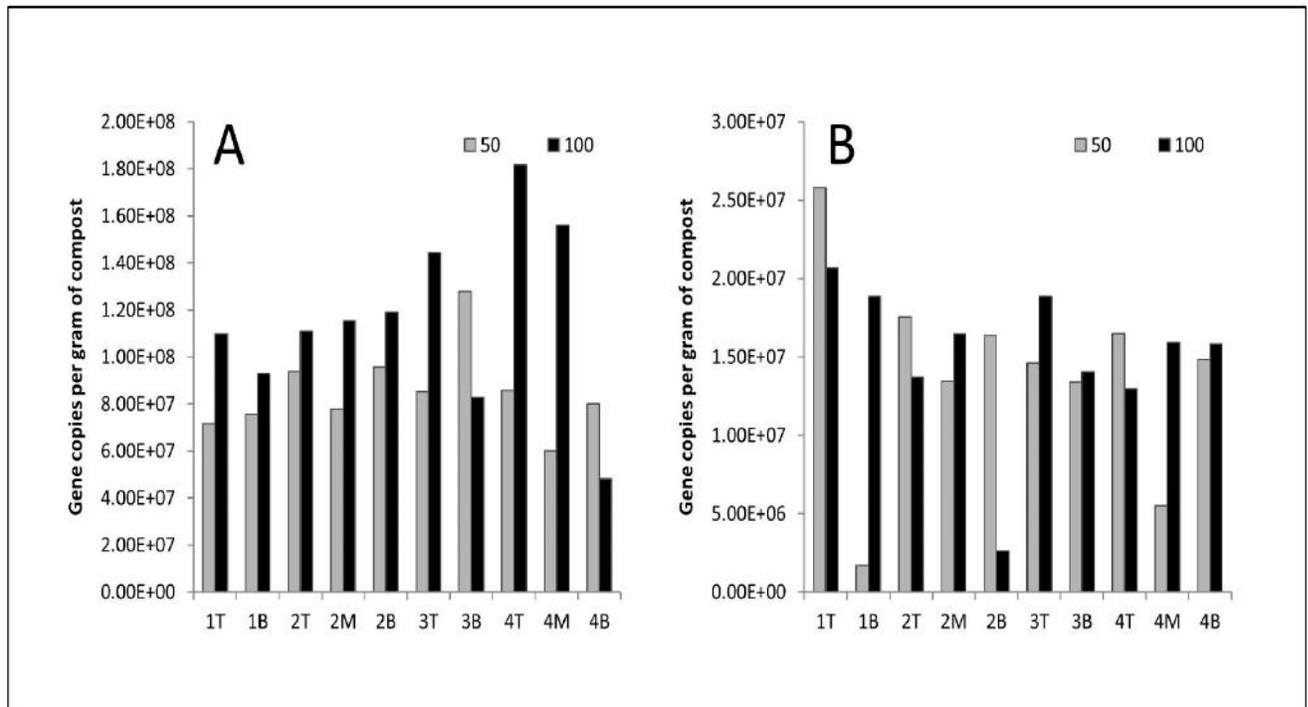


Figure 10. Analysis of methanotrophic community over the time by real-time qPCR in the compost. (A) *Methylococcus* group, and (B) Type II methanotroph group. 1 and 2, Active aeration; 3 and 4, Passive aeration; T, top; M, middle; B, Bottom.

Most probable number counts of methanotrophs. The most probable number (MPN) method was used to quantify methanotrophs, using NMS (Nitrate Mineral Salts) medium. One gram of compost was mixed with 20 ml of NMS medium in a 120-ml serum bottle and sealed with a rubber stopper. The gas atmosphere in the headspace of the bottle was fed with methane by replacing 15 ml air with 10 ml CH₄ and 5 ml of CO₂. The bottle was incubated at room temperature for 4 weeks. Bottle headspaces were sampled every week during the incubation by removing 1 ml of gas, and CH₄ and O₂ concentrations were quantified on a gas chromatography (GC) equipped with the thermal conductivity detector and the flame ionization detector (Agilent; Varian-450). Positive methane consumption in a vial compared to blanks (containing no compost) was scored as positive growth. The MPN of methanotrophs was expressed as MPN per gram of compost.

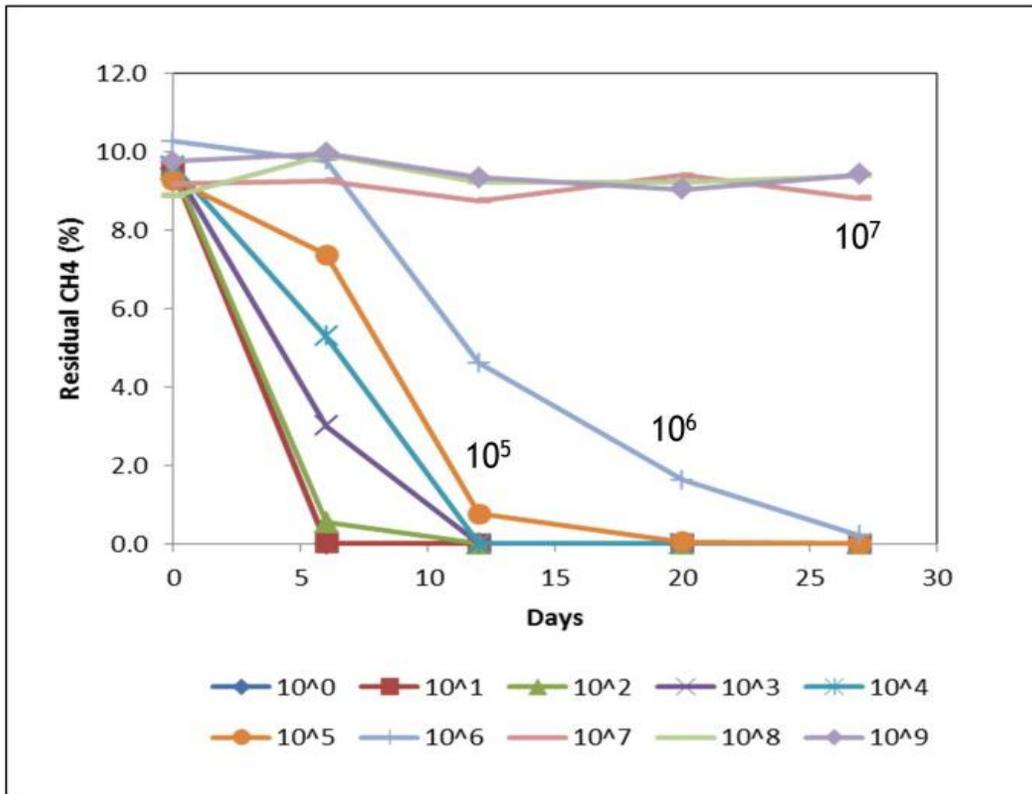


Figure 11. Methane oxidation of a serially diluted compost sample to determine the most probable number (MPN)

To verify the qPCR method, the results from a qPCR experiment were compared with values from a most probable number experiment (as MPN per gram of compost). For the MPN experiment, one of the compost samples from a field site (Fernie, BC) was serially diluted with the NMS medium and incubated under methane enriched

conditions. Methane oxidation (i.e. presence of methanotrophs) was observed in 10^1 - 10^6 dilution samples (Figure 11), which means the most probable number of methanotrophs was in the order of 1.0 - 9.9×10^6 per gram of compost. qPCR result for this particular sample showed 5.2×10^7 *pmoA* gene copies per gram of compost. In this study, the culture dependent MPN method showed a comparable value in methanotrophic population levels compares to the qPCR method, verifying the usefulness of the method.

Conclusions to Section I

Methods fro analysis of methanotrophic populations in the biofilters were optimized. Community analysis (relative ratios of methanotrophs compared to other bacteria) and quantitative PCR results consistently showed an increase of methanotrophic populations in laboratory biofilter microcosms and mesocosms over time during incubation with methane. The molecular monitoring of methanotrophic bacteria therefore tracks the increase in methane oxidising activity in fresh biofilters. The most probable number method confirmed the qPCR was an accurate method. However, initial results suggest that most methanotrophs present are very recalcitrant. They do not die under extended starvation and therefore may not be used effectively as indicators of the present activity of a biofilter. This was tested more closely in Section II.

II. Monitoring protocol development: determination of reliable indicator species of biofilter activity (2015-2016)

Based on laboratory experiments of methanotrophs incubated under actively growing or starvation conditions, we developed a monitoring system that should indicate the current methanotrophic activity of a biofilter using compost as a packing material. This monitoring protocol has been dubbed the MOM (methylotrophs over methanotrophs) protocol. It is based on high-throughput sequencing of community 16S rRNA genes, followed by assessment of the relative abundance of methylotrophic bacteria like Methylophilum spp. that grow and die quickly in response to methanotrophic activity. The ratio of methylotrophs to methanotrophs and other bacteria should be an indicator of activity. This proposed monitoring protocol is ready to be tested on field biofilters, however samples from these are not yet available.

Materials and methods

DNA extraction. DNA was extracted from 0.5 g of compost using the FastDNA SPIN Kit for Soil (MP Biomedicals) following the manufacturer's instruction with the following modification. Since it was difficult to get the high purity DNA from compost, additional purification steps using 5.5 M guanidine thiocyanate (GTC) were introduced in the washing step (Knief et al., 2003). The DNA concentration was determined by using a Qubit Fluorometer with a Quant-iT™ dsDNA HS Assay Kit (Invitrogen) and the purity of DNA was determined by using Nanovalve Plus™ (GE Healthcare).

Illumina sequencing of 16S rRNA genes. The hypervariable V4 region of the 16S rRNA gene were amplified according to the Illumina protocol (http://www.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). The Illumina MiSeq system (Illumina, Inc.) was used to sequence 16S rRNA gene amplicons using standard protocols (http://www.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Communities were analyzed using QIIME software (Caporaso et al, 2010). This allowed the determination of ecological statistics and detection of individual species.

Starvation experiment. Twenty five gram amounts of compost were mixed with either water or nitrate mineral salts (NMS) medium, and added into 1 L media bottles (Figure

1). Bottles were sealed with butyl rubber stopper and 10% (v/v) of CH₄ and 5% of CO₂ added to the headspaces. The bottles were incubated at room temperature and monitored CH₄ and CO₂ levels using gas chromatography (Agilent; Varian-450). Each week microcosms were opened and left to stand on a clean bench for 30 minutes to allow the gases inside the bottle to be replaced with air. Each bottle was then re-sealed with a butyl rubber stopper and CH₄ and CO₂ re-injected at the same concentration as described above.

After 3 weeks of incubation, some incubations (active conditions) were continued with constant methane replacement and others (starvation conditions) were incubated henceforth without any methane in the headspace, either under anoxic or oxic conditions. All treatments were performed in triplicate. Each week a portion of compost was taken from the bottles for DNA extraction and microbial community analysis. The starvation experiment has been continued for several months. Samples from different time points have been analysed.

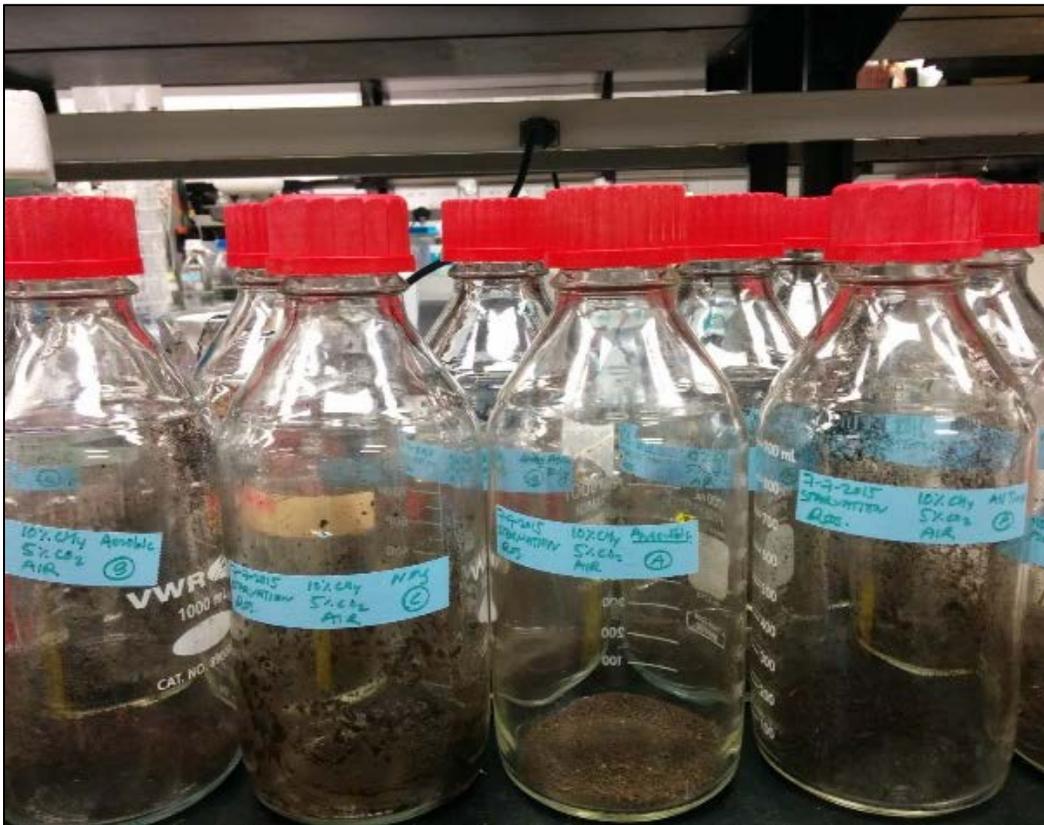


Figure 1. Incubation of compost for the starvation experiment.

Results and Discussion:

Spore / cyst formation of methanotrophs From the literature review, we found that *Methylomonas* spp. rarely form cysts and never spores, and *Methylomicrobium* spp. do not form either spores or cysts (Table 1). This indicates that these two species might be suitable indicator strains to monitor the methanotrophic activity in the methane biofilter system. Since they do not form resting stages, they are likely to die rapidly in compost that is not actively consuming methane, and therefore their presence could be used as an indication of an active biofilter. To verify this hypothesis, we analyzed the microbial community under active versus starvation conditions in laboratory microcosms (figure 1).

Table 1. Summary of spore/cyst formations among the species of methanotrophs.

Group		Spore	Cyst	References
Type Ia	<i>Methylobacter</i>	N/A	Yes	Int. J. Syst. Bacteriol., 1993, 43, 735-753
	<i>Methylomonas</i> *	No	Yes	
	<i>Methylomicrobium</i>	No	No	Int. J. Syst. Bacteriol., 1995, 45, 182-185
Type Ib	<i>Methylococcus</i>	No	Yes	
	<i>Methylocaldum</i>	N/A	Yes	Arch. Microbiol., 1997, 168, 493-503
Type II	<i>Methylocystis</i>	No	Yes	Int. J. Syst. Evol. Microbiol., 2013, 63, 1096-1104
	<i>Methylosinus</i>	Yes	No	Int. J. Syst. Bacteriol., 1993, 43, 735-753
	<i>Methylocapsa</i>	-	Yes	Int J. Syst. Evol. Microbiol., 2002, 52, 251-261
	<i>Methylocella</i>	Yes	N/A	Int. J. Syst. Evol. Microbiol., 2010, 60, 2659-2664
Verruco	<i>Methylacidiphilum</i>	N/A	N/A	

Microbial community structure in response to starvation in compost. Each microcosm was supplied with ~10% CH₄ (v/v) and 5% CO₂ (v/v) in the headspace during the starvation experiment. Starvation treatments (Figure 2) included compost incubated under aerobic or anaerobic conditions. Active treatments (Figure 3) included compost and compost with added nutrients (NMS medium) to stimulate growth.

Headspace was replaced each week. After 3 weeks of an initial growth phase the methane replacement was stopped for the starvation treatments (Figure 2).

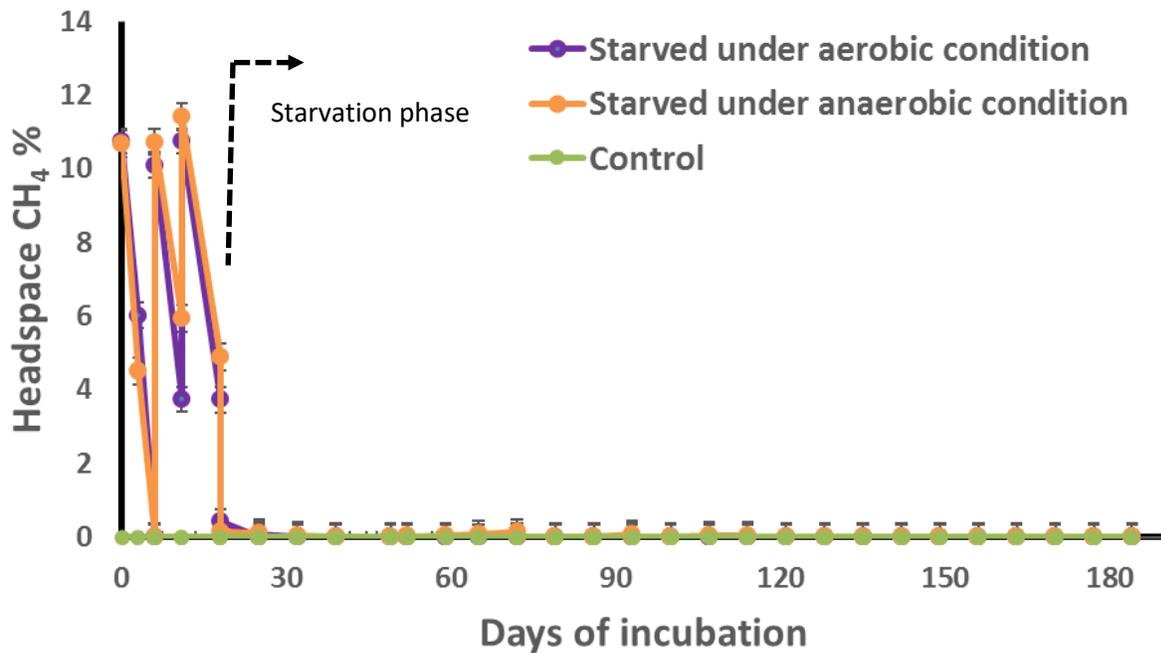


Figure 2. Methane oxidation of compost in the starvation treatment sets. Methane starvation was started after 3 weeks of incubation under aerobic or anaerobic condition. Aerobic condition was generated by replacing the head space of the bottle with air and anaerobic condition was generated by filling with N₂ when the samples were taken.

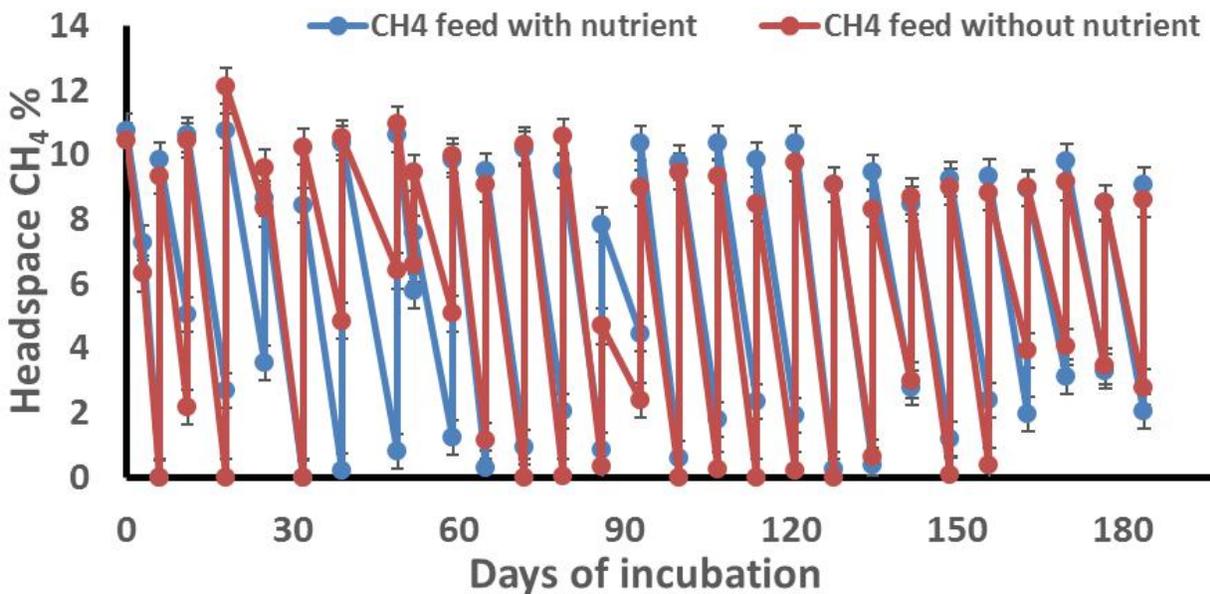
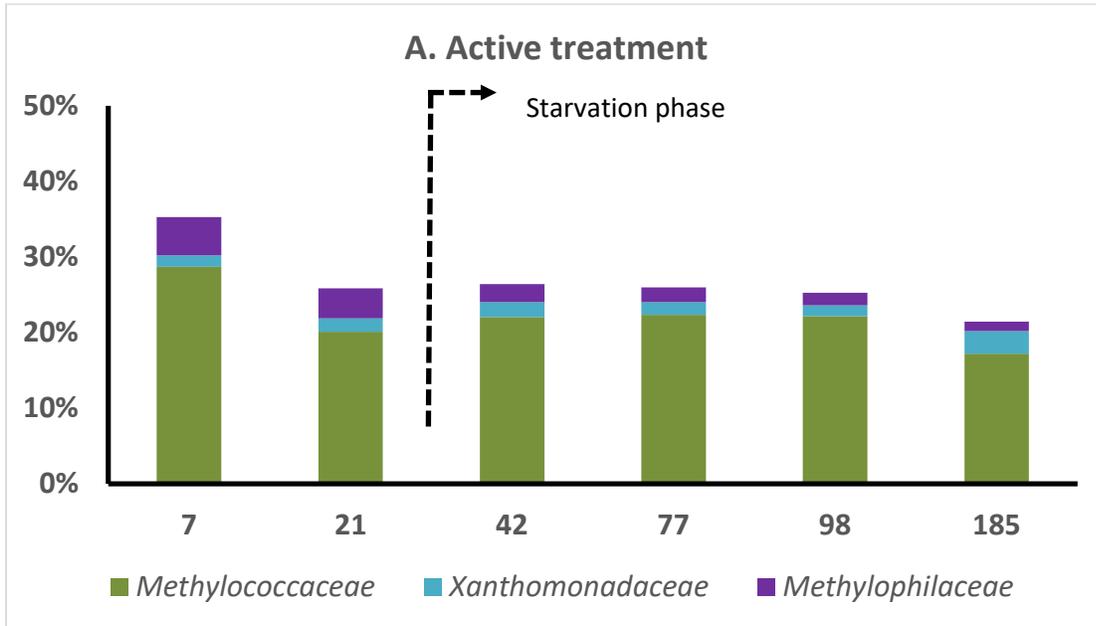


Figure 3. Methane oxidation of compost under different nutrition conditions.

Samples were taken at the beginning of incubation (Day 0) and different time points of incubation and DNA extracted. Illumina sequencing of 16S rRNA genes was performed for the selected samples.

Compost methanotrophic community. Figure 4 represents the methanotrophic and methylotrophic bacteria present in the aerobic and anaerobic starvation treatment compared to active treatment over time. Methanotrophs belonging to the family *Methylococcaceae* were dominant when enriched with methane (3 weeks) in all treatments. These *Methylococcaceae* showed only a very slow decrease of their relative population after the onset of starvation, in accordance with their ability to form resting stages (Figure 6, Table 1). This indicates the overall population of methanotrophs is NOT a useful proxy for estimating the activity of the biofilter. However, *Methylomicrobium* do not form spores and cysts and therefore can not survive when methane is limited or unavailable.



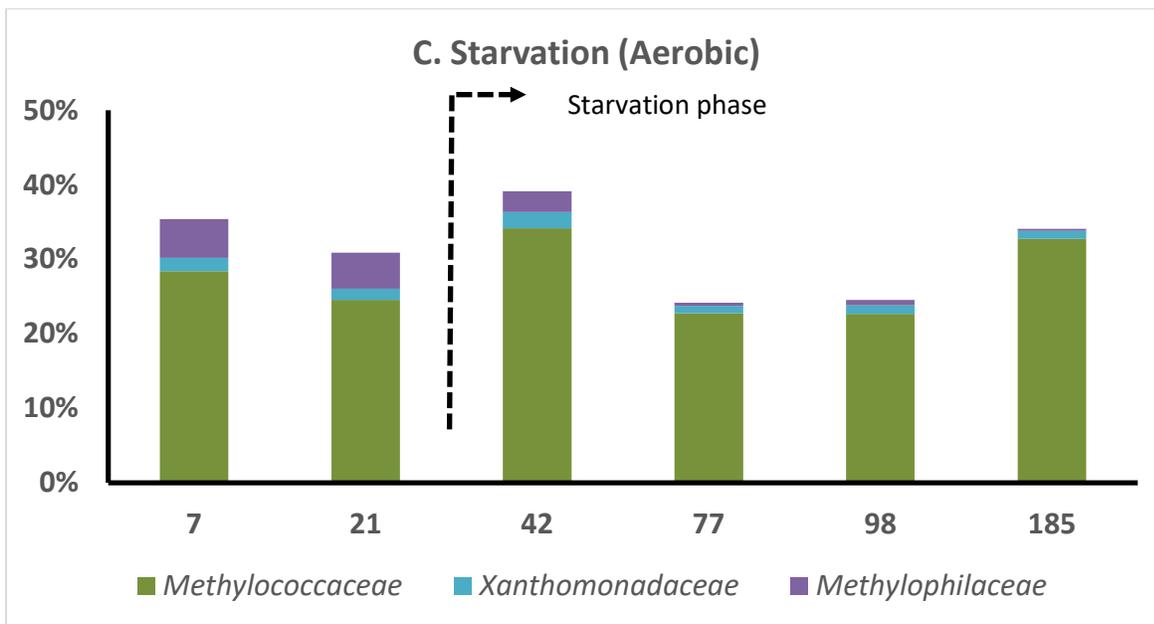
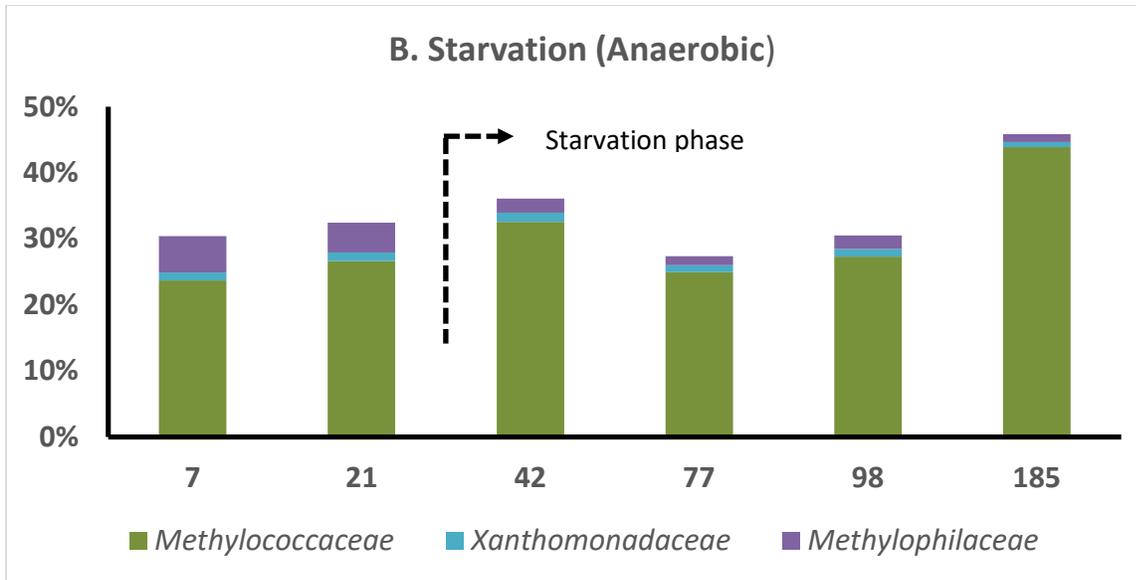


Figure 4. Microbial community analysis of compost using 16S rRNA gene-targeted illumina sequencing. Microbial community dynamics over the incubation time (days) in the A) Active treatment, B) Starvation (Aerobic) and C) Starvation (Anaerobic) of compost.

The original hypothesis to be tested in this experiment was that the ratio of the ephemeral methanotroph *Methylophilum* sp. would be high in active compost but would decrease during starvation. This would indicate that it is indeed a good candidate as an indicator species of biofilter activity. However, this trend is also not evident in the experiment (Figure 5).

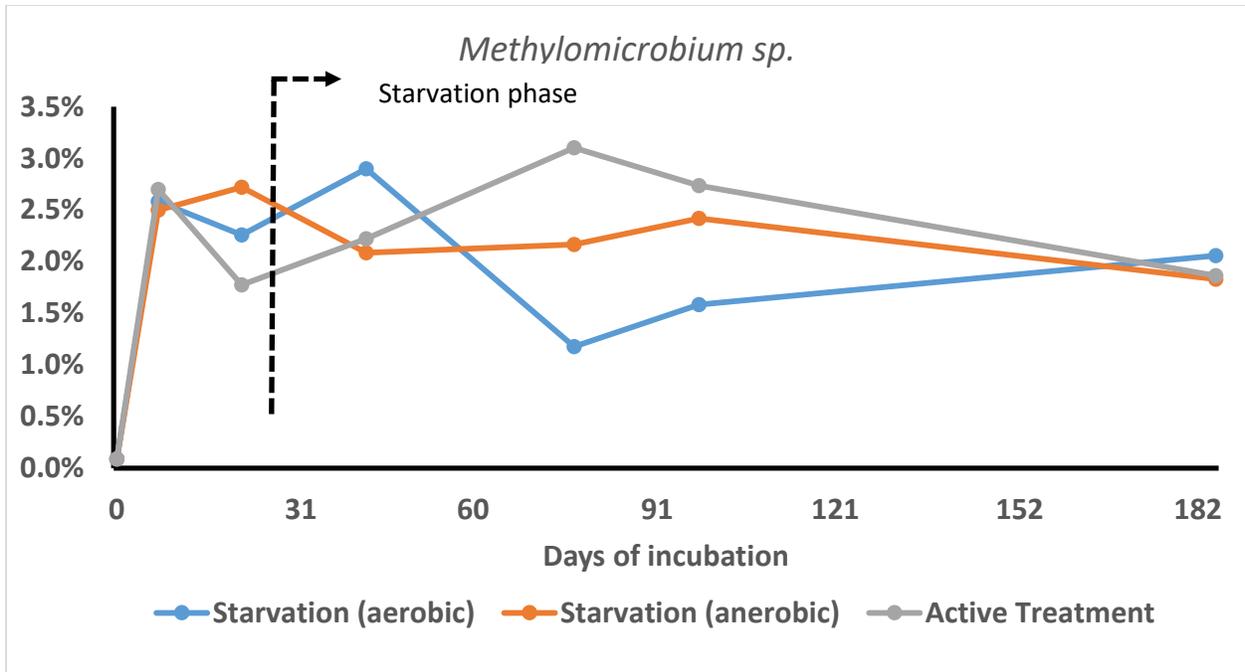


Figure 5. Microbial community analysis of compost using 16S rRNA gene-targeted illumina sequencing. Microbial community dynamics over the incubation time in the starvation treatment (Anaerobic) of compost.

A *pmoCAB* gene database was assembled from all available methanotroph genomes in order to design a quantitative PCR primer to monitor populations of *Methylochromobium*. However, the *pmoA* gene sequence of *Methylochromobium* sp. was highly conserved compared to other methanotrophs (some of which are known to form spores, like *Methylobacter*). It was impossible to design a specific primer set for this genus alone (Figure 6).

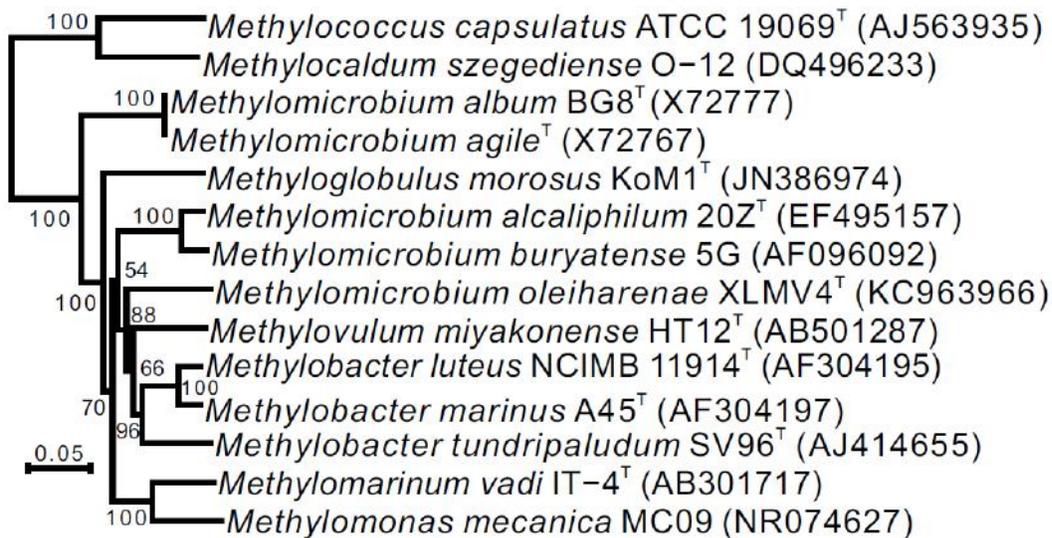


Figure 6. Neighbor-joining concatenated *pmoCAB* gene sequence based phylogeny of methanotrophs.

The conclusion from these data must be that NO single methanotroph species is a good indicator of the present biofilter activity. Although methanotrophs grow as the biofilter becomes more active, they do not decline when the biofilter becomes inactive. Even after extended starvation methanotrophs are abundant. A high population may indicate the potential for activity, but it does not indicate that the activity is actually occurring.

Alternative monitoring protocol targeting methylotrophs. Although methanotroph species did not seem to be a good indicator of biofilter activity, two groups of methylotrophs (the *Methylophilaceae* and *Xanthomonas*) declined far more rapidly with the onset of starvation (Figure 6). Figure 7 shows the trend for one species within the *Methylophilaceae*: *Methylotenera* sp. (Figure 7).

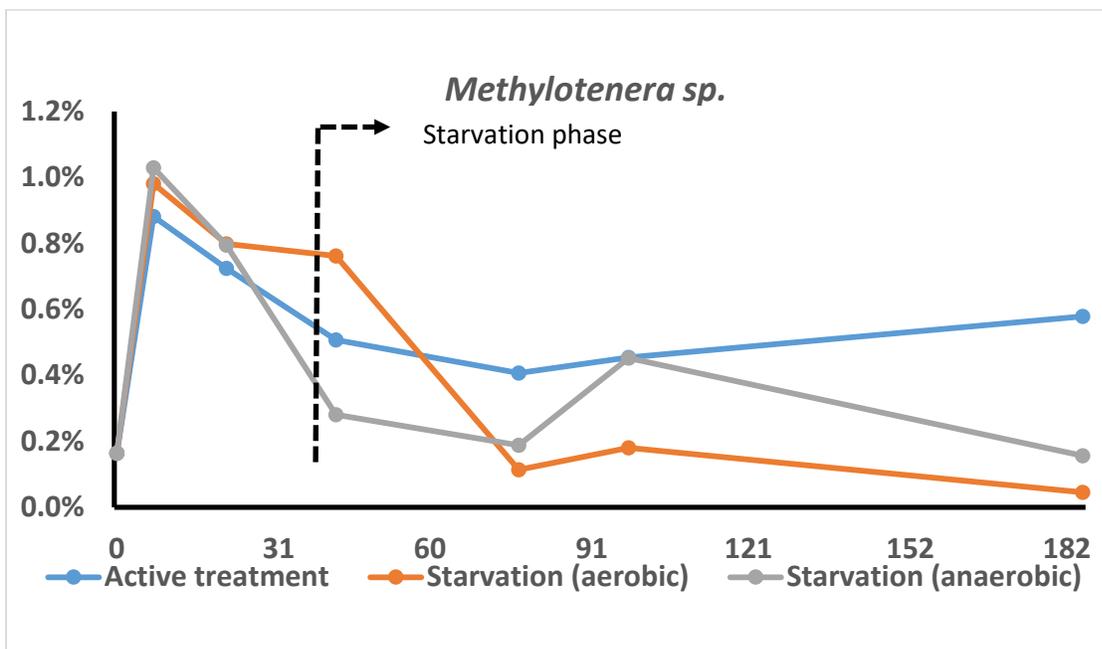


Figure 7. Methylotrophic (*Methylotenera* sp.) population change over time in different treatments.

The probable explanation for this is that these methylotrophic bacteria require active methanotrophic activity to supply them with food. They grow on methanol and formaldehyde, which are byproducts of methane oxidation. They are therefore dependent on methanotrophs in the compost biofilter to provide them with energy.

The methanotrophic population persists for a long time due to their ability to form spores and cysts. However, the methylotrophic co-feeders appear to be short lived and decline when methane is removed from the system (Figure 6). Therefore, we have instituted a monitoring system for these "methanotroph-associated methylotrophs" (the ratio of *Methylotenera* to *Methylococcaceae*) (Figure 8). In active biofilters the ratio was

maximal at around 0.045, and varied from (0.02-0.045). However, this ratio dropped below 0.005 within one and half month of the onset of starvation and continued to decline over time to values 0.001 with extended starvation. We consider this ratio as a rapid test of biofilter activity. If the ratio drops below 0.02, a biofilter should be examined more closely. Of course this hypothesis will require testing in the field.

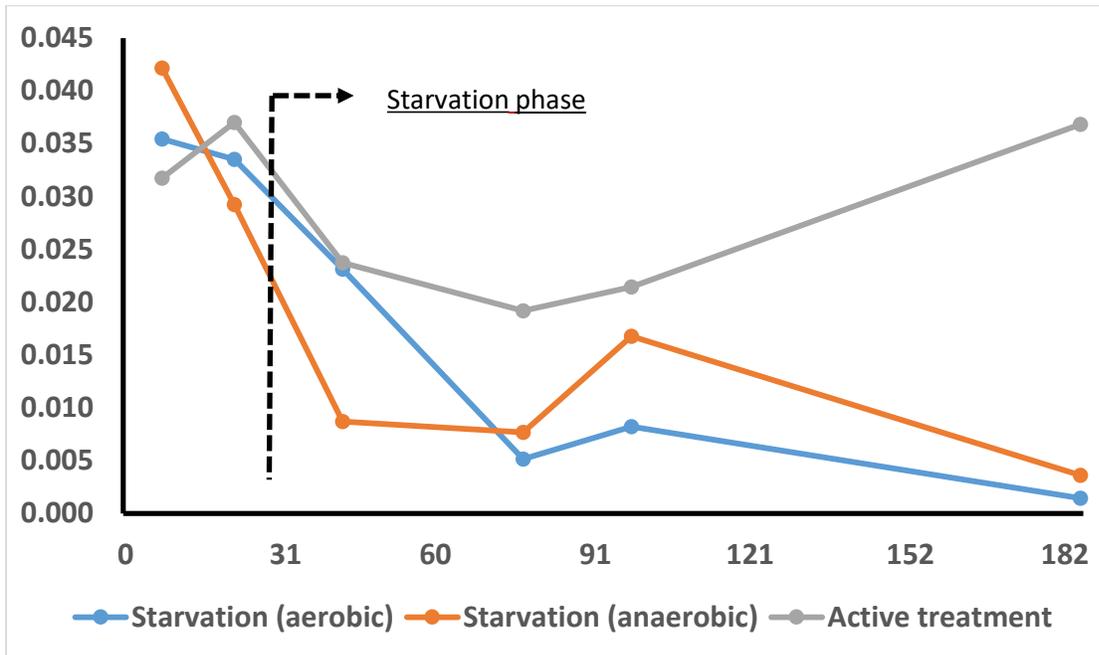


Figure 8: Ratio of overall percentage of methylotrophs (*Methylothera*) to methanotrophs (*Methylococcaceae*).

Conclusions to Section II:

Methylomicrobium was hypothesized to be the best methanotroph species for monitoring biofilter activity, as it does not form resting stages. However, its relative abundance compared to other methanotrophs and other bacteria in general did not markedly decline during starvation and we concluded that a monitoring protocol based on any methanotroph was unlikely to be successful. However, some methylotrophic bacteria do decline very rapidly during methane starvation. *Methylophilaceae* spp. (*Methylothera*). are a clear example. The best overall indicator species for methane-oxidising activity in the compost are in fact not methanotrophs, but rather short-lived methylotrophs dependent on methanotrophic activity for their sustenance. These will be the basis of our monitoring protocol, which we designate MOM (methylotrophs over methanotrophs).

III. Development of microbial consortia for seeding biofilters in acidic environments.

The main objective here is to develop inocula to enhance biofilter methane oxidation rate under conditions where acidification is expected due to H₂S.

Methods and Results.

Different microcosms were set up at pH ranging from 2.5 to 5.5 by spraying 1M H₂SO₄ to 10 g of compost and measurement by pH a meter. We have aerobic methanotrophic isolates, *Methylacidiphilum inferorum* V4 and *Methylacidimicrobium* sp. LP2A that have been isolated in our lab and can grow in extremely acidic conditions. Therefore, acidophilic *Verrucomicrobia* methanotrophs strain V4 and LP2A were grown with ~10% CH₄ (v/v) and 5% CO₂(v/v) in the headspace of 120 ml serum bottles containing 20 ml of a Nitrate Mineral Salts (NMS) medium, incubated at 55 C° or 30 C°, respectively. The acidophilic isolates (1 ml) were added to the compost (25 g) after they reached an optical density (OD₆₀₀) of 0.5. The addition of these isolates did not enhance the methane oxidation rate of the acidified compost, thus it seems that they are unable to survive in a compost environment for some reason.

Therefore, natural acidic peat samples (pH 4.0) from Alberta and acidic soil samples (pH 4.8) from the Paintpots spring in BC, Canada) were used to enrich methanotrophs for seeding acidified biofilters. Peat samples were incubated with ~10% CH₄ (v/v) and 5% CO₂(v/v) in the headspace of 120 ml serum bottles containing 20 ml of Nitrate Mineral Salts (NMS) medium. Oxidation of 10% methane took nearly 5 weeks and the enrichments were again re fueled two more times (Figure 1). Enrichment samples were taken for DNA extraction and 16S rRNA gene community analysis. Nearly 70% of the methanotrophs enriched belonged to the alphaproteobacterial methanotrophs, particularly *Methylosinus* sp. and *Methylocapsa* sp. (Figure 2). Enriched microbial consortia will be added to acidified compost and methane oxidation rates tested.

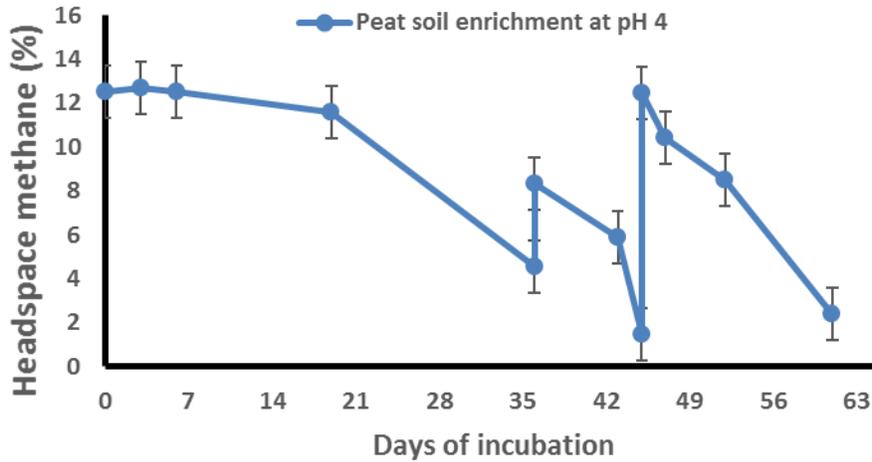


Figure 1. Methane oxidation profile of peat samples over time.

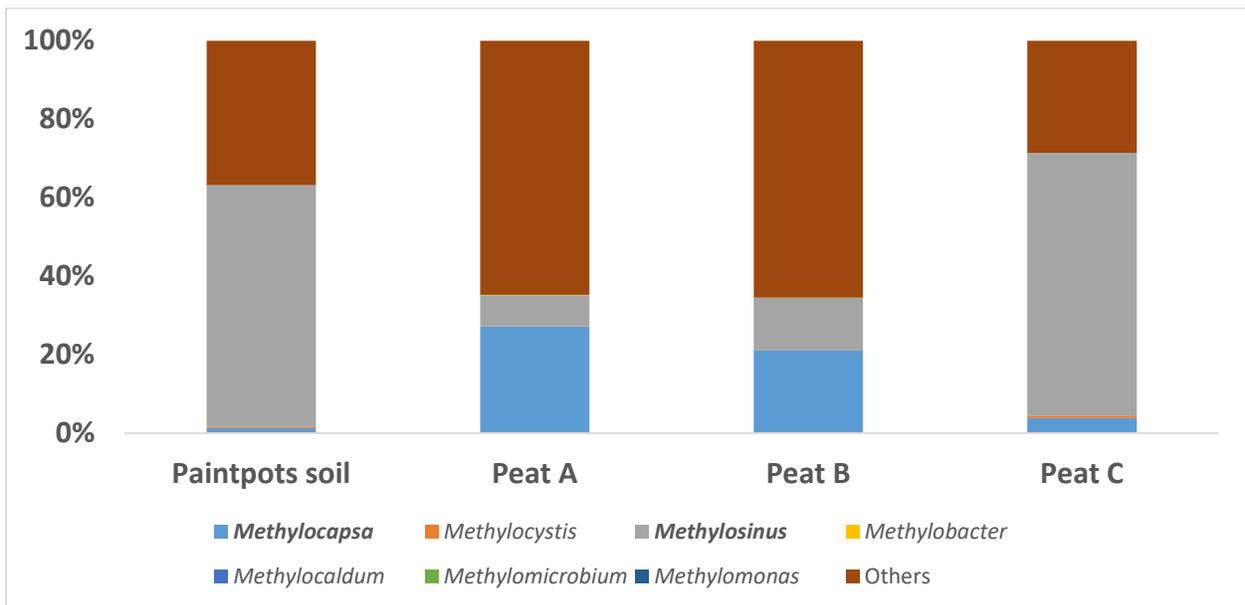


Figure 2. Overall methanotrophic population detected in enrichments by 16S rRNA gene Illumina sequencing.

Conclusions to Section III:

Methylosinus sp. and *Methylocapsa* sp. was enriched to around 70% and 30 % respectively of total bacteria from acidic samples. These groups of methanotrophs have been already isolated and identified to oxidize methane under acidic conditions (Dedysh, Panikov et al. 1998, Dunfield and Dedysh 2010). Microcosms will be set up at varying acidic condition and enriched peat samples will be used as inocula to test the methane oxidation efficiency under acidic conditions.

Future Work

- Test inocula for seeding acidified biofilters
- Develop a qPCR system for methylotrophs
- test the MOM protocol in field filters and mesocosms. The major issue here may be heterogeneity in a large field biofilter, and obtaining a proper sample.
- verify critical values of the MOM protocol by analysing more replicates of the starvation experiment.

Outputs and deliverables:

Conference presentations:

Kim, J.J., Khadka, R., Hettiaratchi J.P., Dunfield, P.F. *Control of point-source low-volume methane emissions using methane biofiltration technology: Development of a molecular monitoring protocol*. The 6th Annual Bioindustrial Meeting: Biorefining Conversions Network – Alberta Innovates Bio (BCN-AI Bio). November, 2015, Edmonton

Dunfield P.F., Kim, J.J., Khadka, R. *Can understanding microbial communities help to design better biofilters?* Application of Biological Methods to reducing Greenhouse Gases: Schulich School of Engineering International Workshop. August 2015, Calgary.

Khadka R., Dunfield P. F. *Phylogenetic history of copper monooxygenase gene family*. ISME, August, 2016, Montreal

References :

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 7(5): 335-6.

Knief C, Lipski A, Dunfield PF, (2003) Diversity and activity of methanotrophic bacteria in different upland soils, *Appl Environ Microbiol*. 69(11): 6703-14.

Mizrahi-Man, O., E. R. Davenport and Y. Gilad (2013). "Taxonomic classification of bacterial 16S rRNA genes using short sequencing reads: evaluation of effective study designs." *PLoS One* 8(1): e53608.

Sinclair, L., O. A. Osman, S. Bertilsson and A. Eiler (2015). "Microbial Community Composition and Diversity via 16S rRNA Gene Amplicons: Evaluating the Illumina Platform." *PLoS ONE* 10(2): e0116955.

Dedysh, S. N., N. S. Panikov and J. M. Tiedje (1998). "Acidophilic Methanotrophic Communities from Sphagnum Peat Bogs." *Applied and Environmental Microbiology* 64(3): 922-929.

Dunfield, P. and S. Dedysh (2010). Acidic environments. *Handbook of Hydrocarbon and Lipid Microbiology*, Springer Berlin Heidelberg: 2181-2192.

Bogner, J., Spokas, K., Burton, E., Sweeney, R., Corona, V. (1995) Landfills as atmospheric methane sources and sinks. *Chemosphere* 31: 4119–4130.

Bourne, D. G., I. R. McDonald and J. C. Murrell (2001) Comparison of pmoA PCR primer sets as tools for investigating methanotroph diversity in three Danish soils. *Appl Environ Microbiol* 67(9): 3802-3809.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 7(5): 335-6.

Costello, A. M. and M. E. Lidstrom (1999) Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. *Appl Environ Microbiol* 65(11): 5066-5074.

- Dunfield, P.F. (2006) The soil methane sink. in D. Reay, C.N. Hewitt, K. Smith, and J. Grace (eds) *Greenhouse Gas Sinks*. pp 152-170. Wallingford, UK. CABI Publishing
- Dunfield, P.F. (2009) Methanotrophy in extreme environments. in John Battista et al (eds) *Encyclopedia of Life Sciences*. John Wiley and Sons, Chichester. www.els.net.
- Dedysh SN, Liesack W, Khmelenina VN, Suzina NE, Trotsenko YA, Semrau JD, Bares AM, Panikov NS, Tiedje JM: *Methylocella palustris* gen. nov., sp nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. *International Journal of Systematic and Evolutionary Microbiology* 2000, 50:955-969.
- Ettwig KF, van Alen T, van de Pas-Schoonen KT, Jetten MSM, Strous M: Enrichment and molecular detection of denitrifying methanotrophic bacteria of the NC10 Phylum. *Applied and Environmental Microbiology* 2009, 75(11):3656-3662
- Ferguson, RBm Gary W. Hergert, Charles A. Shapiro and Charles S. Wortmann, (2007) Guidelines for Soil Sampling, <http://www.ianrpubs.unl.edu/epublic/pages/publicationD.jsp?publicationId=831>
- Gebert, J., Groengroeft, A., and Pfeiffer, E.-M. (2010) Relevance of soil physical properties for the microbial oxidation of methane in landfill covers. *Soil Biol Biochem* 43: 1759-1767.
- Gebert, J., Roewer, I.U., Scharff, H., Roncato, C.D.L., and Cabral, A.R. (2011) Can soil gas profiles be used to assess microbial CH₄ oxidation in landfill covers? *Waste Manag* 31: 987-994.
- Gouy M, Guindon S, Gascuel O. (2010) SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 27: 221-224.
- Hanson, R.S. and Hanson, T.E. (1996) Methanotrophic bacteria. *Microbiol. Rev.* 60: 439-471.
- Henckel, T., Roslev, P., and Conrad, R. (2000) Effects of O₂ and CH₄ on presence and activity of the indigenous methanotrophic community in rice field soil. *Environ Microbiol* 2: 666-79.
- Henneberger, R., Luke, C., Mosberger, L., and Schroth, M.H. (2011) Structure and function of methanotrophic communities in a landfill-cover soil. *FEMS Microbiol Ecol* 81: 52-65.

- Holmes, A. J., A. Costello, M. E. Lidstrom and J. C. Murrell (1995a) Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. *FEMS Microbiol Lett* 132(3): 203-208.
- Holmes, A. J., N. J. Owens and J. C. Murrell (1995b) Detection of novel marine methanotrophs using phylogenetic and functional gene probes after methane enrichment. *Microbiology* 141 (Pt 8): 1947-1955.
- Knief C, Lipski A, Dunfield PF, (2003) Diversity and activity of methanotrophic bacteria in different upland soils, *Appl Environ Microbiol.* 69(11): 6703-14.
- Kolb S, Knief C, Stubner S, Conrad R, (2003) Quantitative detection of methanotrophs in soil by novel pmoA-targeted real-time PCR assays, *Appl Environ Microbiol.* 69(5): 2423-9.
- Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar, Buchner A, Lai T, Steppi S, Jobb G, Förster W, Brettske I, Gerber S, Ginhart AW, Gross O, Grumann S, Hermann S, Jost R, König A, Liss T, Lüßmann R, May M, Nonhoff B, Reichel B, Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A, Schleifer K-H. (2004) ARB: a software environment for sequence data. *Nucleic Acids Res* 32: 1363-1371.
- Luesken, F. A., B. Zhu, T. A. van Alen, M. K. Butler, M. R. Diaz, B. Song, H. J. M. Op den Camp, M. S. M. Jetten and K. F. Ettwig (2011) *pmoA* Primers for Detection of Anaerobic Methanotrophs. *Appl Environ Microbiol.* 77(11): 3877-3880.
- Martineau C, Whyte LG, Greer CW, (2010) Stable Isotope Probing Analysis of the Diversity and Activity of Methanotrophic Bacteria in Soils from the Canadian High Arctic, *Appl Environ Microbiol.* 76(17): 5773–5784.
- McDonald, I. R., L. Bodrossy, et al. (2008) Molecular ecology techniques for the study of aerobic methanotrophs. *Appl Environ Microbiol.* 74(5): 1305-1315.
- Nadkarni MA, Martin FE, Jacques NA, Hunter N, (2002) Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology* 148(Pt 1): 257-66.
- Saidi-Mehrabad A, He Z, Tamas I, Sharp CE, Brady AL, Rochman FF, Bodrossy L, Abell GC, Penner T, Dong X, Sensen CW, Dunfield PF, (2013) Methanotrophic bacteria in oilsands tailings ponds of northern Alberta, *ISME J.* 7(5): 908-21
- Sharp, C., M. Stott and P. Dunfield (2012) Detection of autotrophic verrucomicrobial methanotrophs in a geothermal environment using stable isotope probing. *Front Microbiol.* 3: 303.

Stein, L.Y., Roy R., and Dunfield, P.F. (2012) Aerobic methanotrophy and nitrification: Processes and connections. in Battista et al (eds) *Encyclopedia of Life Sciences*. John Wiley and Sons, Chichester. www.els.net.

9.6 Journal Paper: Methane Biofiltration in the Presence of Non-Methane Organic Compounds in Solution Gas

Methane Biofiltration in the Presence of Non-Methane Organic Compounds in Solution Gas

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Abstract:

Solution gas is a natural gas consisting mainly methane and small amounts of non-methane organic compounds. When the amount of solution gas released at individual locations are relatively small and the quality is low, it is not economically feasible to recover this gas. Therefore, environmentally acceptable methods are needed for their control. This research is focused on assessing the viability of using methane biofiltration technology to control point source, low volume solution gas emissions. The solution gas contains diverse pollutants in addition to methane, particularly hydrogen sulfide and non-methane organic compounds compared to the systems with which the methane biofilters have already been tested. A comprehensive set of laboratory experiments have been undertaken and the results showed that methane oxidation is not affected by the presence of lower compositions of ethane and propane that could presence in solution gas. However, increased ethane loading rates to methane biofilter columns adversely affected the methane oxidation efficiency.

Keywords: Methane Biofiltration, Trace Gases, Solution Gas.

1. INTRODUCTION

When crude oil is extracted from high-pressure reservoirs, the dissolved gases that comes out of the crude oil mixture and the gas trapped in the reservoirs are referred as solution gas [1, 2]. It is a natural gas consisting mainly of methane (CH₄) and small amounts of non-methane organic compounds (NMOC). Typically, the solution gas is flared, vented or conserved at well sites [2]. Although conservation/recovery is the ideal approach, when the quantities of solution gas released at individual locations are relatively small and the quality is low, it is not economically feasible to recover this gas. In such situations, the current practices involve venting the gas into the atmosphere or the process of low-temperature combustion (flaring). For example, Alberta (Canada) produces large amounts of solution gas. The majority of this gas is conserved and a relatively small fractions, added together in a larger context, this still represents a large quantity in absolute volumes, are flared or vented. In 2012, 94.2 % of the solution gas produced in Alberta was conserved, however, the combined volume of flared and vented solution gas was 978 million cubic meters [3]. Furthermore, about 140 billions of cubic meters of natural gas is flared annually at oil production sites worldwide, resulting in about 350 million tons of carbon dioxide (CO₂) in annual emissions [4].

The current practices of venting and flaring of CH₄-rich waste gas are fraught with unacceptable consequences. First, CH₄, the major component of solution gas, is a key greenhouse gas (GHG) with 34 times the global warming potential (GWP) of CO₂ over a 100-year time horizon [5]. Therefore, direct venting contributes to climate change. Second, flaring is known to produce gaseous by-products that are highly toxic, with potential to cause carcinogenic and non-carcinogenic health effects in humans [2]. Currently, there is no cost-effective established technology other than flaring to control low-volume solution gas emissions and therefore the development of environmentally acceptable methods are needed.

Methane biofiltration (MBF) is a biological process-based technology that could be applicable to mitigating point-source, low-volume and low-quality solution gas emissions. Methanotrophic bacteria, when residing in granular media, such as soil and compost, are capable of converting CH₄ to CO₂, thus serving as an important CH₄ sink and reducing the overall amount of GHG released to the atmosphere [6, 7, 8]. Methanotrophs, a part of the physiological group of methylotrophs, are able to produce MMO (methane monooxygenase) enzymes, which catalyze the oxidation of CH₄ [9]. The MBF technology has minimal environmental impact and generally does not require on-site operator presence and is consequently well-suited to oil well sites, small landfills, and other remote

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facilities. The common application of MBF is to attenuate CH₄ emissions from small and old sanitary landfills. Many of the past research efforts were focused on the application of MBF on sanitary landfill covers [9, 10, 11, 12, 13, 14, 15]. To date, only a very few researchers have tested MBF technology to mitigate CH₄ emissions from the oil and gas sector [6]. The overall process is not well understood.

The solution gas streams contain various pollutants in addition to CH₄, particularly non methane organic compounds (NMOCs) and hydrogen sulfide (H₂S). Johnson et al. [2] conducted a solution gas categorization study based on 5614 solution gas samples from different oil well sites in Alberta. Even though the solution gas composition could greatly vary between individual wells, according to the study, the prime constitute of solution gas, CH₄, composes of 70% and the rest being NMOCs (~16%), H₂S (~4%), Nitrogen (N₂~5%) and CO₂ (~5%). The NMOCs include mainly alkanes, about 10% Ethane (C₂H₆), 5% Propane (C₃H₈), and relatively very low compositions of Butane (C₄H₁₀) and Pentane (C₅H₁₂).

Compared to the previously studied methane biofilter systems on landfill gas, solution gas contains higher CH₄ compositions and the trace gases that have not been tested in the past. Some of the recent studies identified the co-metabolic properties of methanotrophs, the CH₄ degrading bacteria, and the various interactions that may occur between the various trace gases in landfill biogas. This particular study also looked at the interactions between landfill biogas and the microorganisms present in the landfill. [10, 11, 16]. The co-metabolism, interactions of trace gases in solution gas, and methanotrophs have not been tested in the past, however.

In this study, a comprehensive set of laboratory experiments were undertaken to assess the impact of higher CH₄ compositions and the presence of NMOCs, particularly Ethane (C₂H₆) and Propane (C₃H₈), on CH₄ biofilter performance; hence to assesses the viability of applying MBF technology in the solution gas sector. This paper presents the results obtained from a series of laboratory batch-scale and pilot-scale (methane biofilter column) experimental studies.

2. MATERIALS AND METHOD

A series of batch experiments and actively-aerated flow-through biofilter column experiments were conducted to determine the effect of both high CH₄ compositions and NMOCs on CH₄ oxidation. Although a variety of NMOCs are present in solution gas, the majority are C₂H₆ and C₃H₈; hence, C₂H₆ and C₃H₈ were used as representative NMOCs in this study.

2.1 Materials

Compost samples collected from the City of Calgary composting plant were used as a biofilter media in batch experiments. In the column experiments, top soil collected from the University of Calgary grounds were used. Prior to the experiments, the moisture content (MC) and water holding capacity (WHC) of those samples were determined according to the standard test methods [17]. The MC and the WHC of the compost samples used in the batch experiments were 19.6% and 72%, respectively, and the MC in the soil samples used in the column experiments were 18%.

High-purity (99.99%) CH₄, C₂H₆, C₃H₈, and oxygen (O₂) gases purchased from Praxair Canada Inc. were used in this study.

2.2 Experimental Design and Methodology – Batch Experiments

A 3³ factorial design was used to ascertain the co-metabolism and interaction effects of the representative NMOCs. Three factors, CH₄, C₂H₆ and C₃H₈ compositions, were tested at three different composition levels, as shown in Table 1.

Table 1: Experimental factors and treatment levels

Factors		Treatment levels		
		-1	0	1
A	CH ₄ composition (%)	70	75	80
B	C ₂ H ₆ composition (%)	0	5	10
C	C ₃ H ₈ composition (%)	0	2.5	5

Batch experiments were conducted in 250mL airtight amber glass bottles. The bottles had a narrow mouth with an open top cap and Teflon-silicone septa. A total of 27 combinations (3³) were used. Furthermore, randomly selected samples were conducted in triplicates. Each glass bottle was provided with 20g of pre-incubated compost at its field capacity. The bottles were then closed and sealed. The pre-determined amounts of CH₄, C₂H₆ and C₃H₈ gas (to make the required composition as shown in Table 1) were then added to the bottles using a syringe through septa caps, after withdrawing the corresponding amounts of headspace air from the bottles. In addition, each bottle was provided with 5% O₂, as the methanotrophs are aerobic bacteria.

All the bottles were kept in room temperature. The headspace gas composition of each bottle was measured after the addition of the gases at day 0 and periodically thereafter. This was done by collecting 0.5 mL of headspace gas using a gas-tight-sample-lock syringe and injecting it into the VARIAN 4900 Micro Gas Chromatograph. The gas compositions were measured until all the CH₄, C₂H₆ and H₂ were consumed. During the monitoring period, only the O₂ was added to the bottles to the rate of its depletion.

2.3 Experimental Design and Methodology – Flow-Through Biofilter Column Experiments

Four biofilter columns with a height of 1 m and a 15.2 cm outer diameter were constructed with Plexiglass. Gas sampling ports were drilled at 5cm intervals along the biofilter column and fitted with 3.8 mm male NPT adapters. A perforated steel plate and a fine steel mesh was placed 10 cm above the base of the biofilter column to facilitate the gas transfer. The biofilter columns were closed at both ends with Plexiglass end caps equipped with rubber O-rings. Figure 1 shows a schematic diagram of the biofilter column.

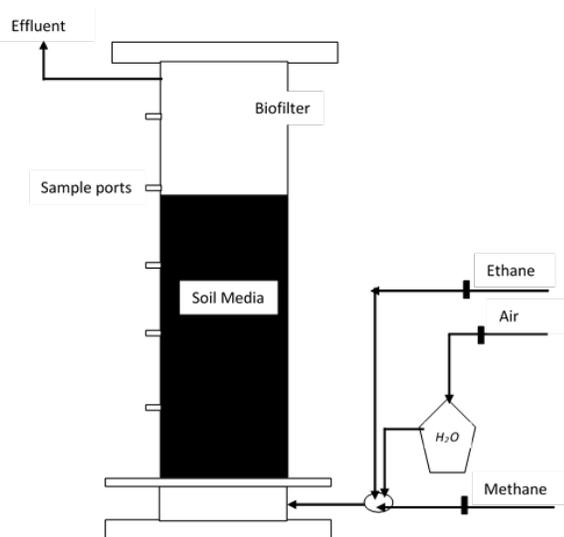


Figure 1: Experimental setup of the biofilter column

Soil that went through a 2.5 mm sieve was placed inside the biofilter columns up to a height of 60 cm. The soil filled in each column was about 10 kg. In the flow-through column experiments, only C₂H₆ was used as the representative NMOC to study the potential interaction effects of trace gases on CH₄ oxidation. CH₄ to C₂H₆ ratios of methane-ethane biofilters were taken as 8:1, 7:2 and 5:4. This was done in order to simulate solution gas and to observe the effect of C₂H₆ when the C₂H₆ concentrations are higher than the C₂H₆ concentrations in solution gas.

CH₄ and C₂H₆ loading rates at different operating stages of biofilter columns are included in Table 2. Biofilter column A was operated with 100% CH₄. Columns B, C and D were operated with different CH₄ to C₂H₆ ratios. The

loading rates of column A to D were increased in steps after reaching the steady state CH₄ oxidation for each step. The air flow rate was determined based on the stoichiometry of CH₄ oxidation.

Table 2: CH₄ and C₂H₆ loading rates at different operating stages of biofilter columns

Stage	Biofilter Column						
	A	B		C		D	
	100%	(CH ₄ :C ₂ H ₆) (8:1)		(CH ₄ :C ₂ H ₆) (7:2)		(CH ₄ :C ₂ H ₆) (5:4)	
	CH ₄	CH ₄	C ₂ H ₆	CH ₄	C ₂ H ₆	CH ₄	C ₂ H ₆
(g/m ² /day)							
I	≤406.8	≤406.	N/A	≤406.8	N/A	≤406.8	N/A
II	406.8	406.8	95.3	406.8	217.9	406.8	610.3
II	502.1	502.1	117.6	502.1	268.9	502.1	753.1
I	601.6	601.6	141.0	601.6	322.3	601.6	902.4
V	711.7	711.7	166.8	711.7	381.2	711.7	1067.5
V	841.9	841.9	197.1	841.9	536.6	711.7	1261.4
V	1001.7	1001.	234.7	1001.7	649.2	711.7	1261.4
V	1211.9	1211.	284.0	1001.7	649.2	711.7	1261.4

During the operational period, gas samples were collected regularly from the sampling ports and analyzed using a portable Hewlett Packard Micro-Gas Chromatograph. CH₄ and C₂H₆ oxidation rates were calculated as shown in Equations 1 and 2 [18, 19].

$$\text{CH}_4 \text{ oxidation efficiency} = \left[\frac{C_{m,in} - C_{m,out} \left(\frac{C_{n,in}}{C_{n,out}} \right)}{C_{m,in}} \right] \times 100\%$$

Equation 1

$$\text{C}_2\text{H}_6 \text{ oxidation efficiency} = \left[\frac{C_{e,in} - C_{e,out} \left(\frac{C_{n,in}}{C_{n,out}} \right)}{C_{e,in}} \right] \times 100\%$$

Equation 2

where:

$C_{n,out}$ and $C_{m,out}$ are N₂ and CH₄ concentration at the outlet respectively,

$C_{n,in}$ and $C_{m,in}$ are N₂ and CH₄ concentrations at the inlet respectively,

$C_{e,in}$ and $C_{e,out}$ are C₂H₆ concentration at the inlet and outlet respectively.

3. RESULTS AND DISCUSSIONS:

3.1 Effect of CH₄ Composition – Batch Experimental Results

Figure 2 shows the headspace CH₄ and CO₂ composition versus time in CH₄-only (70% CH₄ [-1 -1 -1], 75% CH₄ [0 -1 -1] and 80% CH₄ [1 -1 -1]) batch experimental bottles.

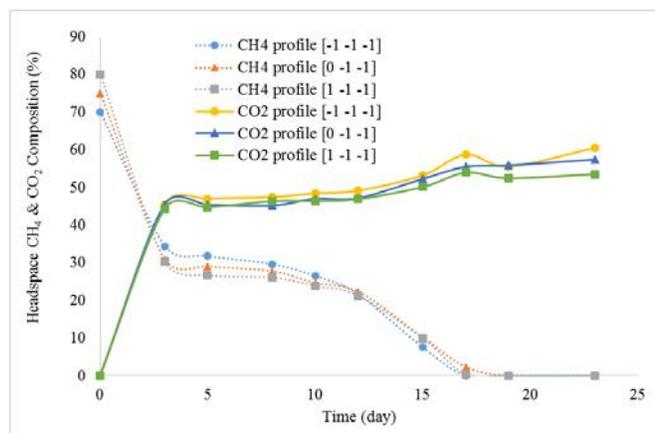


Figure 2: Changes of headspace CH₄ and CO₂ compositions over time

The headspace CH₄ composition decreased over time while CO₂ composition increased, indicating CH₄ oxidation. Nearly identical trends of change in headspace CH₄ and CO₂ were observed for the bottles with different CH₄ compositions. All the headspace CH₄ was converted to CO₂ in about 20 days, and there were no significant changes observed between the different compositions of CH₄. This suggested that higher CH₄ compositions in solution gas could be treated by CH₄ biofilters, compared to the lower CH₄ concentrations in landfill biogas.

3.2 Effects of C₂H₆ and C₃H₈ on CH₄ oxidation- Batch Experimental Results

The headspace CH₄ conversion trends were similar to the trends shown in Figure 2 for all the other samples. Based on the measured headspace CH₄ compositions over time, the average CH₄ oxidation rate of each bottle was estimated. The average CH₄ oxidation rate as $\mu\text{gCH}_4/\text{g}_{\text{dry-wt}}\cdot\text{day}$ was obtained from the slope of the graph of the headspace CH₄ (in $\mu\text{gCH}_4/\text{g}_{\text{dry-wt}}$) versus time (day) for each sample. Similarly, the C₂H₆, and C₃H₈ oxidation rates were also calculated. Table 3 shows the resultant oxidation rates. The methane oxidations rates were lied in the range of 196 to 233 $\mu\text{gCH}_4/\text{g}\cdot\text{d}$. However, the differences in CH₄ oxidation rates among the different samples were not that significant suggesting that the CH₄ oxidation rate is not affected by the presence of given compositions of CH₄, C₂H₆ and C₃H₈.

Table 3: Oxidation rates for different sample

Sample Number	CH ₄ (%)	C ₂ H ₆ (%)	C ₃ H ₈ (%)	CH ₄ Oxidation Rate ($\mu\text{gCH}_4/\text{g}\cdot\text{d}$)	C ₂ H ₆ Oxidation Rate ($\mu\text{gC}_2\text{H}_6/\text{g}\cdot\text{d}$)	C ₃ H ₈ Oxidation Rate ($\mu\text{gC}_3\text{H}_8/\text{g}\cdot\text{d}$)
-1 -1 -1	70	0	0	227.86	0.00	0.00
-1 -1 0	70	0	2.5	224.78	0.00	26.79
-1 -1 1	70	0	5	216.73	0.00	49.30
-1 0 -1	70	5	0	217.90	35.06	0.00
-1 0 0	70	5	2.5	217.12	23.80	24.40
-1 0 1	70	5	5	214.52	28.50	46.32
-1 1 -1	70	10	0	207.67	79.83	0.00
-1 1 0	70	10	2.5	209.79	78.38	25.64
-1 1 1	70	10	5	215.40	74.43	47.55
0 -1 -1	75	0	0	224.02	0.00	0.00
0 -1 0	75	0	2.5	221.74	0.00	22.88
0 -1 1	75	0	5	219.04	0.00	46.10
0 0 -1	75	5	0	210.79	25.33	0.00
0 0 0	75	5	2.5	196.95	17.94	21.84
0 0 1	75	5	5	214.08	28.91	46.25
0 1 -1	75	10	0	209.97	68.47	0.00
0 1 0	75	10	2.5	218.75	74.24	24.76
0 1 1	75	10	5	218.58	75.36	47.52
1 -1 -1	80	0	0	222.39	0.00	0.00
1 -1 0	80	0	2.5	220.57	0.00	25.12
1 -1 1	80	0	5	215.30	0.00	51.02
1 0 -1	80	5	0	215.76	26.07	0.00
1 0 0	80	5	2.5	219.05	32.08	20.72
1 0 1	80	5	5	226.82	34.72	45.59
1 1 -1	80	10	0	215.73	73.58	0.00
1 1 0	80	10	2.5	220.24	74.38	21.99
1 1 1	80	10	5	233.46	74.35	45.89

The average CH₄ oxidation rates from factorial design experiments were further analyzed using the Analysis of Variance (ANOVA) model to evaluate the main factor effect and the interaction effects of CH₄, C₂H₆ and C₃H₈ on CH₄ oxidation. SPSS software was used to solve the ANOVA model. Table 4 shows the ANOVA results.

Table 4: ANOVA results obtained from SPSS Analysis for CH₄ oxidation

Source	Sum of Squares	DF	Mean Square	F _{Calculated}	F _{Table}
A	418.92	2	209.46	14.02	14.91
B	328.00	2	164.00	10.97	14.91
C	13.37	2	6.69	0.45	14.91
A * B	371.27	4	92.82	6.21	11.28
A * C	55.22	4	13.80	0.92	11.28
B * C	350.35	4	87.59	5.86	11.28
A * B * C	283.12	8	35.39	2.37	9.20
Error	149.44	10	14.94		
Total	1785390.4	37			

The degrees of freedom for each individual factor (Factors and levels are included in Table 1) were calculated by subtracting 1 from its number of levels. The degrees of freedom associated with the interactions between factors were calculated by multiplying the degrees of freedom corresponding to each factor. The sums of squares were calculated by the sum of squared differences between the sample means of each group and the overall mean, weighted by the sample size in each group. The mean squares were obtained by dividing the sum of squares by its appropriate degrees of freedom. The F value for each factor and interaction was calculated by dividing the specific mean square by the error mean square. Assuming a 0.001 significance level (i.e. at 99.9% confidence interval) and with the resultant degrees of freedom, a critical F value was obtained from F distribution tables for each factor and interaction. Those calculated F values exceeding the related critical F values obtained from the tables were assumed to be significant factors or interactions at the 0.001 significance level.

From Table 4, it is evident that each single factor, A (CH₄ composition), B (C₂H₆ composition), and C (C₃H₈ composition), was not significant as were the interactions of A*B, A*C, B*C, and A*B*C. From these results, it can be stated that methane oxidation rates are not affected by the presence ethane and the propane compositions that were considered in this study.

The potential reason is the ability of some methanotrophs to co-metabolize the other short chain alkanes in addition to methane. The CH₄ oxidation is catalyzed by methane monooxygenase (MMO) enzyme to form methanol; methanol is then oxidized by methanol dehydrogenase to form formaldehyde, which is then converted into formate and CO₂ [20]. There are two types of MMO enzymes based on the concentration of copper ions in the medium: a membrane-bound, particulate MMO (pMMO) and a cytoplasmic, soluble MMO (sMMO) enzymes [20]. Some methanotrophs such as *Methylococcus capsulatus* contain both pMMO and sMMO [21]. Unlike sMMO, pMMO has a relatively narrow substrate specificity.

It can only oxidize short chain *n*-alkanes (fewer than five carbon atoms) [21]. The oxidation of propane, *n*-butane and *n*-pentane preferentially occurs at the C₂ position [22]. Furthermore, it is reported that pMMO could also epoxidate alkenes at the double bond. Unlike the limited existence of sMMOs, pMMO is ubiquitous in methanotrophic bacteria [21, 23].

3.3 Effects of Ethane on Methane oxidation: Flow-Through Biofilter Column Experimental Results

CH₄ oxidation profiles for biofilter columns A, B, C and D are shown in Figures 3, 4, 5 and 6, respectively.

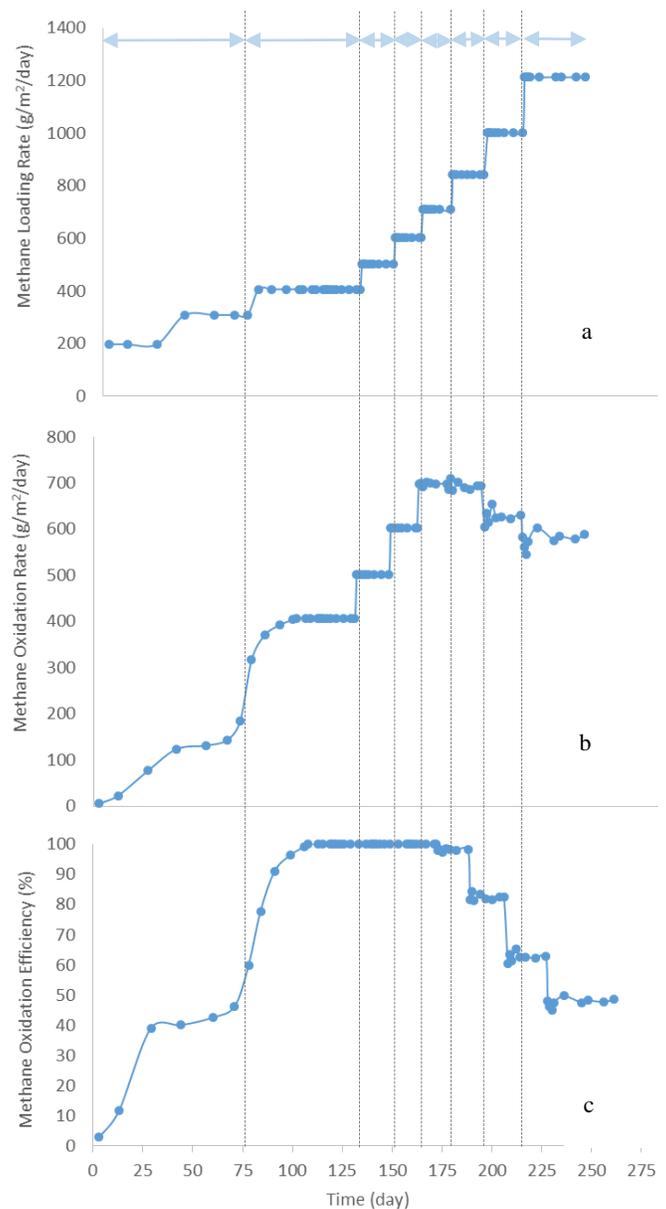


Figure 3: Biofilter CH₄ oxidation profiles over time for Column A (a) CH₄ loading rate (b) CH₄ oxidation rate (c) CH₄ oxidation efficiency

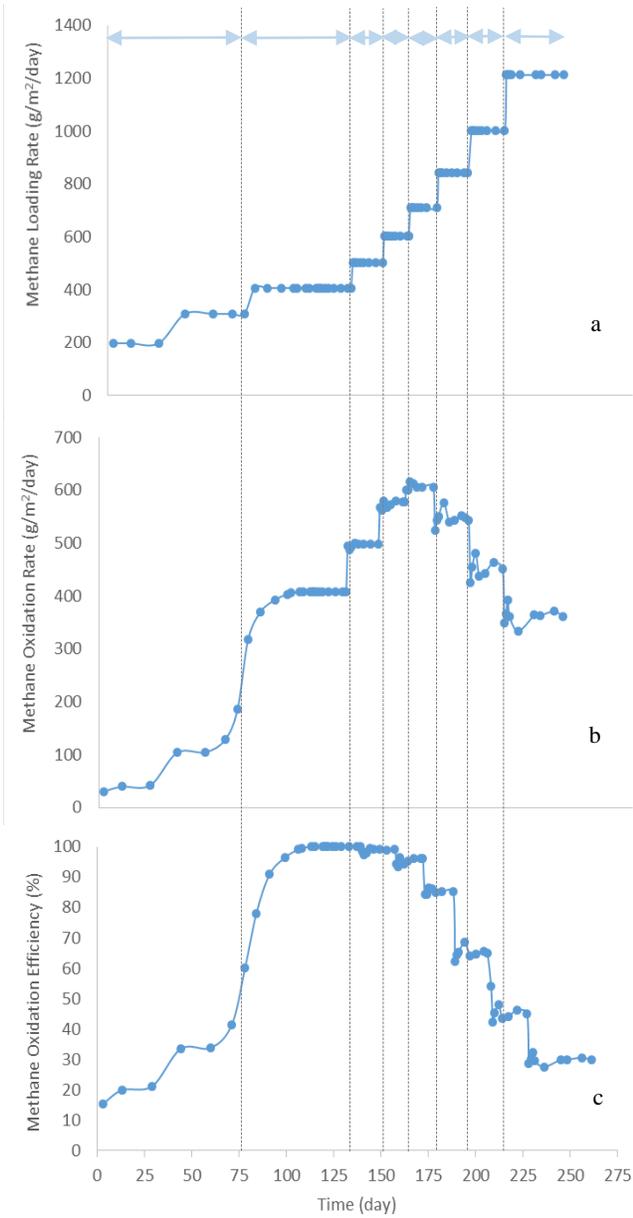


Figure 4: Biofilter CH₄ oxidation profiles over time for Column B (a) CH₄ loading rate (b) CH₄ oxidation rate (c) CH₄ oxidation efficiency

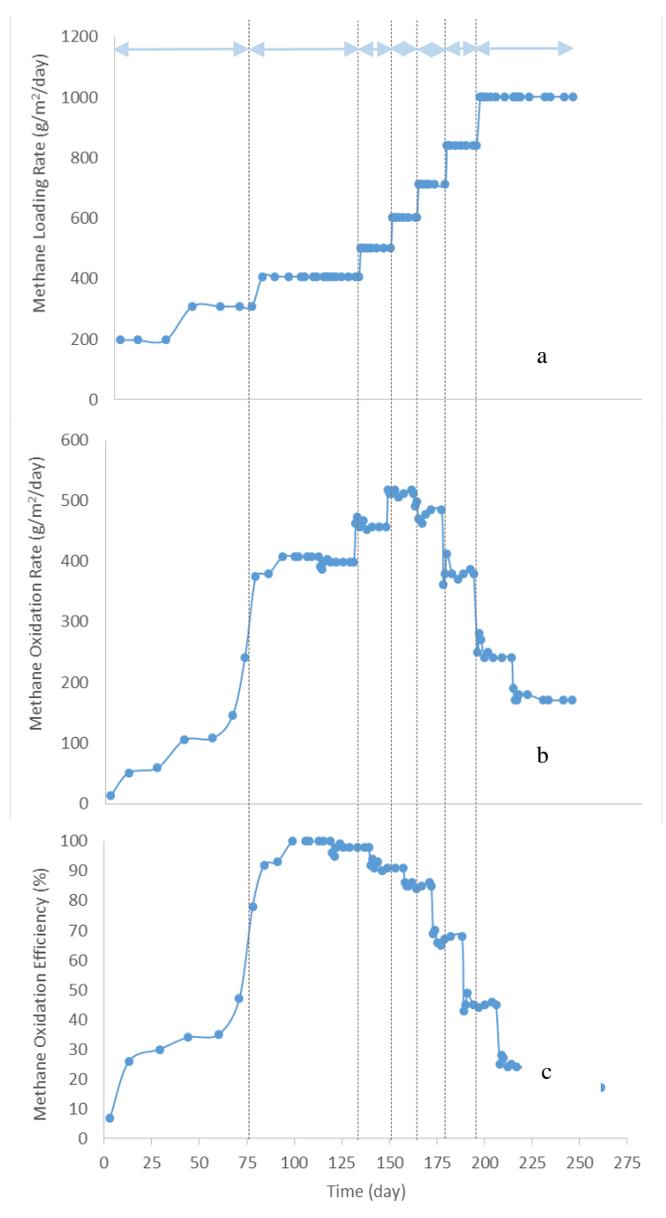


Figure 5: Biofilter CH₄ oxidation profiles over time for Column C (a) CH₄ loading rate (b) CH₄ oxidation rate (c) CH₄ oxidation efficiency

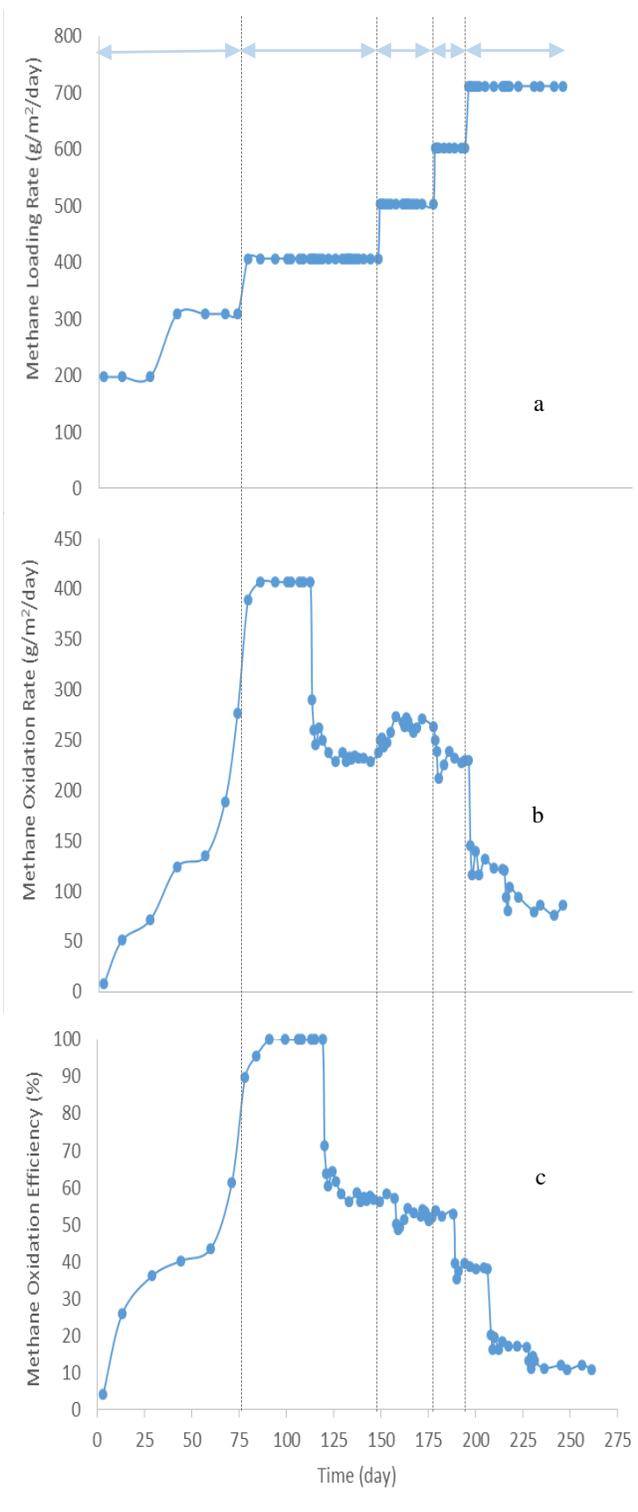


Figure 6: Biofilter CH₄ oxidation profiles over time for Column D (a) CH₄ loading rate (b) CH₄ oxidation rate (c) CH₄ oxidation efficiency

During Stage I, all the biofilter columns showed a similar profile: after a period of 100 days, it appears that the methanotrophs in the biofilter columns have reached their full potential for CH₄ oxidation. Oxidation profiles exhibited

steady state conditions with a levelled off value of 406.9 g/m²/day with a CH₄ oxidation efficiency of 100%.

Biofilter column A, which was treated with only CH₄, maintained 100% oxidation efficiency during the CH₄ loading rates. The loading rates stayed between 406.9 g/m²/day (in Stage II) to 601.7 g/m²/day (in Stage IV). A drop in CH₄ oxidation efficiency was observed when the CH₄ loading rate was increased to 711.7 g/m²/day (i.e. in Stage V) or more. For example, CH₄ oxidation efficiency dropped from 100% to 84.9% when CH₄ loading rate increased from 711.7 g/m²/day to 840.9g/m²/day. Further increase in CH₄ loading rate from 840.9 g/m²/day to 1211.9 g/m²/day in column B resulted in the low CH₄ oxidation efficiency of 33.3 %.

From Stage II onwards, C₂H₆ was introduced into column B, C and D. In column B (CH₄: C₂H₆ ratio of 8:1), the rate of CH₄ oxidation increased by increasing CH₄ loading rates up to 840.9 g/m²/day. Increasing CH₄ loading rates beyond 840.9 g/m²/day resulted in a decrease in CH₄ oxidation. Whereas in column C (CH₄: C₂H₆ ratio of 7:2), the CH₄ oxidation rate was increased by increasing loading rates up to 711.7 g/m²/day, and a further increase in loading rates resulted in a decrease in oxidation. Contrary to previous patterns, in column D, reduction in CH₄ oxidation was observed even at a CH₄ loading rate of 406.9 g/m²/day after introducing C₂H₆ at a CH₄ to C₂H₆ ratio of 5:4.

Table 4 provides a summary of steady state CH₄ and C₂H₆ oxidation efficiency results observed in different columns.

Table 4:CH₄ and C₂H₆ oxidation efficiencies at different loading rates

Stage	Biofilter Column						
	A	B		C		D	
	CH ₄ - 100 %	(CH ₄ :C ₂ H ₆) (8:1)		(CH ₄ :C ₂ H ₆) (7:2)		(CH ₄ :C ₂ H ₆) (5:4)	
	CH ₄ %	CH ₄ %	C ₂ H ₆ %	CH ₄ %	C ₂ H ₆ %	CH ₄ %	C ₂ H ₆ %
II	100	100	99.2	98.1	89	57.2	41
III	100	99.1	95.2	91.1	82.2	53.1	32.2
IV	100	96.1	86.2	85.3	66.1	38.1	15.2
V	98.2	85.1	67.1	67.1	37.1	17.1	9.1
VI	82.2	65.1	49.1	45.1	23.2	11.2	7.1
VII	41.8	30.9	27.7	18.2	18.1	NA	NA
VIII	33.3	23.9	21.9	15	13.9	NA	NA

In Stage I, the column fed only with CH₄ (CH₄ loading rate of 406.9 g/m²/d or less) showed steady state CH₄ oxidation efficiency of 100%. The CH₄ oxidation efficiency of column A dropped from 100% to 98.2% in Stage V and reached a low of 33% in Stage VIII. In column B, the drop of CH₄ oxidation efficiency was observed beyond Stage III,

and reached a low of 24% at Stage VIII. The columns with high C₂H₆ compositions behaved differently. In column C and D, the oxidation efficiency decreased after introducing C₂H₆ in Stage II. Even though the CH₄ oxidation efficiency reduction in column C is not significant, a substantial reduction in CH₄ oxidation efficiency from 100% to 57.2% was recorded in column D.

Furthermore, C₂H₆ oxidation in CH₄-C₂H₆ fed biofilter columns showed similar oxidation patterns to those of CH₄ oxidation. Higher C₂H₆ oxidation occurs (in the range of 65 to 100%) at low CH₄ loading rates in the range of 406.7 to 711.7 g/m²/d, and for CH₄ to C₂H₆ ratio of 8:1 and 7:2.

The results revealed that inlet CH₄ to C₂H₆ ratio of 8:1 and 7:2 had no significant effect on CH₄ oxidation performance below a CH₄ loading rate of 502.1 g/m²/d. This observation could be due to the presence of adequate methanotrophic colonies and co-metabolic activity of C₂H₆ [20, 23, 24]. However, increased CH₄ loading rates and CH₄ to C₂H₆ ratios adversely affected methanotrophic activity. Furthermore, when the inlet gas flow rates of the biofilters were higher, certain percentages of gas could pass through the biofilter column without degradation because of the lower retention time, resulting in decreased oxidation.

CONCLUSION

A series of batch experiments and actively aerated bio-filter columns were operated with various combinations of CH₄ loading rates and selected CH₄ to C₂H₆ ratios. This was done to investigate the CH₄ oxidation performance and the effect of NMOCs on biofilter performance. The results from this study confirmed that the presence of low compositions of C₂H₆ and/or C₃H₈ does not affect the CH₄ oxidation rate. Therefore, it could be concluded that C₂H₆ and C₃H₈ compositions in solution gas can be easily treated with an actively aerated compost/soil biofilter. However, when the ethane compositions are higher than those present in the solution gas, the methane oxidation rates were started to decline.

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REFERENCES

- [1] Alberta Environment, Quantification protocol for solution gas conservation, Report 2012.
- [2] Johnson, MR, Kostiuk, LW, Spangelo, JL, A characterization of solution gas flaring in Alberta, *J Air Waste Manag Assoc* 2001; 51: 1167-1177.
- [3] Alberta Energy Regulator, ST60B-2013: Upstream Petroleum Industry Flaring and Venting Report 2013.
- [4] World Bank, Time to end routine gas flaring, feature Story 2014, (<http://www.worldbank.org/en/news/feature/2014/07/15/gas-flaring-reduction-takes-center-stage-at-global-event>).
- [5] IPCC Intergovernmental Panel on Climate Change, Climate change: The physical science basis 2013. <https://www.ipcc.ch/report/ar5/wg1/>
- [6] Hettiarachchi, VC, Hettiaratchi, JPA, Mehrotra, AK, Kumar, S, Field-scale operation of methane biofiltration systems to mitigate point source methane emissions. *Environ Pollut* 2011; 159(6): 1715–1720.
- [7] Wilshusen, JH, Hettiaratchi, JPA, and Stein, VB, Long-term behavior of passively aerated compost methanotrophic biofilter columns. *Waste Manage* 2004; 24(7): 643–653.
- [8] Stein, VB., Hettiaratchi, JPA, Achari, G, Numerical model for biological oxidation and migration of methane in soils. *J. hazard. toxic radioact. waste* 2001; 5: 225–234.
- [9] Scheutz, C, Kjeldsen, P, Bogner, JE, Visscher, AD, Gebert, J, Hilger, HA, Huber-Humer, M, and Spokas, K, Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions, *Waste Manage Res* 2009; 27: 409-455.
- [10] Menard, C, Ramirez, AA, Nikiema, J, Heitz, M, Effect of trace gases, toluene and chlorobenzene, on methane biofiltration: an experimental study, *Chem Eng J* 2012; 204-206: 8-15.
- [11] Albanna, M, Warith, M, Fernandes, L, Kinetics of biological methane oxidation in the presence of non-methane organic compounds in landfill bio-covers, *Waste Manage* 2010; 30: 219-227.

- [12] Haubriches, R, Widmann, R, Evaluation of aerated biofilter systems for microbial oxidation of poor landfill gas, *Waste Manage* 2006; 26: 408-416.
- [13] Gebert, J, Grongroft, A, Performance of a passively vented field scale biofilter for the microbial oxidation of landfill methane, *Waste Manage* 2006; 26: 399-407.
- [14] Strees, J, Stegmann, R, Microbial oxidation of methane from old landfills in biofilters, *Waste Manage* 2003; 23: 573-580.
- [15] Visvanathan, C, Pokhrel, D, Cheimchaisri, W, Hettiaratchi, JPA, & Wu, JS, Methanotrophic activities in tropical landfill cover soils: effects of temperature, moisture content and methane concentration. *Waste Manage Res* 2009; 17(4): 313-323.
- [16] Su, Y, Zhang, X, Wei, XM, Kong, JY, Xia, FF, Li, W, He, R, Evaluation of simultaneous biodegradation of methane and toluene in landfill covers, *J Hazard Mate* 2014; 274: 367-375.
- [17] APHA, Standard methods for the examination of water & wastewater. 21st Edition, American Public Health Association. Washington, D.C 2005.
- [18] Haththotuwa, CK, Control of Greenhouse Gas Emissions from Oil Production. Master of Science Thesis 2005, University of Calgary, Calgary, Alberta, Canada.
- [19] Haththotuwa, CK, Hettiaratchi, JPA, Hunte, CH, Actively Aerated Methanobiofilters to Control Methane Emissions from Landfills. *Int J Environ Technol Manage* 2012; 15 (3/4/5/6): 333 - 345.
- [20] Das, N, Chandran, P, Microbial degradation of petroleum hydrocarbon contaminants: An overview, *Biotechnol Res Int* 2011; 2011: 1-13.
- [21] Ji, Y, Mao, G, Wang, Y, Bartlam, M, Structural insights into diversity and *n*-alkane biodegradation mechanisms of alkane hydroxylases, *Front Microbiol* 2013; 4: 1-13
- [22] Chan, SI, Chen, HC, Yu, SS, Chen, CL and Kuo, SS, Toward Delineating the Structure and Function of the Particulate Methane Monooxygenase from Methanotrophic Bacteria, *Biochem* 2004; 43 (15): 4421-4430.
- [23] Wang, W, Shao, Z, Enzymes and genes involved in aerobic alkane degradation, *Front Microbiol* 2013; 4 (article 116): 1-7.
- [24] Semrau, JD, Bioremediation vis methanotrophy: overview of recent findings and suggestions for future research, *Front Microbiol* 2011; 2 (article 209): 1-7.

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9.7 Report: Field Work



FIELD WORK REPORT

CONTROL OF POINT-SOURCE LOW-VOLUME METHANE EMISSIONS USING METHANE BIOFILTRATION TECHNOLOGY

Contents

Project: Passive MBF Monitoring and Evaluation; Skimikin, BC (2014-2016).....	3
Summary:	3
Background.....	3
Progress of Work:	3
Objective 1: MBF Performance Analysis Using Inlet and Exit flow conditions (2014-2016)	3
Objective 2: Temperature monitoring to determine the MBF activity (2014-2016).....	13
Objective 3: Building a temporary roof over the two biofilters (2014)	17
Future Work Suggested from work in Skimikin, MBF.....	18
Project: Active MBF Development; Lloydminster, AB; Bonneyville AB (2015-2016)	19
Summary:	19
Background.....	19
Progress of Work:	19
Objective 1: Developing the conceptual design of the field MBF.....	19
Objective 2: Laboratory testing of the design and theoretical simulation.....	21
Objective 3: Selection and identification of the material for field application	25
Current Status of the Project	27
Project: Active MBF Development and construction; Hanna, AB (2016)	29
Summary:	29
Background:	29
Progress of Work:	30
Design Considerations	30
Objective 1: Conceptual Design	30
Objective 2: Construction, Transportation, Installation.....	36
Results and Discussion:	37
Appendix 1: DBM Devon Project.....	42
Appendix 2: Appendices for Bering Project.....	53

Project: Passive MBF Monitoring and Evaluation; Skimikin, BC (2014-2016)

Project Conducted by: *Eranda Bartholameuz, Samadhi Gunasekera, Santosh Yadev, Poornima Jayasinghe, Eamonn Irvine*

Summary: *Three field trips were made to the biofilter site in Skimikin, BC in August, September and October 2014. An abundance of inlet gas was detected, and some outlet gas. 15 temperature sensors were installed into the biofilters in order to measure the temperature change during the winter period. A simple roof was set up for the two biofilters to prevent any snow entry into the filters as well. In year 2015 additional field trips were made to the MBF site in Skimikin, BC between March and August. The data was downloaded from the 15 temperature sensors installed into the MBFs in the previous year. In year 2016 two field trips were made to the MBF site in Salmon Arm in March and August. In addition to the regular analysis of surface fluxes, concentration measurements were taken to identify the surface hot spots. The data was downloaded from the 15 temperature sensors installed. The analysis of the inlet and outlet flux was continued from previous year and is presented below.*

Background

A MBF constructed by Speling Hansen Associates and Columbia-Shuswap Regional District in collaboration with University of Calgary in 2011, located in Skimikin BC was selected to evaluate their performance in this study. This MBF is currently under operation, and installed in a closed landfill. The Skimikin landfill is over 30 years old and was capped and closed in 2005. Both landfills were first started as open dumps and subsequently converted to sanitary landfills.

The MBFs are 1 m in height with a surface area of 25 m² (5 m x 5 m). The biofilter medium is matured compost. Skimikin MBF was constructed and commenced in May 2011.

Progress of Work:

Objective 1: MBF Performance Analysis Using Inlet and Exit flow conditions (2014-2016)

Measuring Inlet flow rate

Equipment and methods to monitor inlet flow and conditions were studied, tested and evaluated

Materials and methods:

- Available flow measurement techniques.
 - Install an inline flow measurement device. However these are very expensive (> \$5000) and application is cumbersome.
 - Pitot tubes coupled with a digital manometer could be used to measure the differential pressure between two points in the inlet flow, which can be used to calculate the velocity, and then the volumetric flow. However, for low flow pipes such as flow coming from a landfill the pressure difference would be too low and will not provide sufficient accuracy.

- Anemometers, air velocity measurement devices, could be used to measure the velocity of a given point in a pipe, and then to calculate the total flow rate.
- A Vane-Anemometer with a Rotating Mini-Vane was selected as the most feasible flow measurement device as it was within the correct measuring range, with sufficient accuracy and resolution.

Device specifications: Air Velocity Range/Resolution: 0.2 to 12.0 m/s / 0.1 m/s; Temperature Range/Resolution: 0 to 80°C / 0.1°C

- The anemometer was calibrated against a high accuracy pitot static tube inside a wind tunnel with variable velocities to obtain the following calibration curve.

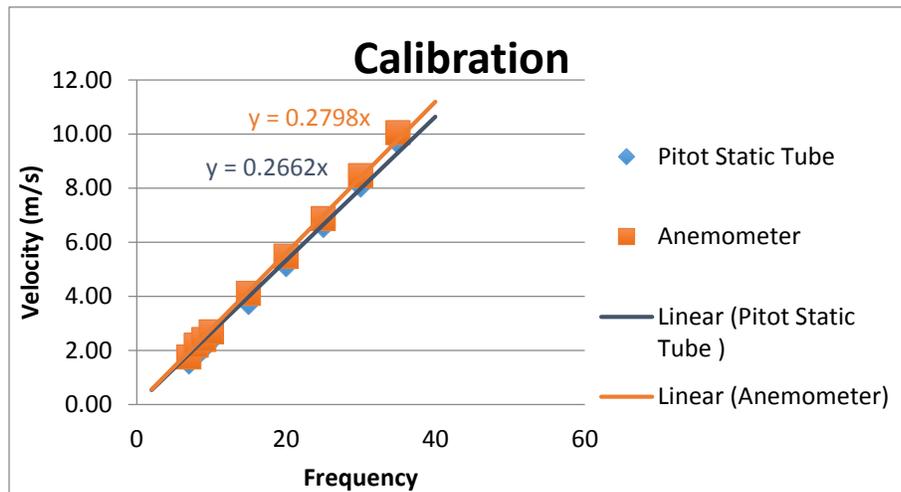
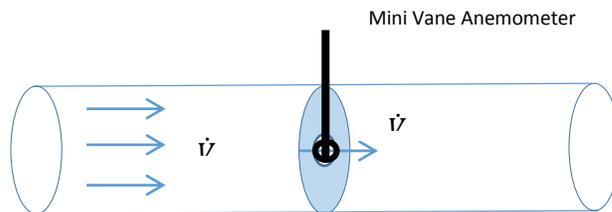


Figure 1: Calibration of the Anemometer

- The anemometer was inserted into a 2” pipe and secured precisely at the centre line, thus to capture the maximum velocity of the flow inside the pipe.



- An equal section of the original pipe will be cut to install the anemometer-pipe device, and sealed with inline pipe fittings. Inlet measurements were taken for 3 hours in 15 minute time intervals to get a better average on the fluid flow throughout the day.



2014 Inlet Measurements

Inlet Gas Composition

<u>CH₄%</u>	23
<u>CO₂%</u>	10.2
<u>O₂%</u>	12.9
<u>Balance</u>	53.9

	Inlet to Biofilter A	Inlet to Biofilter B
Measured Velocity (m/s)	4.35	2.912
Error compensated velocity	4.140042903	2.77144941
Vane Diameter (mm)	0.0127	0.0127
Flow Area (m ²)	0.000506451	0.000506451
Volumetric Flow Rate (m ³ /s)	000.002096727	0.001403602
Volumetric Flow Rate (m ³ /day)	181.1572311	121.2712315

Notes:

- Inlet flow is much higher than expected, showing that there is an abundance of gas flowing in.
- The difference in the flowrates between the two biofilters is due to the difference in the filter bed thickness. Filter B is 0.5m thick and has a lower flow rate due to a smaller pressure differential, while filter A is only 0.3 m thick and has a higher flow rate.
- The 12.9% oxygen detected in the inlet gas might have been due to a leak while inserting the eagle into the measuring port.

2015 Inlet Measurements

Inlet Gas Concentrations – Average

CH ₄ %	49.9
CO ₂ %	27.6
O ₂ %	0
Balance	22.5

Inlet landfill gas flow rates

Biofilter A	Biofilter B
23.99 m ³ /day	19.51 m ³ /day

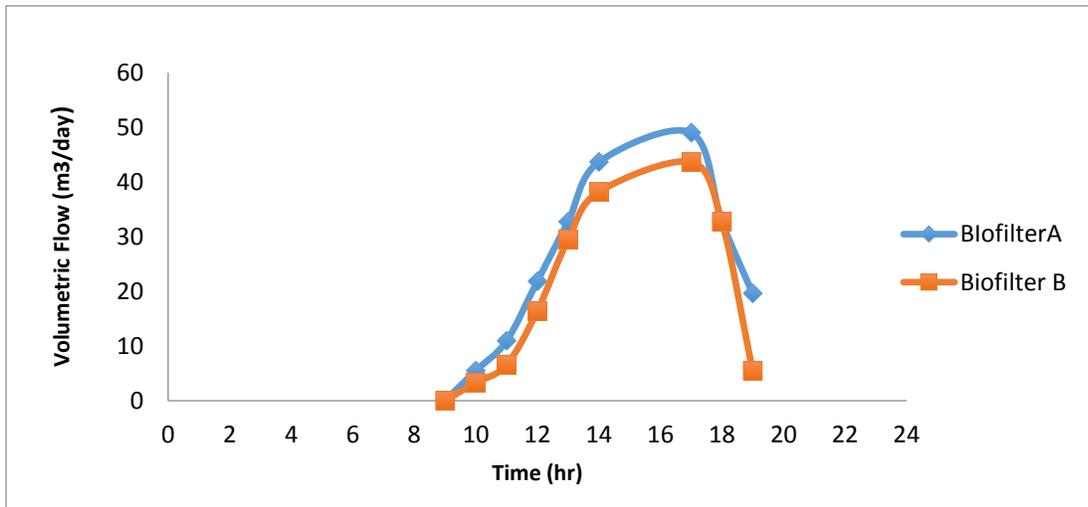


Figure 2: Daily Volumetric inlet volumetric flow variation

Outlet flow rates (methane)

Biofilter A	Biofilter B
0 m ³ /day	0 m ³ /day

No CH₄ detected

Notes

- Inlet flow rate was not constant throughout the day. This could be correlated to the ambient pressure and temperature changes.
- The graph below shows the variation of inlet flow rate during a day.
- Biofilter A consistently has a higher flow rate. The depth of the filter is lower than MBF B, resulting in less head loss.
- Since the inlet flow rates observed are relatively low it could be concluded that the MBF was capable of oxidizing the lower amount of inlet CH₄ observed.
- Hence a 100% oxidation efficiency was assumed. This suggested that there is a significant relationship between inlet flow rate and MBF efficiency. Similar results are reported for other types of biofilters, as can be found in literature.
- Further study is recommended on the CO₂ output by methanotrophs, as the output CO₂ consists of both methanotrophic and respiration CO₂

Measuring Exit Flux

A number of methods are available in literature to estimate exit flux rate, ranging from, surface emission surveys to encapsulating the whole MBF to estimate the exit flow rate. However, most feasible method to estimate exit flux is using flux chambers on a surface grid. Another similar method used in landfills, SES combined with flux chambers can also be used.

Materials and methods:

- Past literature was used to identify optimum flux chamber measurements.
 - When the diameter of the chamber is higher; the circumference area is larger increasing the chance for leaks.
 - If the height of the chamber is too large, the volume is higher, and therefore the time taken to fill up the chamber increases, which in turn requires longer measurement times.
 - Therefore, it was concluded that the geometry of the flux chamber should be symmetrical.
- Using this knowledge two new flux chambers were designed that were 8" diameter and 8" height.
- Flux measurements were taken at 18 different points from each bio filter, over the course of the two days.

2014 Results and Discussion:

- Exit flux measurements closely follow the patterns of vegetation seen at the surface of the biofilter. Gasses were detected at the areas with no vegetation, while no gas was detected at the areas where there was vegetation.

The contour plots below show the results of the flux measurements for CH₄ and CO₂ in biofilters A and B where quantities displayed are in units of g/m²/day.

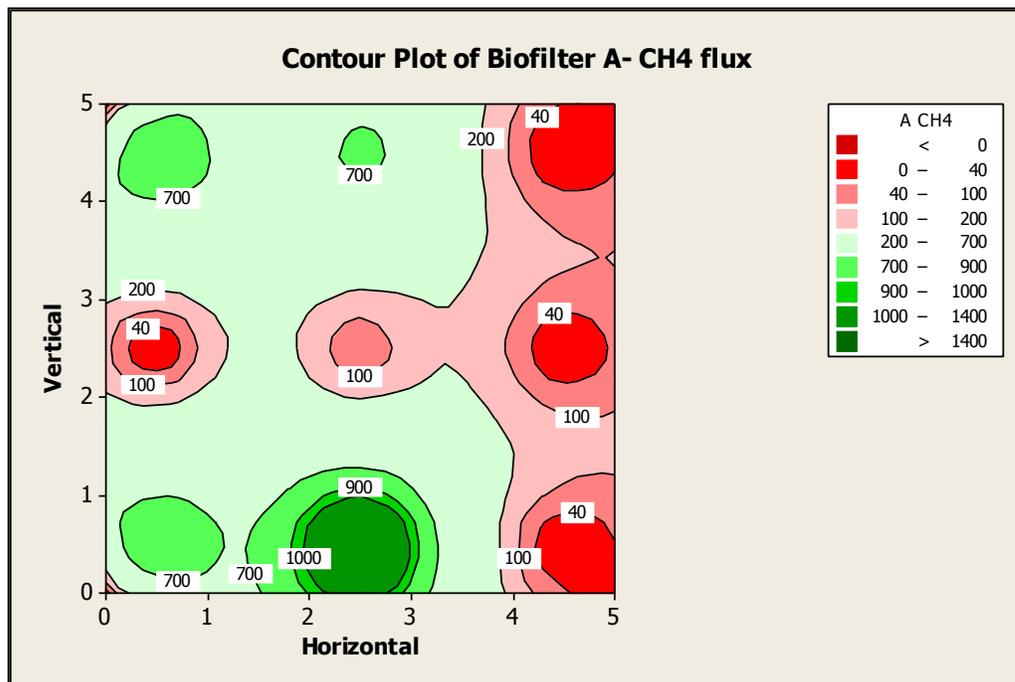


Figure 3: Surface Flux Contour plot MBF-A CH₄

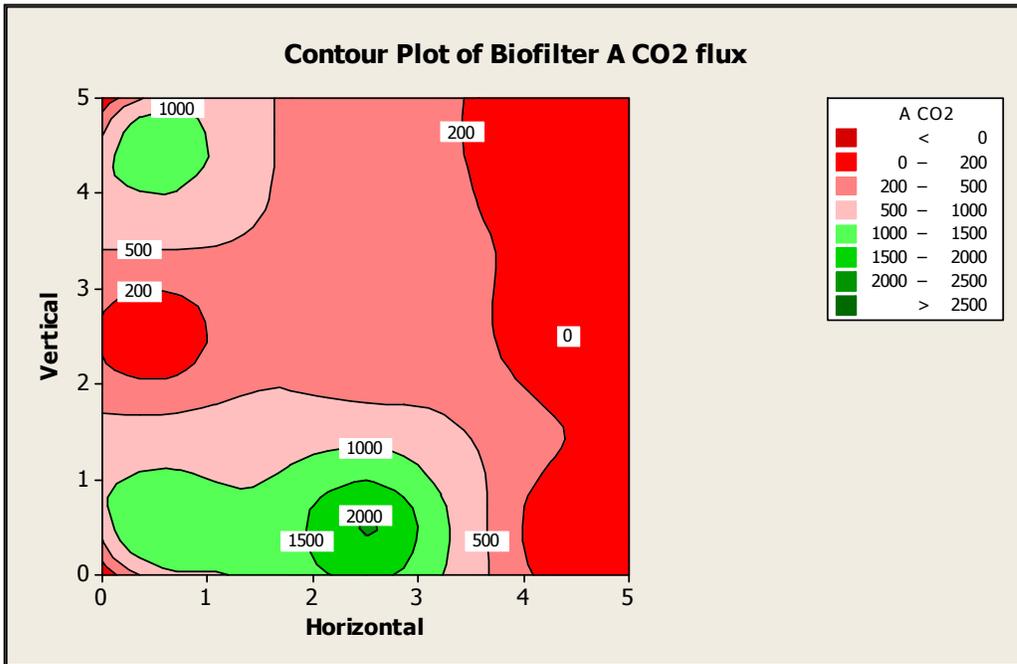


Figure 4: Surface Flux Contour plot MBF-A CO₂

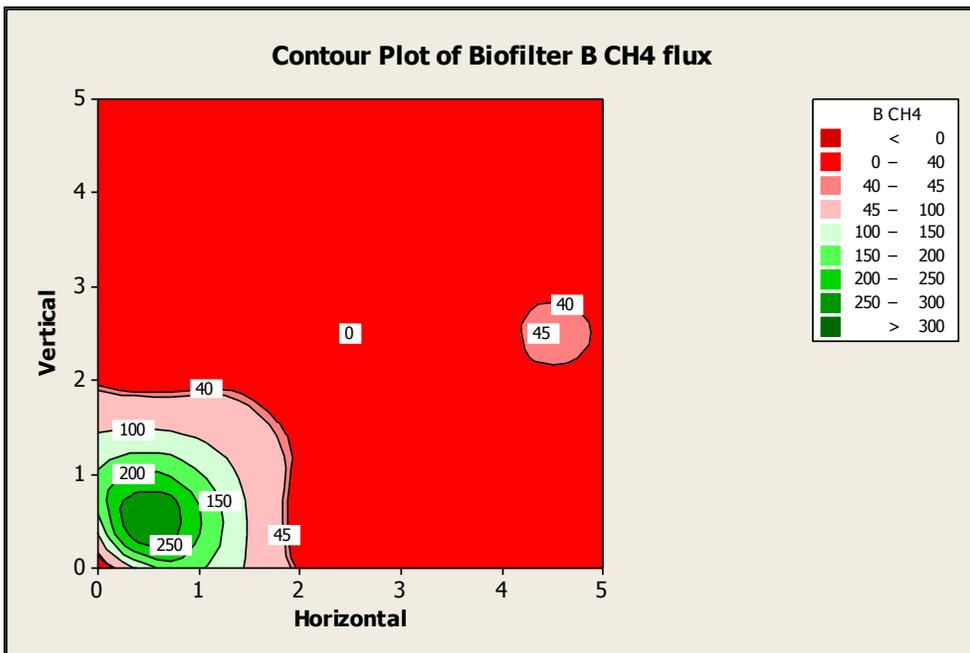


Figure 5: Surface Flux Contour plot MBF-B CH₄

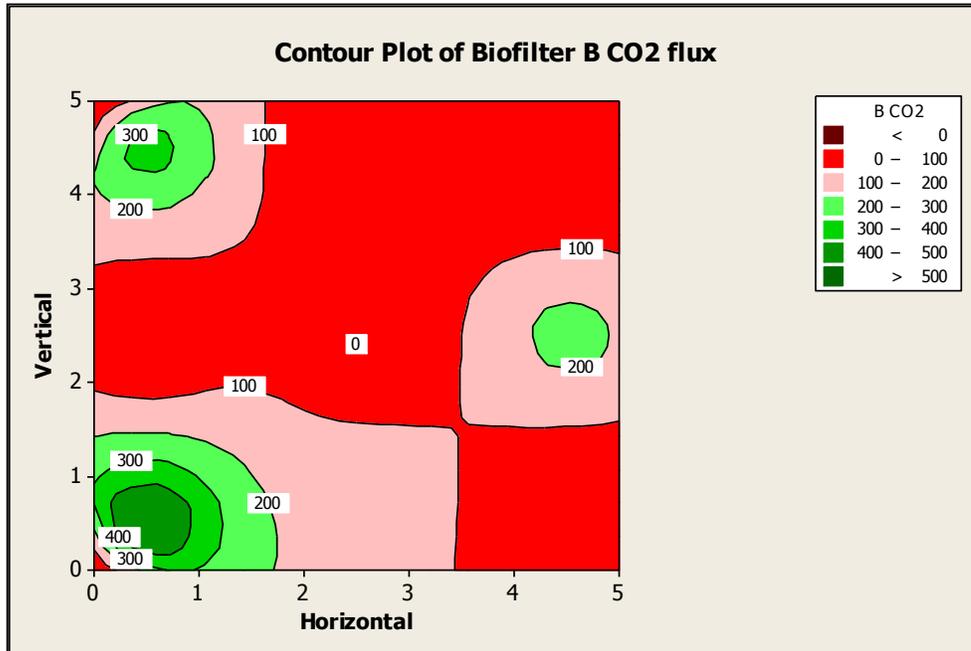


Figure 6: Surface Flux Contour plot MBF-B CO₂

Notes:

- The CO₂ exit flux detected is less than the expected amount of exit CO₂.

Sources of error:

- Exit flow is no longer uniformly distributed across the area, therefore taking flux measurements of a representative area and assuming uniform distribution across the whole area is no longer adequate.
- Number of readings taken is insufficient to identify all the hot spots through which gas exits and therefore is not a conclusive representation of the exit flux.
- The gas analyser has a suction effect, which interferes with the rate at which gas exits naturally.
- Most flux chamber designs incorporate a pressure vent and sweep air inlet. The pressure vent is to maintain the pressure of the chamber at atmospheric pressure, and compensate for the pressure build up due to the increasing concentration of gas inside the chamber. The sweep air inlet is used to make up for the the gas that is taken up by the gas analyzer. The lack of these features might add to the error.

Development of an exit flux measurement protocol

In open biofilters, the exit flux is typically measured through chamber methods. Although chamber methods are simple, there are many impracticalities of using this method in the field. When an MBF has been operational for a long time, it forms channels within the filter bed disrupting the uniform distribution of gas across the surface. This creates a need for flux measurements at all points of the surface since the use of a representative area is no longer accurate. Since each flux measurement takes a significant amount of time, there is a limitation on the number of locations that could be measured. A new exit flux measurement protocol was implemented in order to maximize the information yielded using minimum number of flux measurements. Surface emissions were measured using three methods, surface hot spot identification, subsurface emissions, and flux measurements.

Surface hot spot identification

Surface hot spots were identified using a portable FID (Photovac MicroFID, Figure 2-A). Readings were taken in a grid pattern. At each measurement location, the FID was placed on the ground surface to keep the sensor within one inch of the surface. Measurements were taken for a minimum of 30 seconds and an average value was recorded. Wind gusts prevented accurate readings at some points. Since this is the fastest method of recording emissions, it was used to cover the whole area.

Subsurface emission measurements

A subsurface probe attached to an Eagle 2 Gas Analyzer (Figure 2-B) was used to measure emissions at locations where the FID was unable to achieve an accurate measurement. All subsurface readings were taken for a minimum of thirty seconds at a depth greater than four inches below the surface.

Surface flux measurements

Gas fluxes at selected locations were determined by measuring the change in concentration of gas inside an acrylic chamber with respect to time. A closed-flux chamber, 260 mm in diameter and 260 mm in depth, was used to measure surface gas emissions. The concentrations of methane and carbon dioxide in the chamber were measured with a portable Rki Eagle 2 Gas Analyzer with a PID. Prior to measuring emissions, soil was placed around chamber to create a hermetic seal between the surface and the chamber's edge.

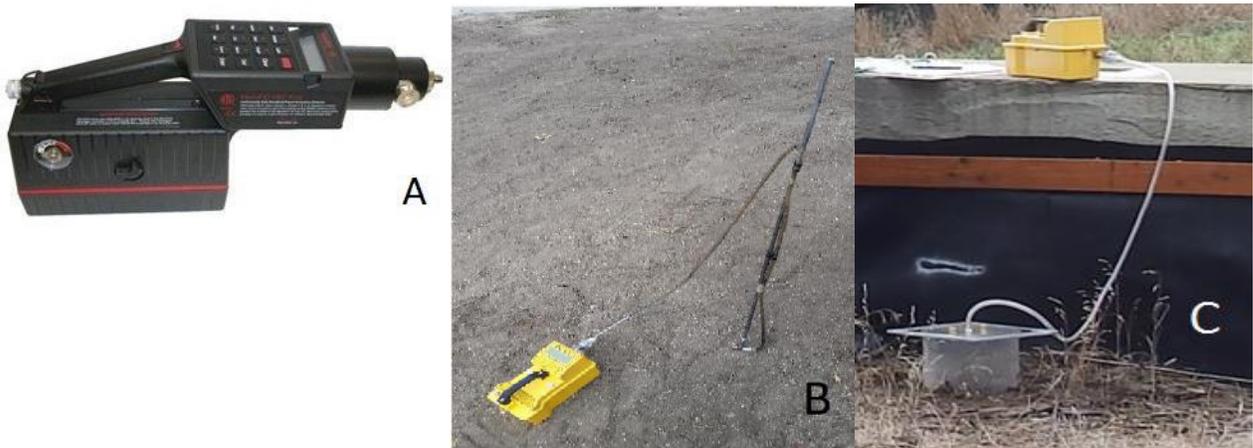


Figure 7

(A) Photovac microFID (B) Subsurface probe attached to an Eagle 2 Gas Analyzer. (C) Closed flux chamber attached to Eagle 2 Gas Analyzer.

Since surface hotspot identification takes the least amount of time, it was used to cover the majority of the area through a larger grid. Sub surface emissions, and surface flux measurements were taken at areas where more gas was seen, in order to define a finer grid to pin point the exact locations and quantities of gas. This proposed method was successfully tested for landfill gas emission in Ryley, AB in 2015.

Flux and Concentration Measurements Comparison (2016 Survey)

A flux and concentration survey was conducted in 2016 to further evaluate the deficiencies in evaluation methods. Several equipment was also tested, namely GEM 5000 and RKI Eagle II.

- Flux measurements indicated somewhat different results for different equipment used. However, it was determined the differences are minimum
- Biofilter A consistently has a higher flow rate. This is because the depth of the filter is lower, which means lower head loss, and therefore a higher-pressure difference to drive the gas flow into the filter.
- Concentration measurements indicated a positive correlation with the flux.

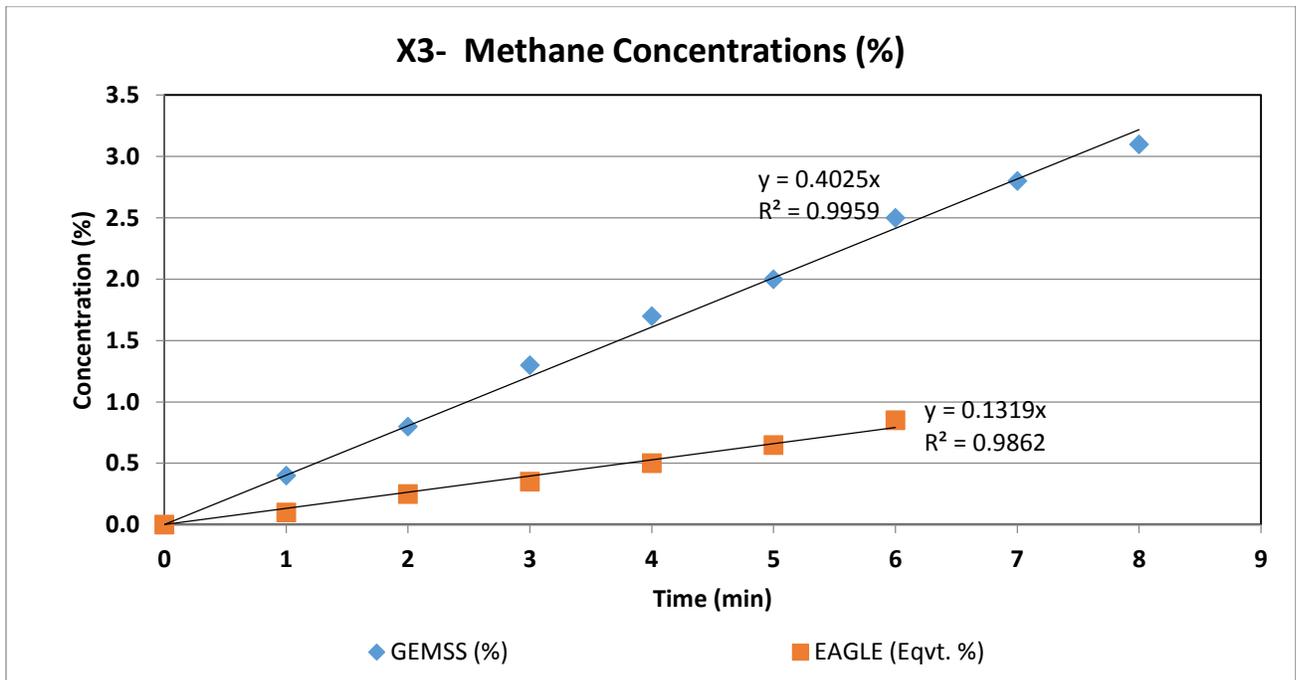


Figure 8: Concentration vs time in a flux chamber using different equipment

	X3=6		1.1		2.3		
							1.3
				X2=2.2	X1=1.0		1.3
							1.6
							4.8
2							8
1.5		3		1.6	1.3	2	1.7

Figure 9: Concentration map in Biofilter A (Concentration values in % v/v)

Surface Flux (g/m ² /d)							
	630						
				300	75		

Figure 10: Evidence of high surface flux areas in Biofilter A

Notes

- There is a considerable difference between the measurements taken from different equipment. It is recommended to develop calibration curves for each of the equipment in the lab with known flux values.
- Surf concentrations indicated higher values at the walls and no significant measurements in the middle part of the biofilter. This could be evidence that the biofilter media is aging and has created wall channels which can impact the efficiency of the system.
- Surface concentrations illustrated in Fig 13 further justifies the presence of high flux sections, near wall also in certain places in the middle of the biofilter.

Conclusions

Based on these evidence it was decided that biofilter media needs to be replaced.

Recommendations were communicated to Columbia Shuswap Regional District (CSRD), the facility owner.

Objective 2: Temperature monitoring to determine the MBF activity (2014-2016)

Installing temperature sensors

Materials and methods:

A total of 15 sensors were installed in two MBFs in Skimikin, BC. 12 sensors were installed in MBF B with a height of 1m and 2 sensors were installed in MBF A which had a height of 0.5 m. One sensor is used to measure atmospheric temperature. In MBF B, 12 temperature sensors were installed at 6 locations, 2 sensors at each location at different surface levels to monitor MBF activity and temperature variations during the winter.

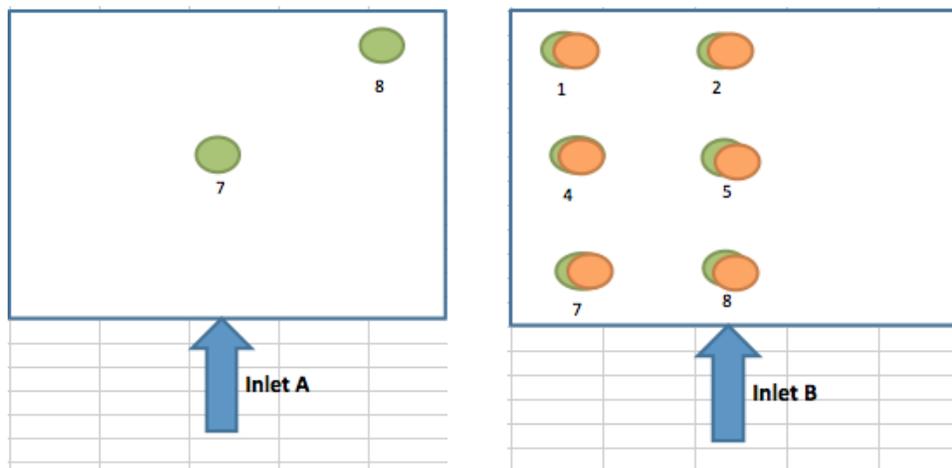


Figure 11: Temperature sensor locations

Notes



Figure 12: Installing Temperature Sensors

The sensors were installed in points that seemed to have more flux, i.e. at areas with no vegetation. The wires were shielded with conduit and connected to two data loggers which are securely placed inside a plastic box and sealed.

Temperature Data and Analysis

Below is a summary of the results obtained for each month.

Month	Average Temperature at surface level/°C	Average Temperature 25cm below surface level/°C	Average Temperature 50cm below surface level/°C
November	7.69	16.25	18.25
December	2.58	5	10
January	2.3	5	6
February	3.18	5	7.5

As can be seen, the MBF did not freeze during the winter, and retained some of its heat within, showing that there is some activity inside the MBF even during the winter period.

The contour plots below show the temperature distribution at each surface level inside the MBF.

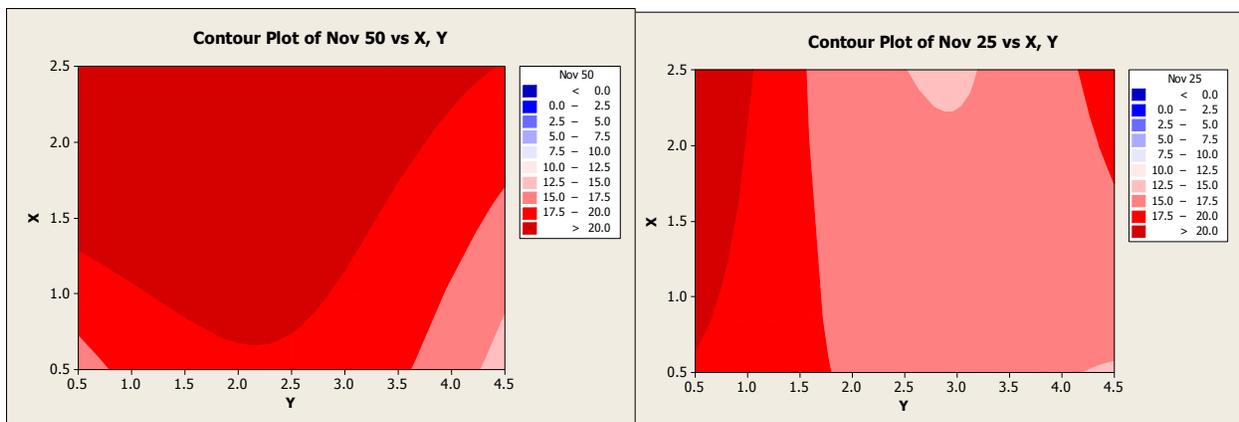


Figure 13: Temperature distribution for December 2014 (50 cm, 25 cm below surface level)

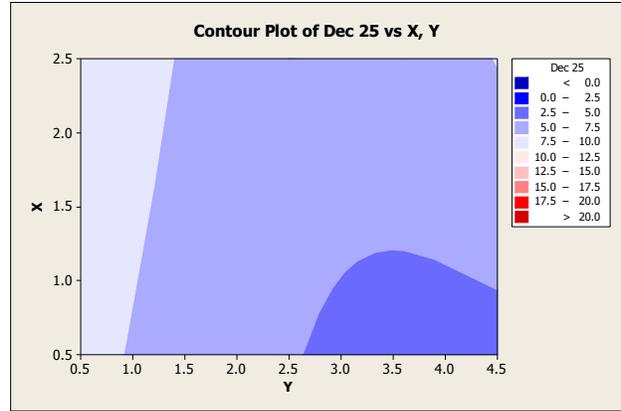
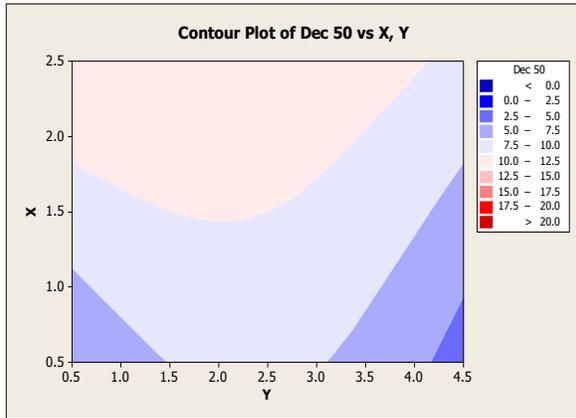


Figure 14: Temperature distribution for December 2014 (50 cm, 25 cm below surface level)

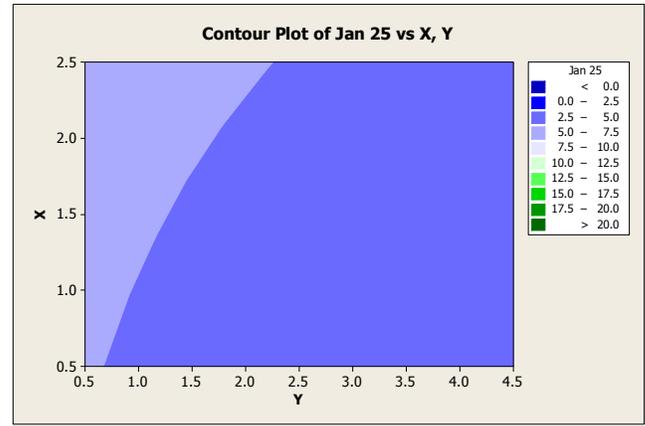
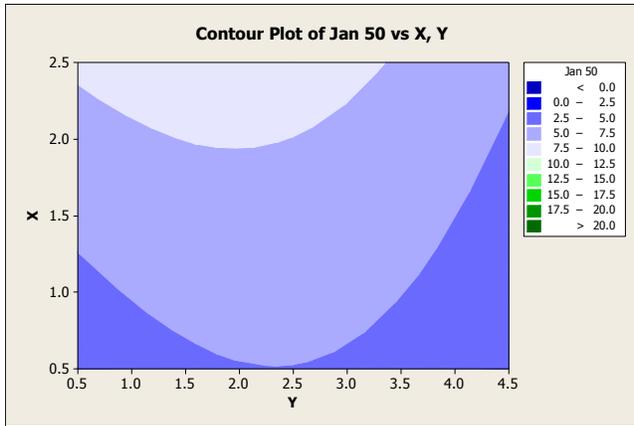


Figure 15: Temperature distribution for January 2015 (50 cm, 25 cm below surface level)

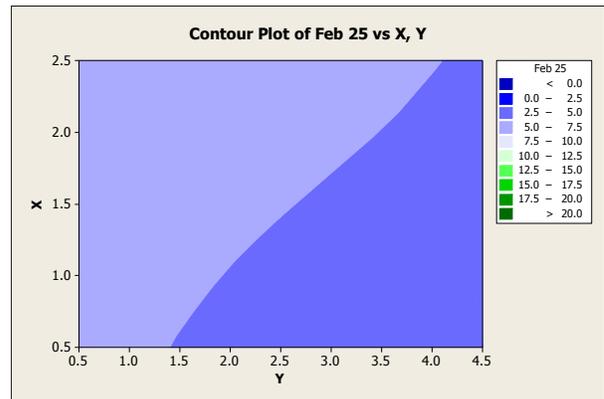
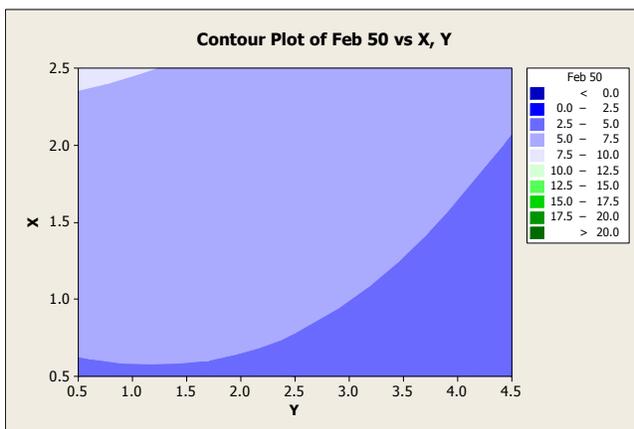


Figure 16: Temperature distribution for February 2015 (50 cm, 25 cm below surface level)

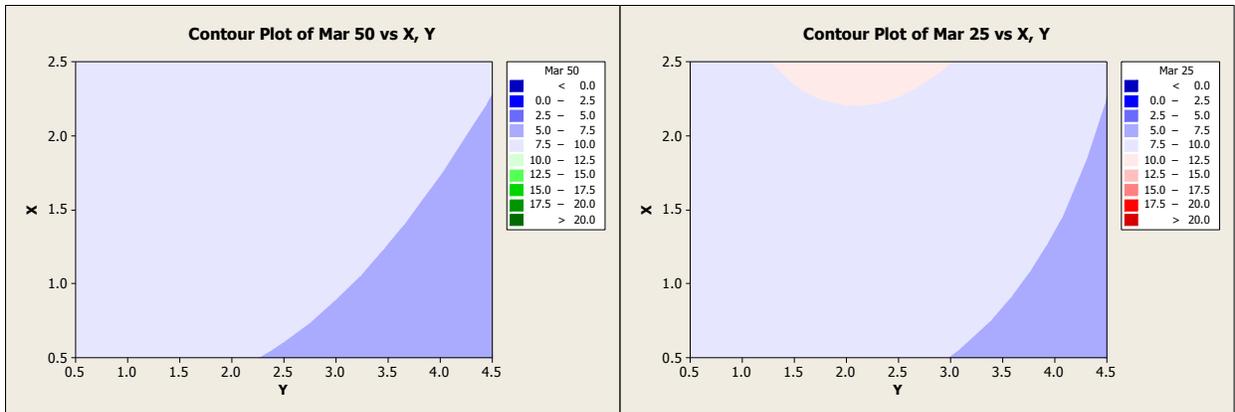


Figure 17: Temperature distribution for March 2015 (50 cm, 25 cm below surface level)

Temperature data summary

It can be seen that at both 25 cm and 50 cm below surface level, the MBF temperature varies in a similar pattern, with point 5 being the most active.

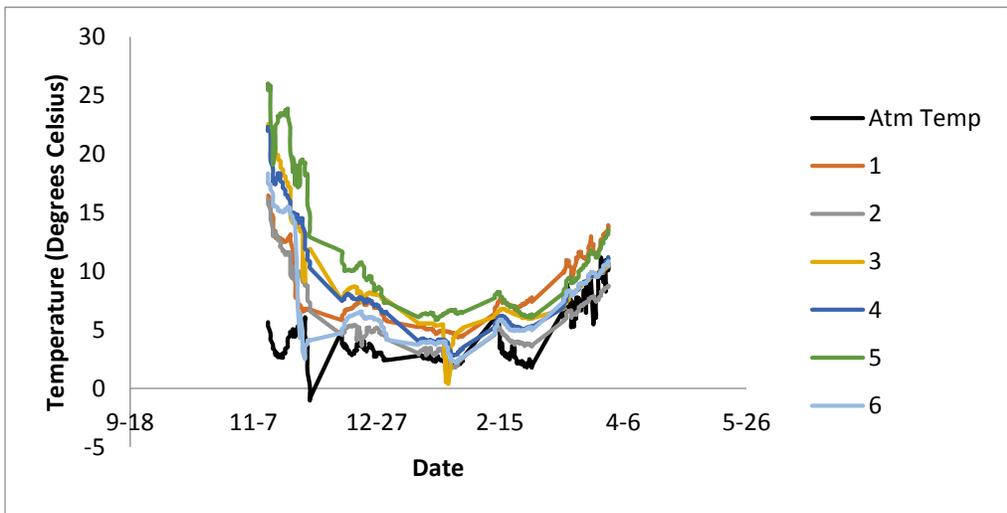


Figure 18: Ambient Temperature vs Biofilter Temperature 25cm below ground surface

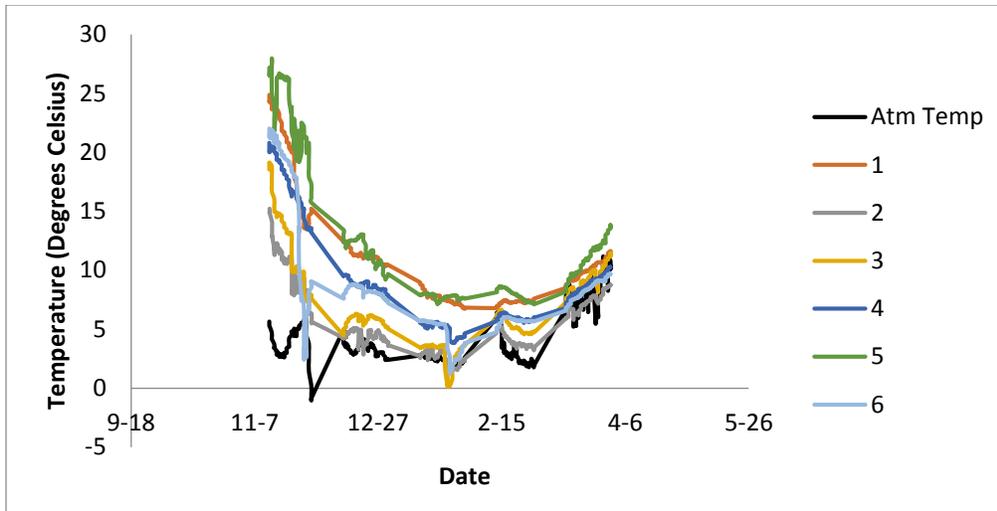


Figure 19: Ambient Temperature vs Biofilter Temperature 50cm below ground surface

Further analysis and continuous data collection is conducted to determine the relationship between temperature and oxidation efficiency.

Objective 3: Building a temporary roof over the two biofilters (2014)

Materials and methods:

- Long wooden blocks were used to construct a structure as shown below.



Figure 20: Roof Construction for MBF

- The connections between two wooden blocks were tied with rope to strengthen the structure.
- The two tarps were wrapped over the wooden structure and tied onto the hooks along the four sides of the two biofilters to ensure proper placement throughout the time duration.



Figure 21: Roof Construction for MBF -2

- This would help keep the snow out of the biofilter while allowing any gas to pass through the edges of the tarp.

Future Work Suggested from work in Skimikin, MBF

Potential Lab and Field Work for Correlation and Relationship Curve Development

Step 1; Sand Box Experiment with CH₄ (need to bring Sand Box outside)

- FID, GEMSS and EAGLE measurement
- Generate lab based correlation curves and correction factors

Step2. Pilot experiment with CH₄ (Olds experimental cells can be used for this)

Validate lab based curve and correction factors and refine coefficients

Step3. Large Scale Field Monitoring

Project: Active MBF Development; Lloydminster, AB; Bonneyville AB (2015-2016)

Project Conducted by: *Eranda Bartholameuz, Samadhi Gunasekera, Matthew Steel, Eamonne Irvine*

Summary: *In 2015 the process was initiated to install the active MBFs in oil fields, several wellsite's were analysed in collaboration with Devon Energy. The conceptual design was developed, discussed and finalized with Devon Energy. A lab scale model of the conceptual design was developed at UofC and tested. Also, industrial MBF material was selected and collected from potential suppliers for active MBFs and analysed in the lab. The media was selected based on the bulk availability, location and physical and chemical characteristics. The physical and chemical characteristics were matched with the optimum media developed in the lab. In 2016 the Project was moved to Bonneyville, AB from Lloydminster, AB. New wellsite's were identified. The projects roles between Devon Inc. and University of Calgary were identified and agreed upon. Design of the piping system and biofilter were finalized. Due to changes in the project financial and technical parameters the final implementation is scheduled for year 2017.*

Background

In the oil/gas industry sector, MBFs could be applied to control:

- casing gas (with intermittent or continuous emissions)
- solution gas (sweet and sour)
- emissions associated with pneumatic instruments
- natural gas metering stations

Among the above mentioned potential applications installing an active MBF for casing gas is the most challenging application. Designing a MBF for these application potential sites were first identified with consultation of Devon Energy. Several sites were identified on Bonneyville, AB and Lloydminster, AB.

Progress of Work:

Objective 1: Developing the conceptual design of the field MBF

The well sites in question release CH₄ at high pressures and flow rates, which a typical MBF cannot handle. Also, since the emissions from the vents are not continuous, the intermittent flow and unpredictable flow rates have called for a new customized design. The proposed design addresses the challenges above by capturing the gas and releasing it at a steady, low velocity. Figure 22 shows a schematic diagram of the design prototype proposed for the Devon Energy well sites.

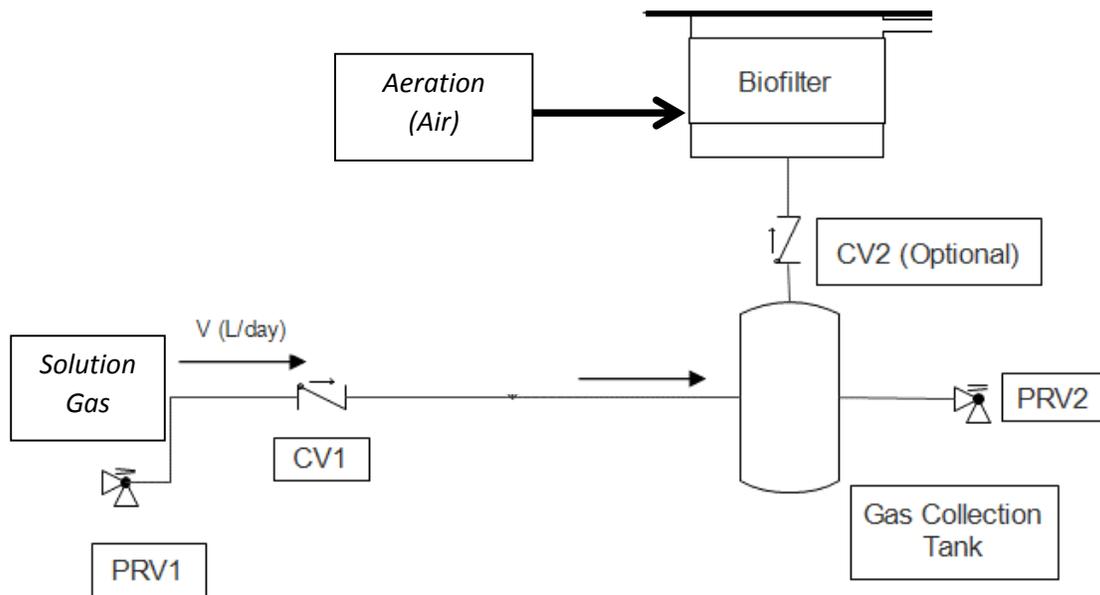


Figure 22: Initial conceptual design for Devon Energy Oil well Casing Gas MBF

Biofilter

The biofilter contains methanotrophic bacteria that can oxidise methane to carbon dioxide. Passing methane at a low flowrate into the biofilter is vital to have an efficient system, since the methanotrophs need sufficient time to complete the oxidation process. The biofilter height and permeability of the media are important parameters in controlling retention time. The biofilter will be kept closed at the top to prevent the entry of snow, which would in turn preserve the uniformity of the filter media.

Gas Collection Tank

The gas collection tank acts as a flow regulation tank that converts the high velocity intermittent methane flow into a more constant low velocity flow, which can then be sent into the biofilter. The tank volume and inside pressure are important parameters to control the flowrate.

Aeration

An active aeration system will be incorporated into the biofilter to distribute air uniformly throughout the biofilter. It will involve two layers of piping one at the bottom that will feed the methane from the well as shown through the red piping in the Figure 23, and the second will circulate air throughout the biofilter which is shown through the grey piping in Figure 23. This aeration system will be made out of PVC as it will be insulated by compost keeping the piping warm so that it won't crack. Also the PVC pipe allows for easy alterations as holes can be drilled easily to allow for improvements of airflow

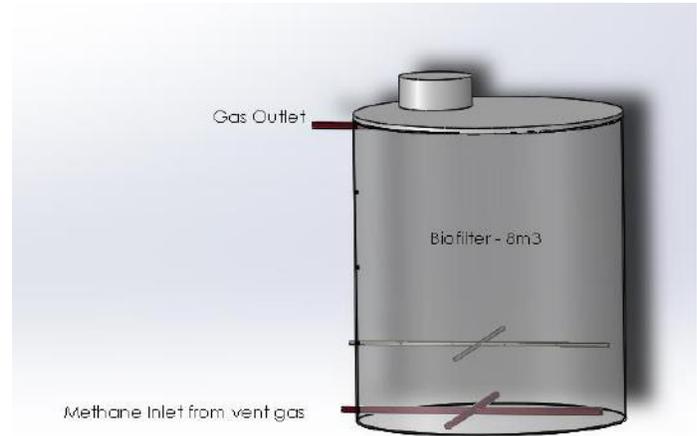


Figure 23: MBF with aeration pipes

The proposed site layout of the MBF and associated assembly is give in Figure 24.

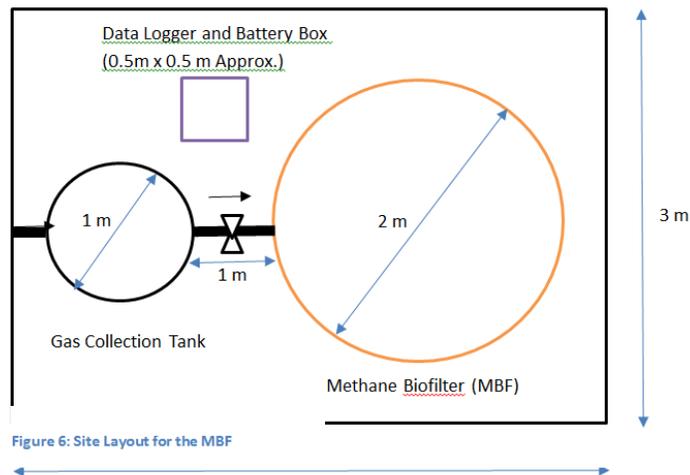


Figure 6: Site Layout for the MBF

Figure 24: Proposed site layout

Objective 2: Laboratory testing of the design and theoretical simulation

The proposed conceptual design is first theoretically analysed using standard mass transfer equations. With this analysis the general behaviour of the system was determined. Actual flow patterns from two well sites was used for the analysis; 11A-13-64-06W4 and 13B-12-64-06W4. The flow patterns for the two wells are illustrated in Figure 25 and Figure 26 respectfully.

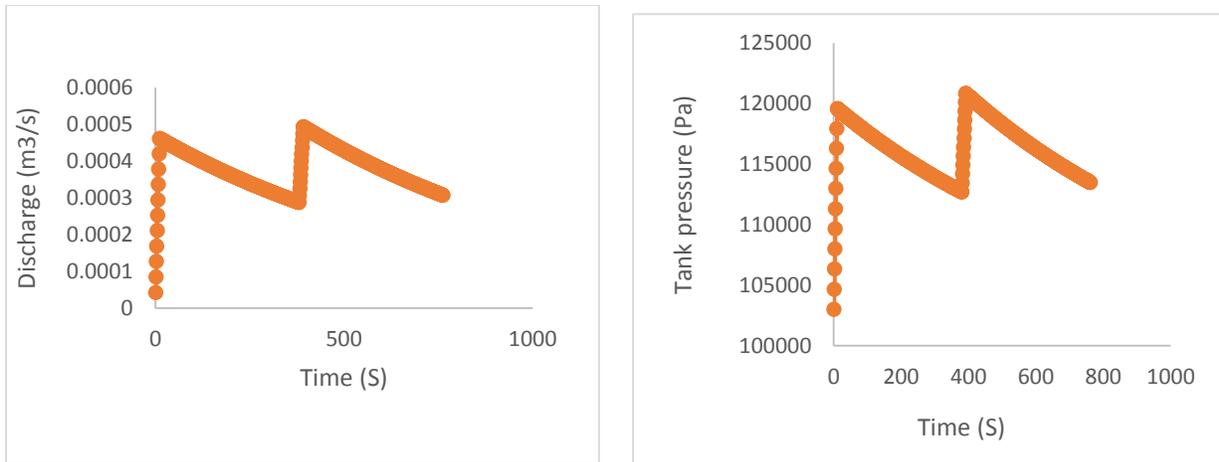


Figure 25: Theoretical Simulation for well # 11A-13-64-06W4

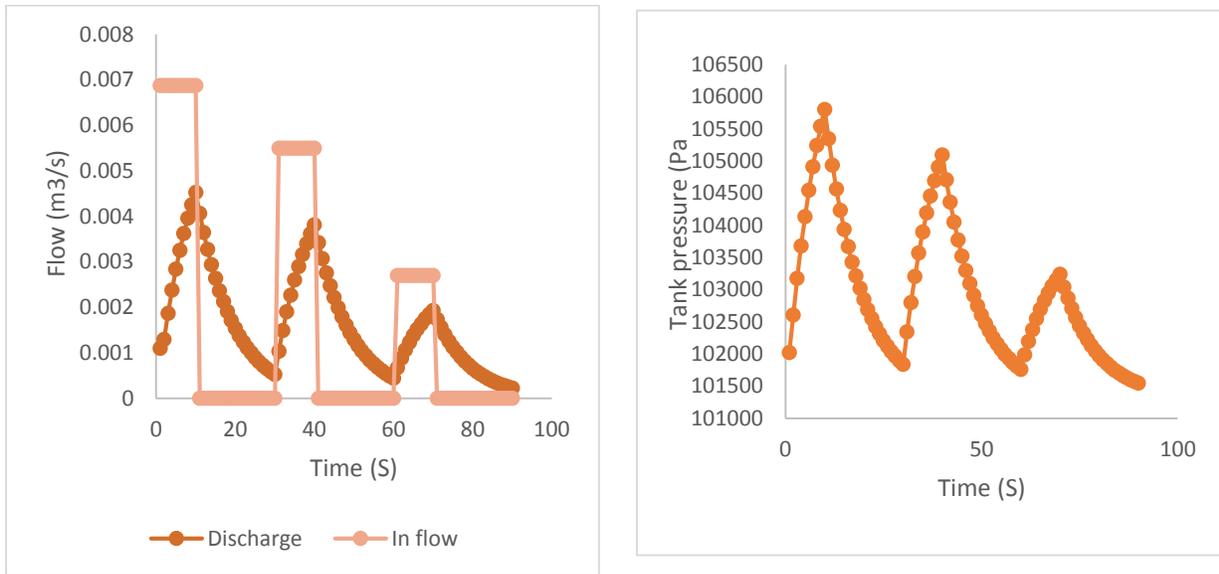


Figure 26: Theoretical Simulation for well # 13B-12-64-06W4

Based on these evidence it was determined that the concept can be applied in the field. However, to further confirm this the conceptual design needed to be analysed in the lab, for this purpose a comprehensive scaled down (similitude) criteria was developed and used. The purpose of the laboratory experiment was to verify the fluid dynamic behaviour of the conceptual design. Several nondimensional parameters are used for developing scaled down laboratory experiment. The developed similitude criteria parameters and analysis is illustrated in table below.

Parameter	Unit	Field Value	Lab Value
Bulk density	kg/m ³	500	640
Particle diameter	m	0.0025	0.0008
Height	m	2	0.4
Superficial velocity	m/s	0.114	0.211

Pressure difference	Pa	6500	12000
Permeability	m ²	6.96 x 10 ⁻¹⁰	1.39 x 10 ⁻¹⁰
Hydraulic conductivity	m/s	4.29 x 10 ⁻⁶	8.57 x 10 ⁻⁷
Effective radius	m	1.17	0.20
Porosity		0.20	0.15
Reynolds number		21.60	12.01
Shape Factor		800	500
Euler Number		4.16 x 10⁵	2.26 x 10⁵
Geometrical Ratio		1.71	2

In the above table, the nondimensional parameters highlighted in bold are the parameters that is matched between field and lab scale.

Based on these values a laboratory scale lysimeter was set up as illustrated in the Figure 27. The lysimeter was filled with play sand to simulate compost. The bulk density of the same particle diameter compost was calculated for the similitude analysis, instead of using the bulk density of sand.

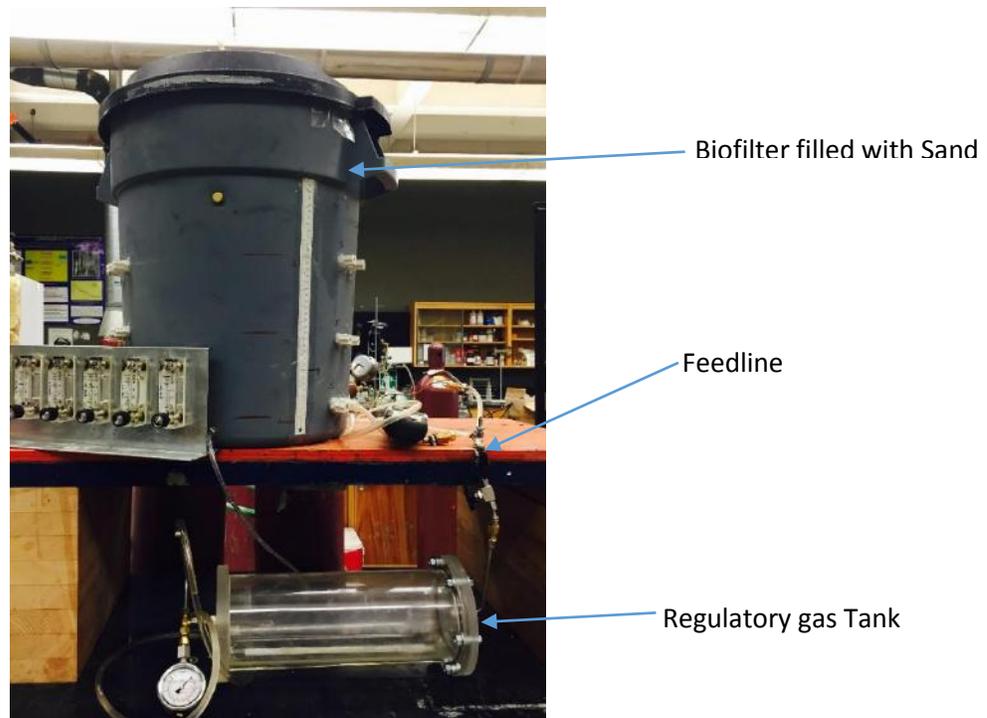


Figure 27: Laboratory setup

Based on the analysis of the scaled down system, it was determined that system is capable of increasing residence time in the field application about 60 times. The detailed analysis data is presented in the table below

Pressure Difference (k Pa)	Time (min)		Pressure Difference (k Pa)	Time (min)
Pressure In Tank 5 kPa			Pressure In Tank 5 kPa with Back pressure	
0.05	0.2		0.05	0.2
0.04	0.4		0.04	0.44
0.03	0.6		0.03	1.42
0.02	1.3		0.02	2.3
0.01	2.45		0.01	4.07
0	4.19		0	5
Pressure In Tank 10 kPa			Pressure In Tank 10 kPa with Back pressure	
0.07	0.3		0.05	0.2
0.06	0.4		0.04	0.45
0.05	1.1		0.03	1.01
0.04	1.22		0.02	3
0.02	1.4		0.01	3.55
0.01	2.05		0	4.3
0	3.09			
Pressure In Tank 15 kPa			Pressure In Tank 15 kPa with Back pressure	
0.05	0.3		0.05	0.2
0.04	0.5		0.04	0.5
0.03	1.44		0.03	1.22
0.03	2.2		0.02	2.3
0.02	2.52		0.01	3.3
0.01	3.34		0	5.06
0	4.3			
Pressure In Tank 20 kPa			Pressure In Tank 20 kPa with Back pressure	
0.05	0.3		0.05	0.2

0.04	1.13		0.04	1.1
0.03	1.3		0.03	1.55
0.02	2		0.02	2.21
0.01	3.3		0.01	3.2
0	5.22		0	6.1

Objective 3: Selection and identification of the material for field application

Based on the study conducted by Goya (2016), it was determined that a material mixture of compost and wood shavings is optimum for passively aerated MBFs. However, this is only applicable to lower flow rates. Hence it was decided to use compost or top soil only for the field applications.

Several potential compost and top soil suppliers were identified near Bonnyville, Lloydminster area where the MBF will be placed. The samples from these suppliers are analysed in the lab to determine the better material for MBF application. A total of 6 suppliers were identified, however samples from only two suppliers were selected for the analysis. These two suppliers are selected based on material availability, and logistics.

Identified suppliers and selected material

- P1 - Pioneer Landscaping – Wetted compost
- P2 - Pioneer Landscaping – Non-wetted compost
- W1 - Wickems Landscaping – Compost sifted
- W2 - Wickems Landscaping – Compost un-sifted

The Samples of the selected material were analyzed for Volatile Solids (VS), Resident Moisture Content (MC), Ash Content and C:H:N elements. The details are illustrated in the table below

P1	Dry Solid					Ash		
	C %	H %	N %	O%	Ash %	C %	H %	N %
1	5.73	0.93	0.39			0.33	0.22	0.03
Mean	5.73	0.93	0.39			0.33	0.22	0.03
Volatile solids	5.40	0.71	0.37	10.99	82.54			
# of moles per 100 g	0.45	0.71	0.03	0.69				
Chemical ratio	17.26	27.23	1.00	26.33				
Moisture Content =	61%							

P2	Dry Solid					Ash		
	<i>C %</i>	<i>H %</i>	<i>N %</i>	<i>O%</i>	<i>Ash %</i>	<i>C %</i>	<i>H %</i>	<i>N %</i>
1	5.08	0.84	0.38			0.51	0.26	0.01
Mean	5.08	0.84	0.38			0.51	0.26	0.01
Volatile solids	4.57	0.58	0.37	11.94	82.54			
# of moles per 100 g	0.38	0.58	0.03	0.75				
Chemical ratio	14.41	21.95	1.00	28.24				
Moisture Content =	56%							

W1	Dry Solid					Ash		
	<i>C %</i>	<i>H %</i>	<i>N %</i>	<i>O%</i>	<i>Ash %</i>	<i>C %</i>	<i>H %</i>	<i>N %</i>
1	2.98	0.58	0.18			0.16	0.15	0.03
Mean	2.98	0.58	0.18			0.16	0.15	0.03
Volatile solids	2.82	0.43	0.16	6.07	90.53			
# of moles per 100 g	0.24	0.43	0.01	0.38				
Chemical ratio	21.23	38.84	1.00	34.24				
Moisture Content =	31%							

W2	Dry Solid					Ash		
	<i>C %</i>	<i>H %</i>	<i>N %</i>	<i>O%</i>	<i>Ash %</i>	<i>C %</i>	<i>H %</i>	<i>N %</i>
1	6.15	1.01	0.36			0.27	0.28	0.04
Mean	6.15	1.01	0.36			0.27	0.28	0.04
Volatile solids	5.88	0.73	0.32	11.56	81.51			
# of moles per 100 g	0.49	0.73	0.02	0.72				
Chemical ratio	21.44	31.94	1.00	31.61				
Moisture Content =	42%							

An ideal C:N ratio of 21 was observed in W2 and W1 samples. However, in W1 samples the ash content was much higher, resulting in lower overall nutrient content. The overall nutrient content in samples collected from Pioneer Landscaping; P1 and P2, was higher than W1. Based on these observations, W2 is selected as the best MBF filter media for this application.

In addition to compost media following other equipment is also selected, based on UofC and Devon Energy requirements/specifications.

1. MBF vessel
2. Sensors to be installed
3. Gas analysis equipment

Current Status of the Project

The technical feasibility of the project is finalized in collaboration with Devon Energy. Different tasks that is carried out by UofC and Devon Energy was identified.

A design based memorandum was drafted in July 2016 for the project. The document is attached as an Appendix at the end of this document.

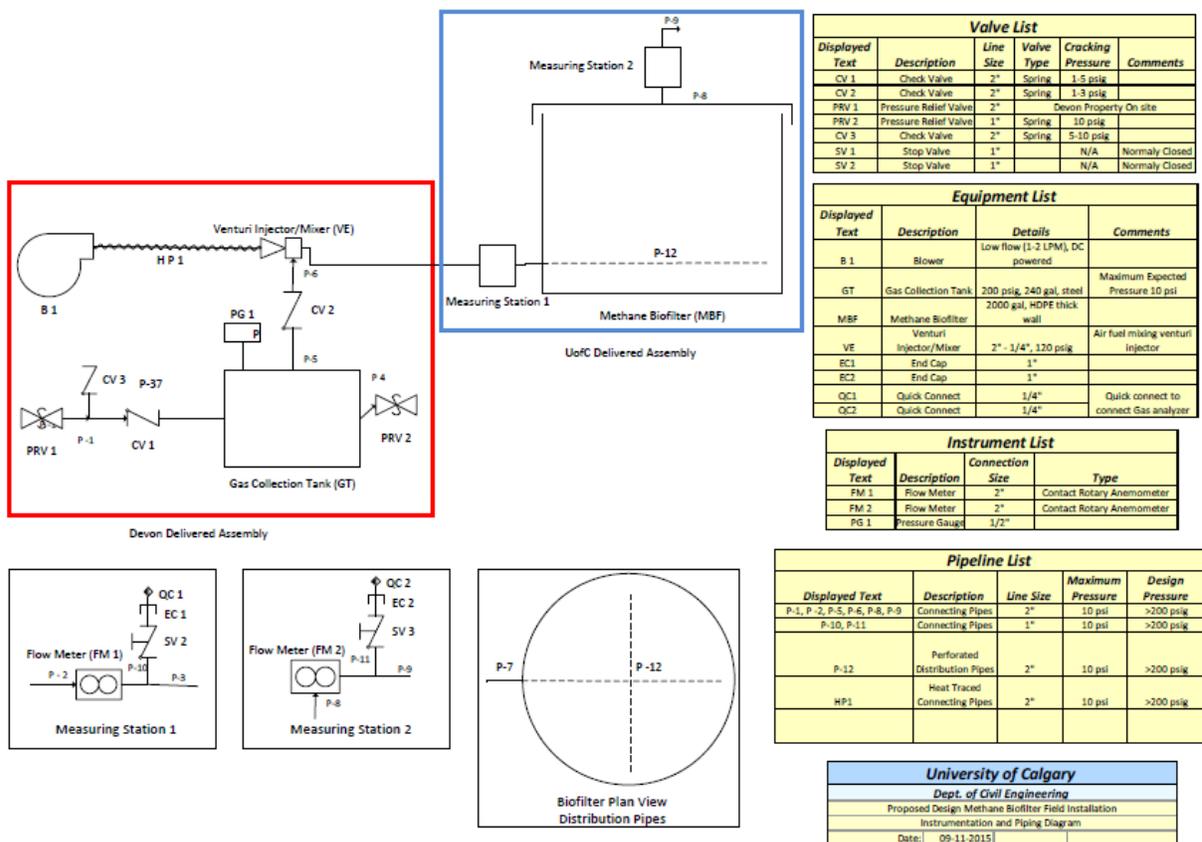


Figure 28: Detailed PID for oil well site MBF assembly components

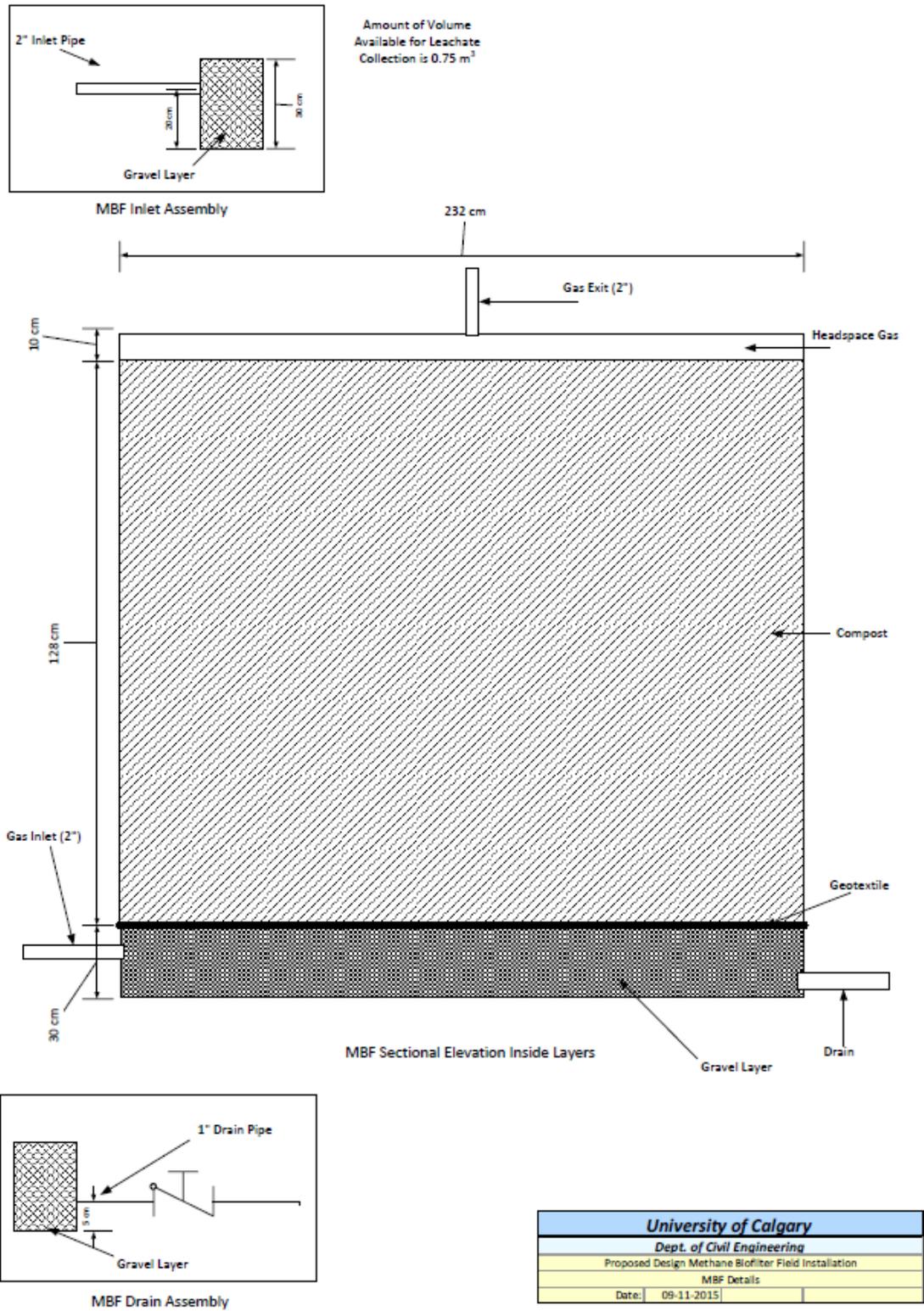


Figure 29: Design Details for MBF

Project: Active MBF Development and construction; Hanna, AB (2016)

Project Conducted by: Eranda Bartholameuz, Samadhi Gunasekera, Eamonn Irvine

Summary: An active aeration closed methane biofilter (MBF) was designed, constructed and installed at a single well battery site in Hannah, AB, with collaboration Bering Exploration Inc. during the summer of 2016. The MBF was designed and constructed at the University of Calgary and then transported to the well location.

Background:

In the oil and gas industry, excess or unwanted flammable gases are often disposed by flaring or venting. Flaring is the process of combusting the gases in an open atmosphere, to dispose flammable gases such as methane cost-effectively. However, if the emission flowrates are too low or too intermittent, or the heating value of gas is too low to sustain combustion, it is not economically feasible to burn surplus gases in a stable method. In such instances, these gases are vented directly to the atmosphere. MBF technology could be applied to attenuate such low volume point sources.

The oil produced from oil reservoirs via an oil well usually exists as a fluid mixture consisting of formation gas, crude oil emulsion (i.e., a mixture of oil and water bonded together) and salt water. This mixture is usually known as reservoir fluid or production fluid. Production fluid is treated to separate salt water at the bottom, crude oil emulsion in the middle and natural gas on top. The process of gathering and treating production fluid in a central location takes place at a facility called a battery.

The battery site under consideration in Hanna, AB, is located north of Hwy #36 22 miles to Twp Rd 34-4, at 12-21-34-13W4M coordinates to the well as shown in figure 1.

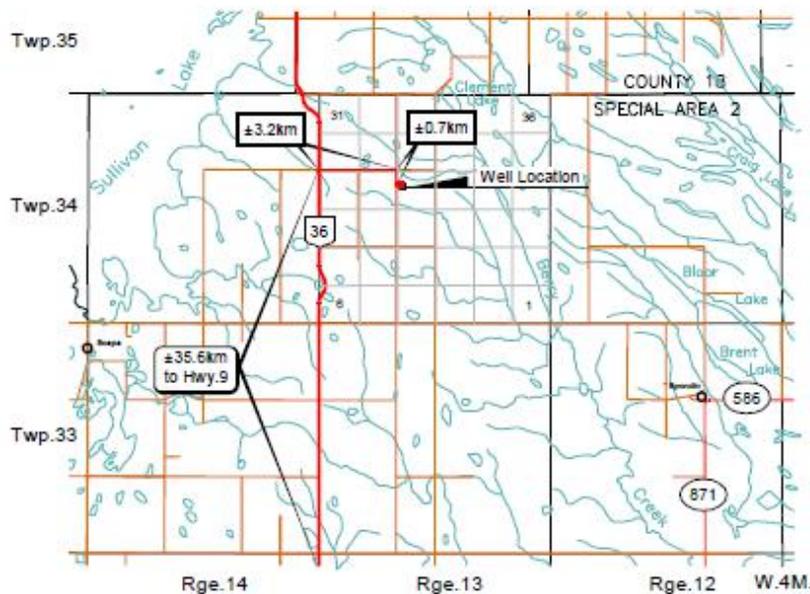


Figure 30: Well Location

It is a single well battery site, where the separation of production fluid from the well into gas, oil and water is carried out at the site itself. A storage tank is used as a treater where the separation process occurs. The gas at the treater consists of mostly natural gas from the production fluid, and may also contain some solution gas released from the crude oil and salt water when the fluid pressure is lowered at the treater. Further details of the wellsite are given in Appendix D. The methane biofilter discussed in this report was designed to receive a line of natural gas from the storage tank as the source of methane and would therefore receive 98% CH₄ with 2% impurities consisting of salt water and crude oil.

Progress of Work:

Design Considerations

- The location of the wellsite is supposed to reach extreme weather conditions during the winter, and receives high winds very often. Therefore, it was concluded that a closed MBF was the only feasible option for this site.
- Past research has shown that active aeration could yield efficiency three times higher than passive aeration and since natural gas received from the storage tank, constitutes 98% CH₄, the system should maintain a high efficiency during its operational period. Therefore a closed MBF with active aeration was proposed.
- According to stoichiometry, an air to methane ratio of 10:1 (v/v) is optimum for methane oxidation. However, past research has shown that even a ratio of 5:1 is sufficient to yield acceptable efficiencies. The gas mixture with air and fuel at a 5:1 ratio is flammable. Therefore preventive measures are required to ensure there are no leaks or heat sources (static electricity build up). Furthermore, proper mixing of air and gas, before it enters the system, should be enabled.
- The wellsite does not have access to a power source. Therefore, the proposed system should be self-sufficient.
- Methane oxidation is an exothermic reaction which generates heat. This heat helps to increase the temperature inside the MBF, thus providing optimum conditions for bacteria. If the MBF freezes, the bacteria may get dormant and reduce the efficiency of the MBF drastically during cold periods. Therefore, in order to maintain activity inside the bio filter during very cold months, it was important to insulate the boundaries of the system. The air and gas fed into the system should be at acceptable temperatures as well, as it is a major constituent of the MBF.
- The system will be constructed at university premises, and would have to be transported to the site and installed.

Objective 1: Conceptual Design

The conceptual design is based on the conceptual design highlighted in Appendix and specific site conditions for this project

As can be seen in figure 31, the biofilter is constructed using a conical frustum shaped tank accompanied by a housing structure, which are both placed on a wooden skid. The cylindrical shape offers less wall resistance during gas migration as opposed to a box tank. The housing structure could store all monitoring equipment and the air blower to pump air into the biofilter. This provides a confined space to heat thus ensuring that the air sent into the tank is at a desired temperature.

The use of the wooden skid has two main advantages. It provides a stable structure to transport the setup and allow for the possibility of relocation in a later time. In addition, it ensures that the structure remains levelled over time. If some settling was to occur due to the weight of the tank, this would still be uniform.

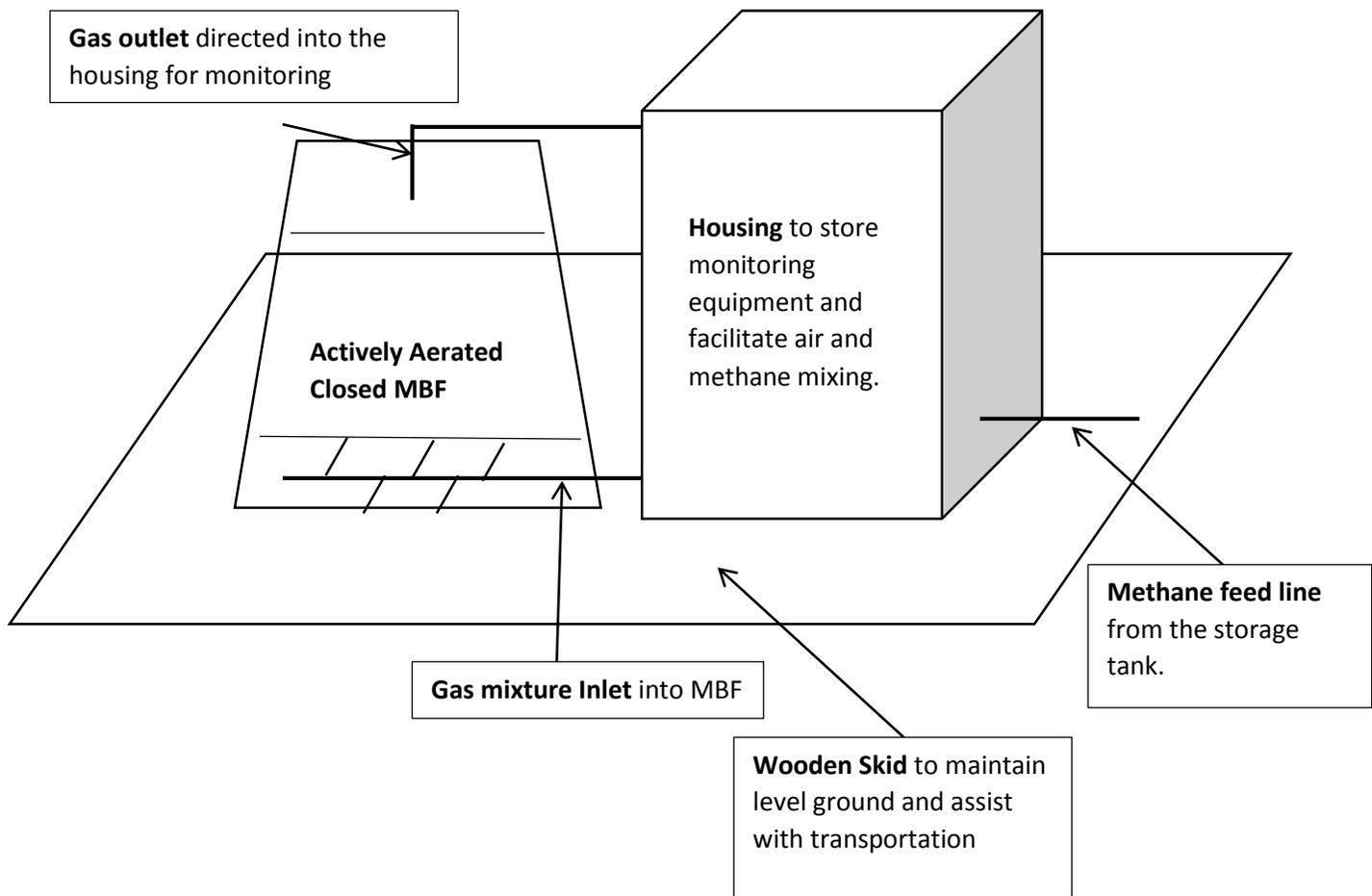


Figure 31: Schematic of proposed design

Design Details

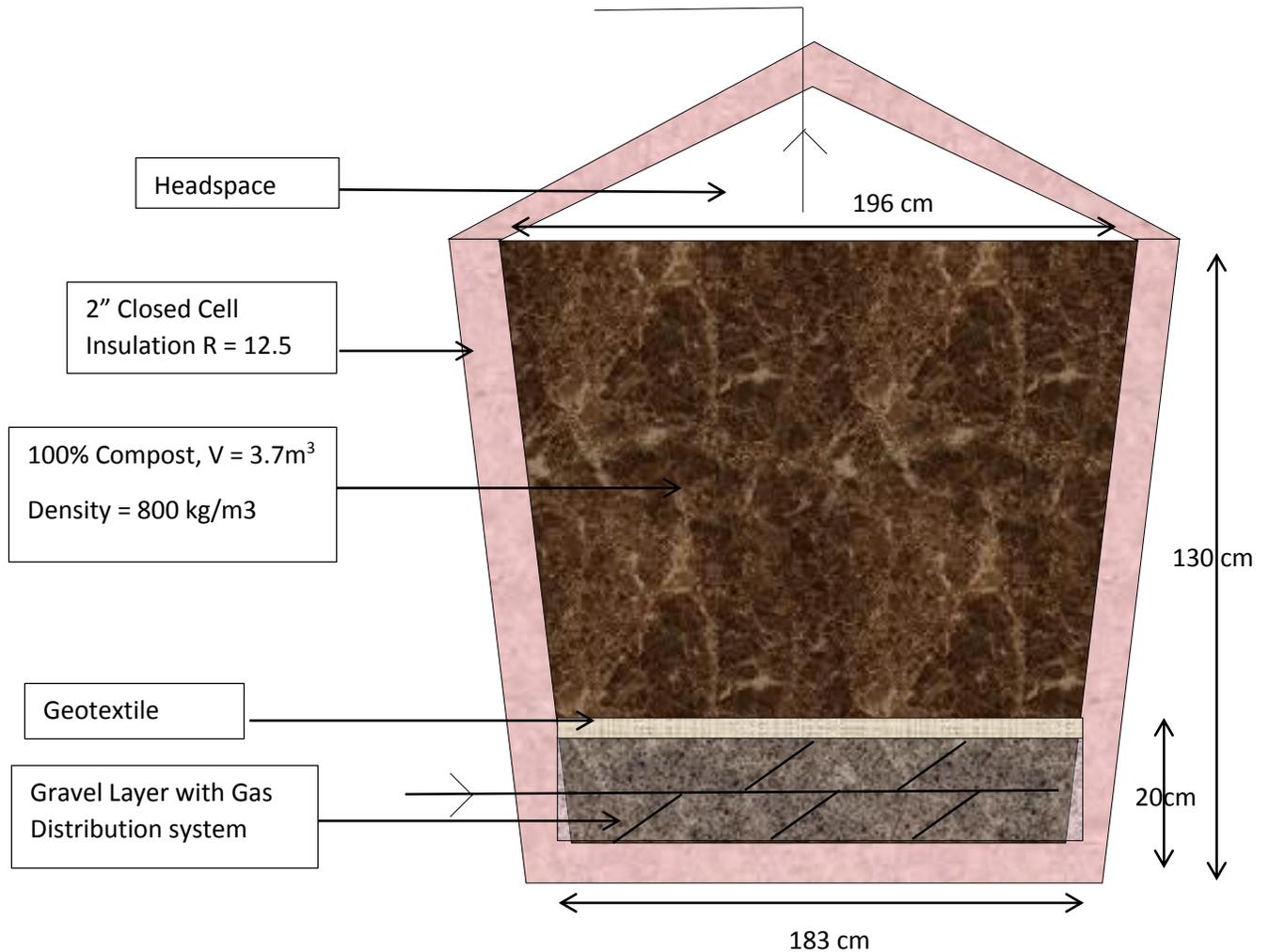


Figure 32: Detailed Schematic of MBF

MBF Sizing

Previous laboratory column experiments show that active aeration is three times more efficient than passive aeration. Therefore, an active MBF could be designed with a much smaller volume than a passive MBF. Due to this reason, the current MBF was designed to have a volume of 4 m³ and an intake of about 40 m³/day. This would provide an empty bed retention time of 2.4 hours. The actual retention time will be further lowered as the tank is packed with media and compacted, thus providing sufficient time for the microbes to act on the methane. Figure 3 shows the exact dimensions of the tank that was chosen.

MBF Media

This MBF was designed to contain 100% compost as compost has a high organic content, nutrients, water holding capacity providing an ideal environment for methanotrophs and yields the best oxidation efficiencies during laboratory experiments.

A density of 800kg/m³ was specified based on previous literature and the moisture content will be 80% of field capacity.

Gas Distribution System

When the MBF is running at its design capacity, it would receive 40 m³ of methane and 200 m³ of air, adding up to a total of 240 m³ of air-methane mixture per day. This gas should be well mixed and uniformly distributed across cross section of the MBF. The gas distribution system consists of a piping network, gravel, and a geotextile to undertake this task. First, the distribution system will be constructed using 2” ABS pipes, with holes drilled into its surface to distribute the gas. The hole sizes are calculated to systematically increase in diameter, thus ensuring that the rear end of the MBF will receive equal amounts of gas as the front end. Details of these calculations are presented in Appendix A. The holes will be drilled at 45 degree angles facing the bottom to allow the gas to first reach the bottom of the tank, and seep up uniformly. The gravel layer provides the space to allow for maximum mixing and distribution. For this purpose, 20 mm gravel will be used. Finally, a geotextile is placed on top of the gravel to prevent compost from clogging the gravel layer.

Insulation

The MBF is insulated with 2 inches of closed cell foam insulation, providing an R value of 12.5.

Piping and Instrumentation

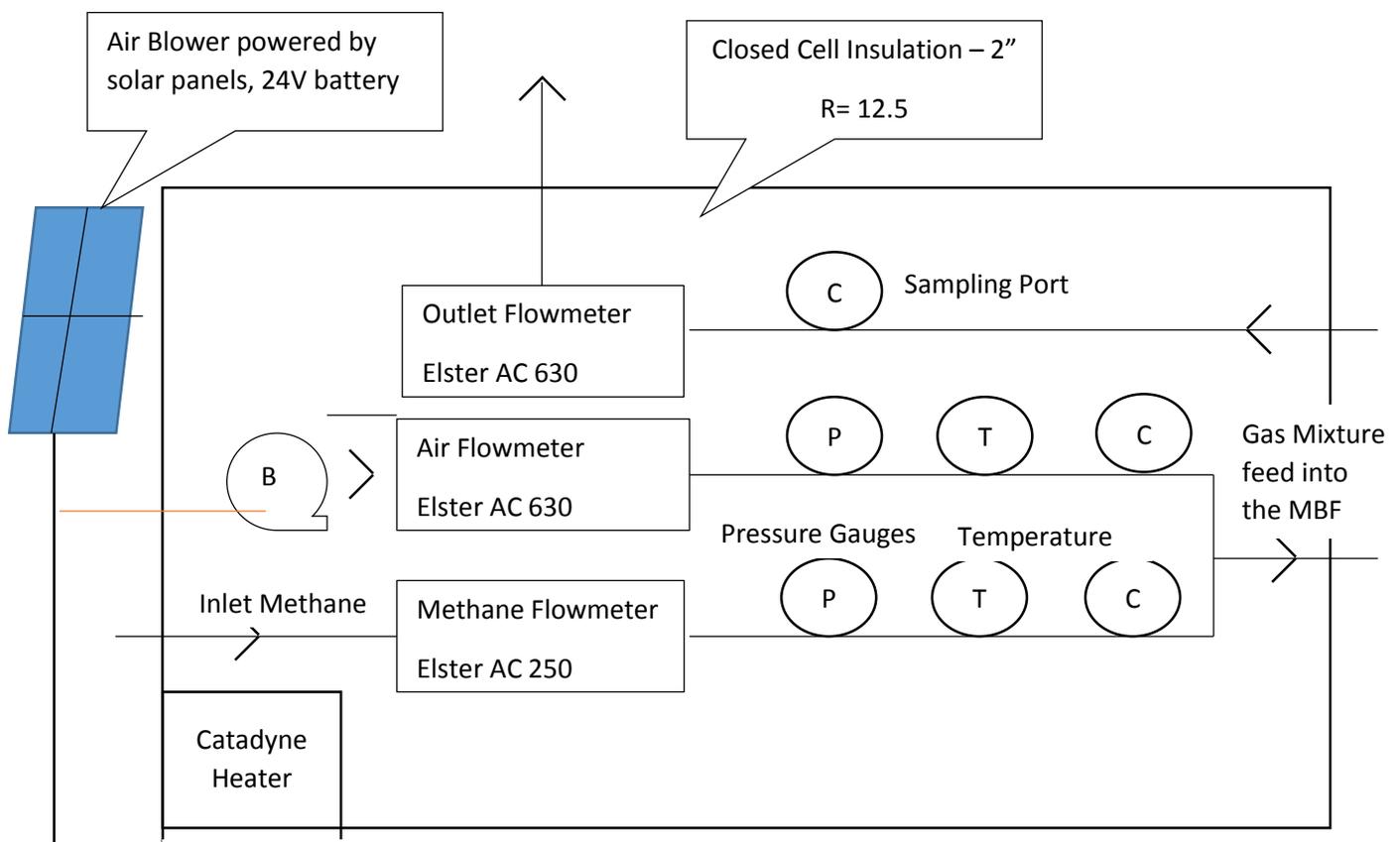


Figure 33: Detailed Schematic of Housing

Air supply

During operation the MBF should receive 5 times more air than methane. The stoichiometric ratio for air to methane is 10:1. However, the increased flowrate of air will increase the retention time of the gases and may result in adverse effects. Also, we expect that even a 5:1 ratio of air to methane would yield acceptable efficiencies. Thus, the system is designed to receive 200m³/day of air, with the option to increase if need be.

A 24 V DC, air blower which could send in up to 650m³/day (16 CFM) will be used to pump air. This blower will be powered by 24V battery and solar panels as the site does not have a power source. A catadyne heater, which uses natural gas as its power source, will be installed to heat the housing structure, thereby heating the air that will be pumped into the MBF.

Monitoring

Since an active aeration closed MBF has not been installed in the field before, due consideration was given to the monitoring aspects of the MBF. Inlet and outlet methane flowrates will be measured using flowmeters as you can see in Figure 4. Provided that there is no leakage in the system, this together with the concentration would enable us to calculate the efficiency of the MBF with ease. A third flowmeter was installed to the air line, to measure the volume of air entering the system. This would allow us to maintain the desired air to methane ratio at all times.

Backpressure is another major concern during the design of the piping system. This MBF, when packed to design criteria will have a pressure of 8.6 kPa. Therefore, the methane and air entering the MBF should have a pressure greater than this value in order to reach the top of the MBF. Furthermore, the methane inlet should have a higher pressure than the air blower to prevent methane from entering the air line. This could have severe consequences as methane and air together is flammable, and its contact with electricity could be very dangerous. Three way valves will be installed to divert the flow to take pressure, temperature and concentration measurements from the corresponding gas lines.

The following parameters will be measured intermittently to evaluate the efficiency of the MBF.

1. Inlet flowrate of methane
2. Inlet flowrate of air
3. Outlet flowrate of the gas mixture
4. Inlet gas composition
5. Outlet gas composition

In addition, the following temperature data will be logged daily to monitor the changes within the MBF.

1. Room temperature of the housing structure
2. Temperature at the headspace
3. Ambient temperature
4. Temperature inside the MBF at 14 different locations as shown in figure 34.

Temperature modelling

As mentioned earlier methane oxidation is an exothermic reaction and generates heat. Therefore, for a given period of time, there is a direct correlation between the temperature rise inside an MBF and the methane oxidation that took place during that time. Temperature sensors will be installed in three levels as shown in figure 5 to monitor the oxidation profiles inside an MBF, and generate a model that predicts the methane oxidation.

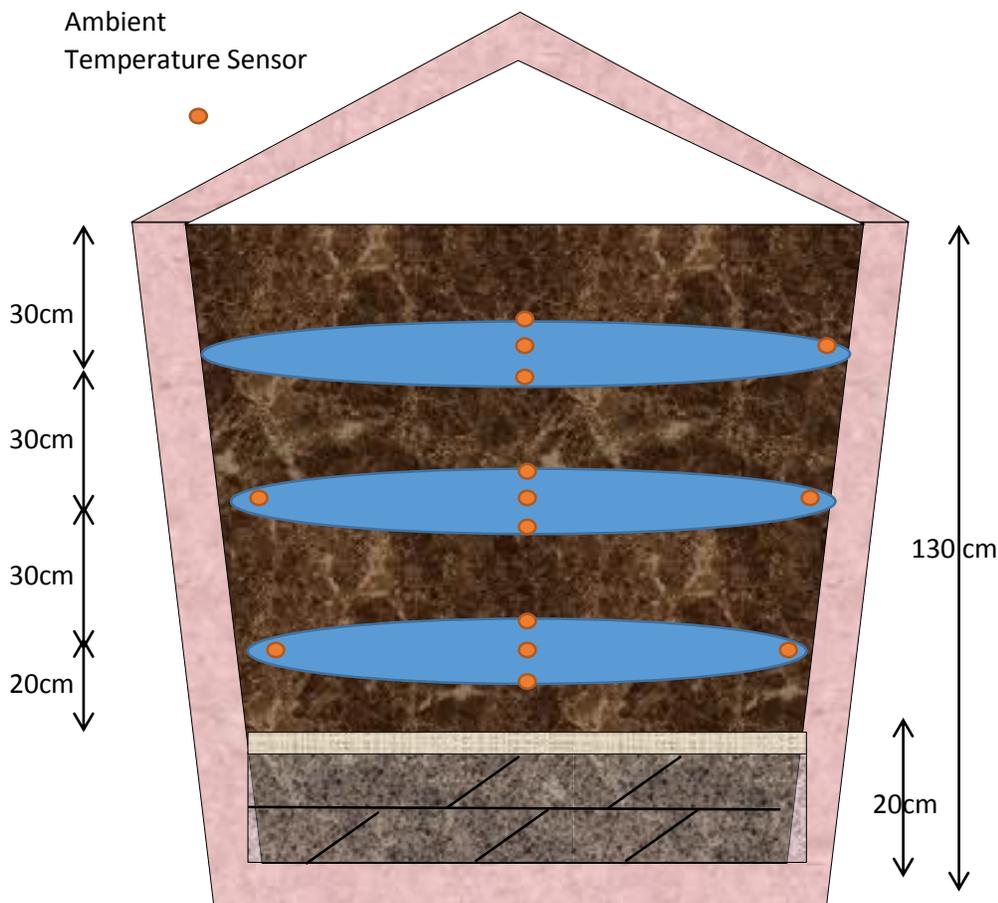


Figure 34: Temperature Sensor Locations

As seen in figure 34, there are 15 temperature sensors installed in the following locations of the MBF. One sensor will be kept outside to record the ambient temperature data. These sensors should give sufficient data to plot vertical temperature profiles, and the heat transfer within the cross section of the MBF as well. The 15 sensors will be connected to two data loggers, with the capacity to log 15 data ports and will be stored inside the housing structure. Details of the equipment used are given in Appendix C.

Objective 2: Construction, Transportation, Installation

Construction

Construction of the MBF took place in university premises, in front of the Schulic School of Engineering F block on the 20th of July, 2016, and was completed on the 22nd of August, 2016. The steps that were carried out during the construction process is outlines below. Detailed pictures of the process can be seen in Appendix B.

1. Housing structure was built on the wooden skid next to the biofilter tank.
2. The internal walls of the housing and the external walls of the tank were insulated using spray foam insulation. The walls were later painted in order to protect the insulation foam from UV damages.
3. The flowmeters were mounted onto the walls inside the housing structure, and steel pipe connections were installed along with the three way valves and the air blower.
4. A pressure test was conducted to eliminate the possibility of leakage by holding the pipes at 30 psi for 24 hours. The first test failed, as the pressure dropped slightly overnight. However, the source of the leak was identified and fixed.
5. The inlet and outlet into the MBF were installed using 2" ABC pipes.
6. The gas distribution system was fabricated and the holes were drilled into the assigned positions.
7. A test run was conducted by connecting an air blower into the pipe distribution system to confirm that air was passing through all sides of the distribution system uniformly.
8. 20mm crushed concrete gravel was filled up to about 10cm. Next, the pipe distribution system was placed on top of the gravel. Small wooden blocks were used to hold the distribution system in its levelled position. More gravel was filled on top of the distribution system to tightly secure it's positioning, and finally, the geotextile was placed on top of the gravel later.
9. A compressed CO₂ cylinder was used to send gas through the distribution system, and flux measurements were taken at the surface of the geotextile. Uniform distribution throughout the perimeter of the tank was confirmed.
10. Compost was collected from the East Calgary Landfill, and tested to measure the moisture content. The compost was at 100% FC as it was raining during this time of the year. So it was dried up to achieve 80% FC.
11. Compost was shoveled into the tank systematically to achieve the desired density, i.e. 800kg/m³. Details of the packing method are given in Appendix A.
12. CO₂ was passed through the tank to measure equal distribution of gas in 20 cm increments. The flux measurements were lower as the tank was filled with more and more compost, due to the increasing retention time. However, we were able to confirm that the gas reached the top of the filter in all corners of the tank.
13. Temperature sensors were installed as per the design while adding compost.
14. The lid of the tank was sealed by weather stripping the top perimeter of the tank and then by wrapping coper pipe insulation around the edge. Silicone was passed inside the insulation to tighten the seal.

Transportation

The well location in Hannah was about a 3-hour drive from Calgary. An industrial truck was hired to transport the entire unit to the site location. Due to the large weight of the setup, precautions were taken to ensure stable elevation. Ratchet straps were used to strap the lid onto the wooden skid. The wooden skid was lifted using a spreader bar. 4 steel hooks rated for 4400 lb were attached to the four corners of the skid, with grade 70 chains attached to it. These chains were connected to either sides of the spreader bar and lifted. Pictures of the assembly are given in Appendix B.

Installation

The following steps were carried out to install the system and run gas through it.

1. Levelled the ground where the setup will be placed.
2. Unload the setup in the levelled ground.
3. Install solar panels and battery.
4. Install catedyne heater.
5. Connect the air blower to the power supply.
6. Connect the temperature sensors to the data logger and obtain baseline data for the temperature inside the MBF before methane oxidation takes place.
7. Connect the methane supply into the setup on September 20th. It is expected to see a lead time during the seeding period of an MBF. Therefore, the methane supply was set to flow at 25 m³/day during this period.
8. Due to the unexpected resistance caused by the packed tank, the air blowers capped at about 100m³/day. Therefore, currently air is set in at a flowrate of 100m³/day.

Results and Discussion:

The following mass flowrates were recorded using the three mass flow meters on the 28th of November, 2016. As can be seen, air is sent in at 10 times the volume of methane to provide an optimum CH₄ to O₂ ratio. There is also a mass balance since the outlet gas mixture is approximately equal to the summation of the inlet air and CH₄ gas flowrates.

	Mass Flow rate (m ³ /day)
Inlet Air	212.01
Inlet CH ₄	21.84
Outlet gas mixture	233.45

However, the gas concentration measurements gave contradictory results showing 33.5% CH₄, 20.4% Oxygen and 0.7% CO₂ at both the inlet and outlet measurement ports.

The temperature fluctuations between the September 20th (Day on which Methane was connected) to the 1st of December at three cross sections of the MBF can be seen in figures 6, 7 and 8.

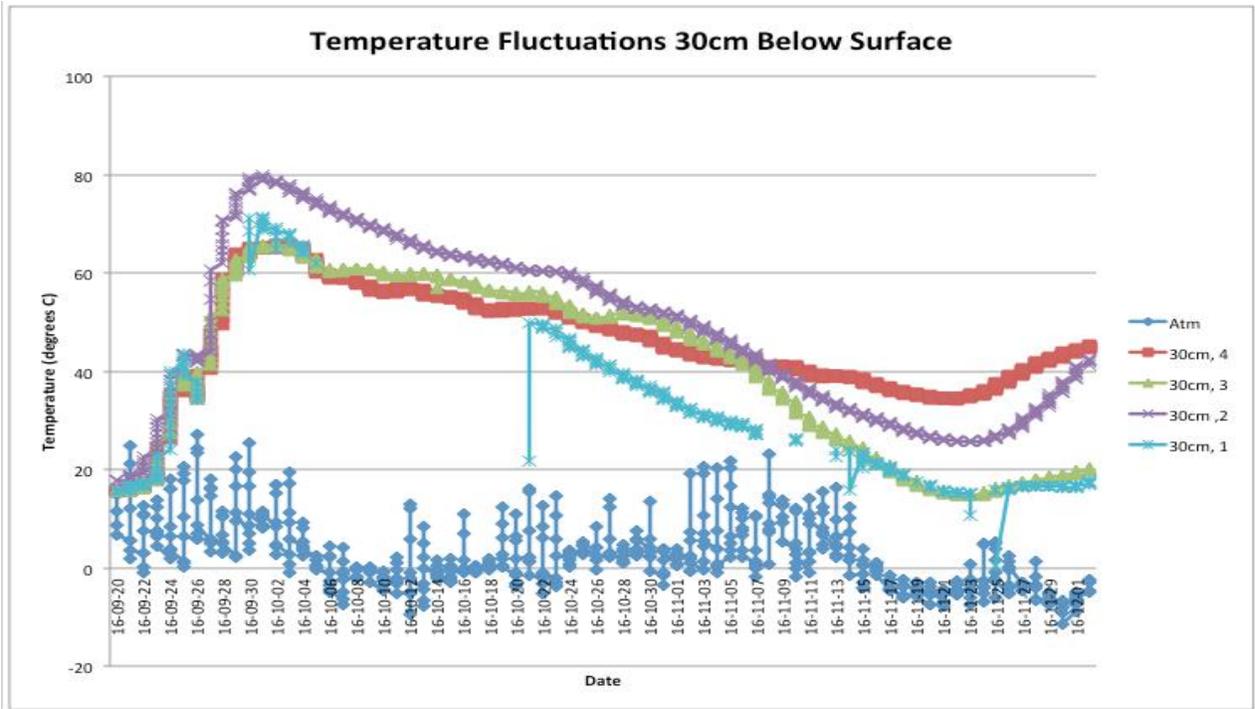


Figure 35: Temperature Fluctuations 30cm Below Surface

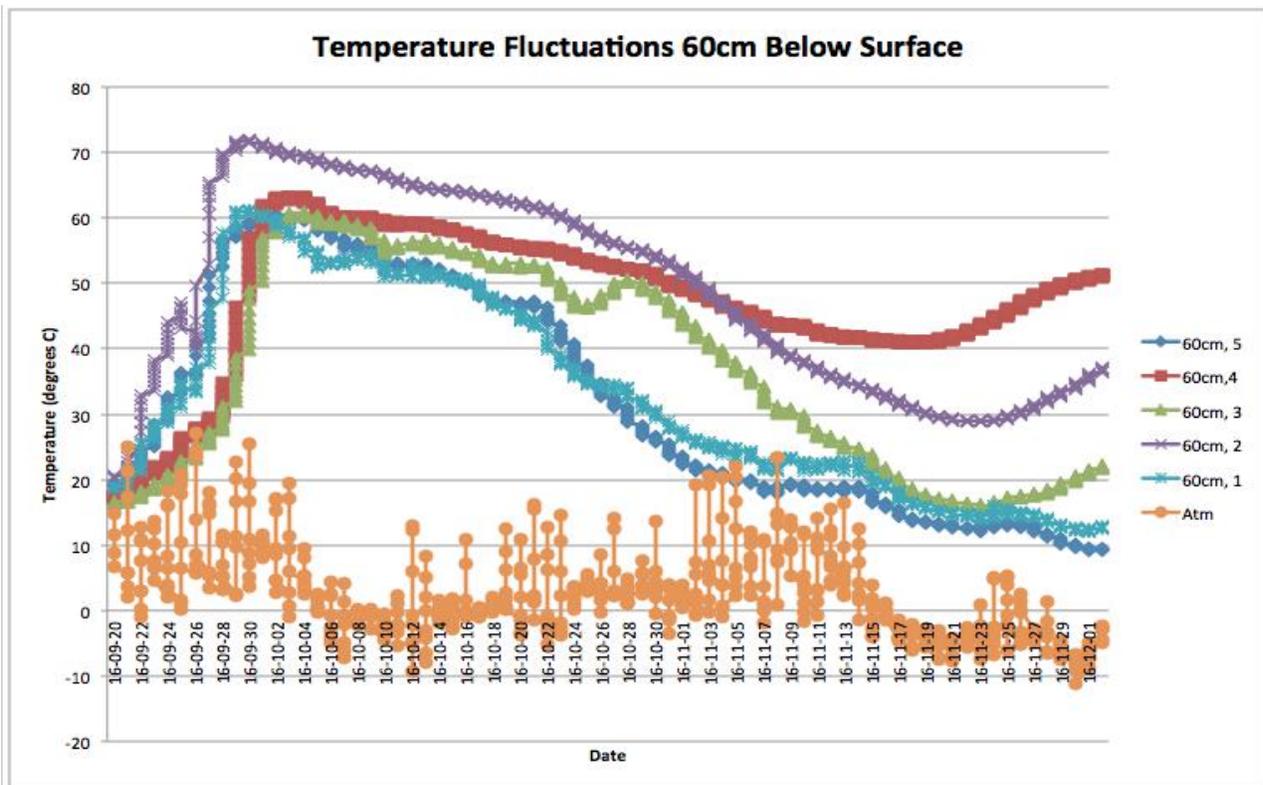


Figure 36: Temperature Fluctuations 60cm Below Surface

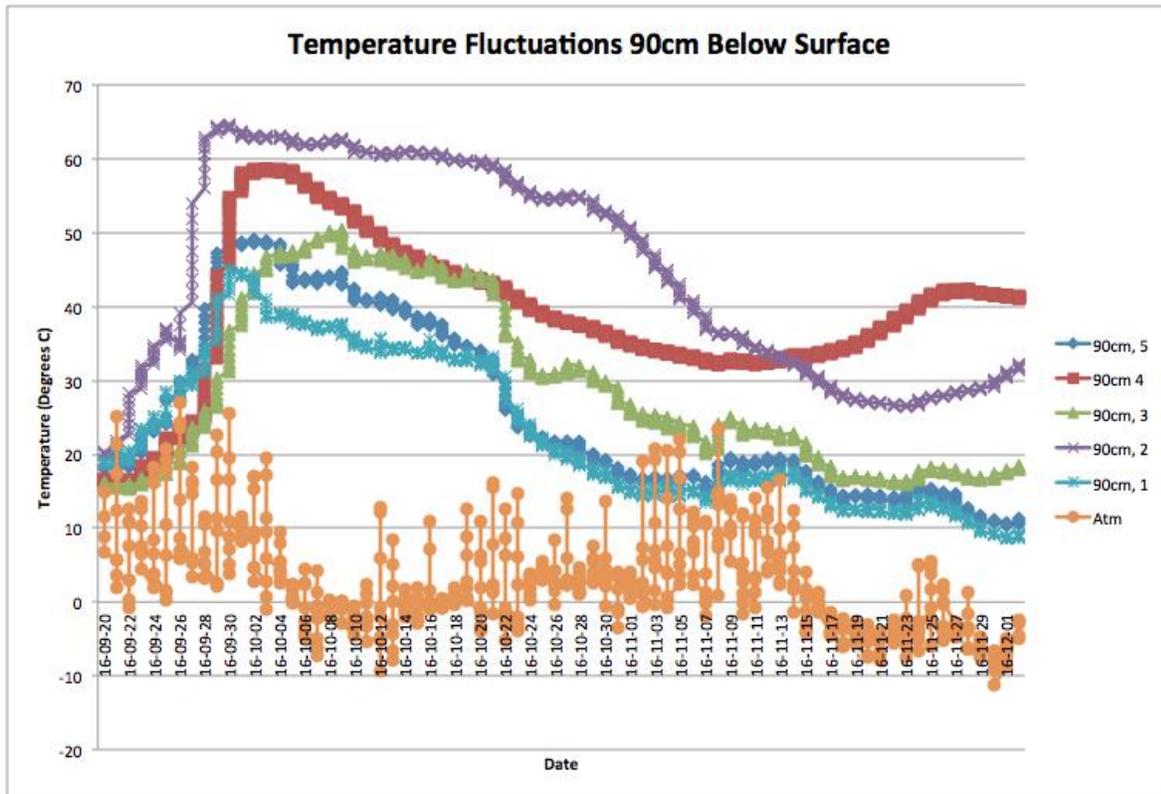


Figure 37: Temperature Fluctuations 90cm Below Surface

As can be seen from all three graphs, the temperature inside the MBF is higher than atmospheric temperature at all times. However, during the mid start of operation, the temperature inside the MBF rises to toxic levels and may have caused harmful effects to the bacteria.

This rise in temperature in the MBF could be corresponded to a malfunctioning of the housing heater during October 4th to October 22nd as it was overheating the room to a temperature of 42 °C. This translates to a temperature of 60 °C to 80 °C inside the biofilter. After October 22nd, the heater was fixed to maintain room temperature between 12 °C – 15 °C. This was later increased to 15 °C – 20 °C as the ambient temperature reduced drastically.

The contour plots on figures 9 and 10 show the distribution of heat across the cross section of the MBF at 60cm and 90cm on December 1st 2016.

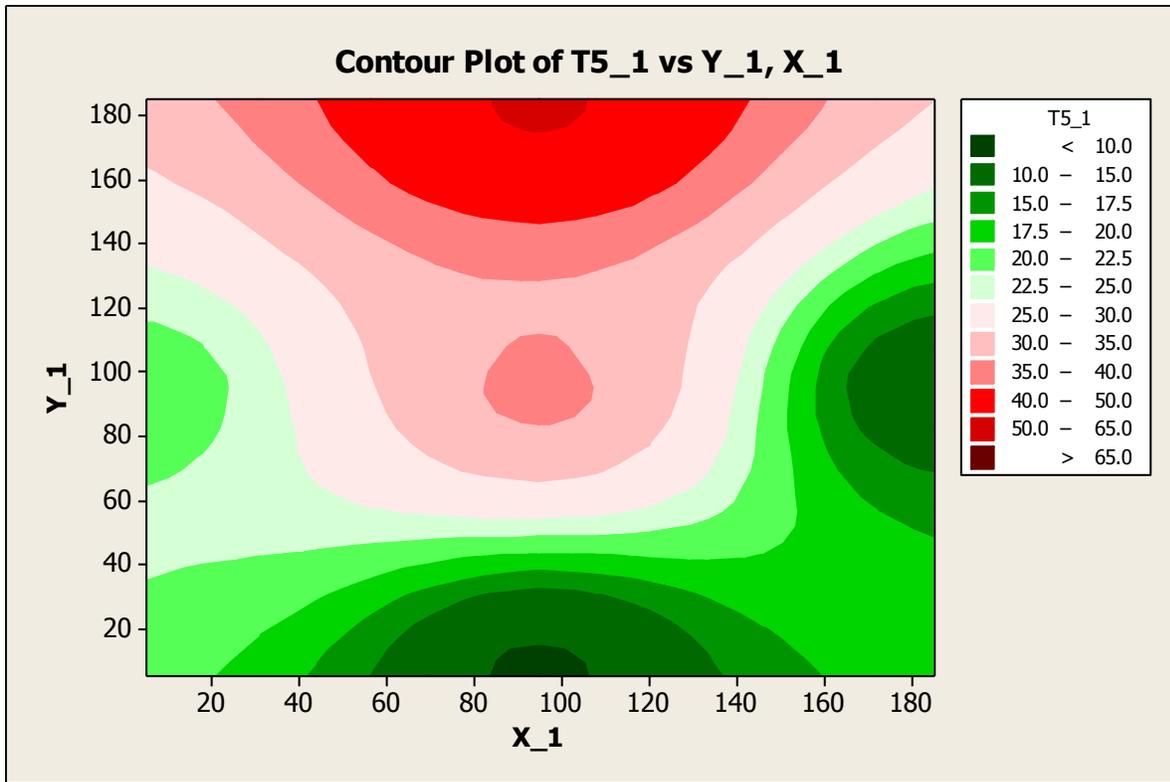


Figure 38: 60cm below surface level, December 1st

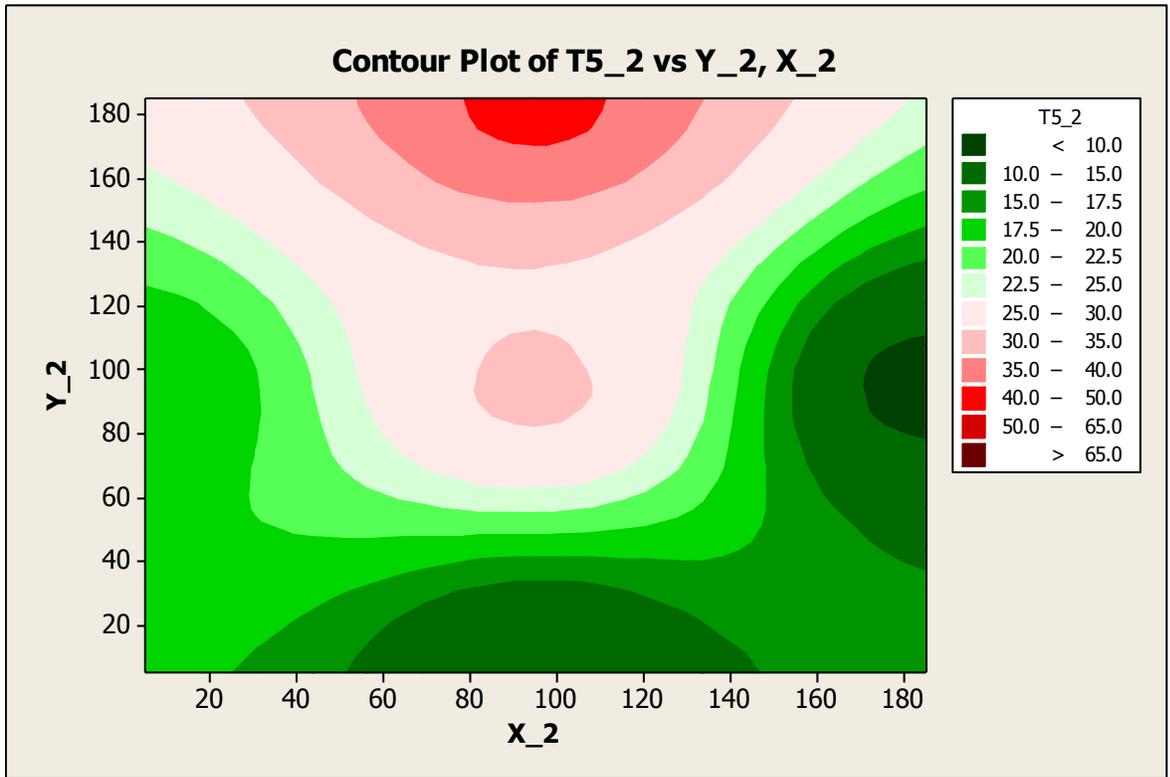


Figure 39: 90cm below surface level, December 1st

It is evident that the temperature follows the exact pattern at both the surface levels although there is a slightly higher average temperature at 60cm.

Further steps must be taken to recalibrate all three flowmeters, and measure gas samples with different equipment. Also, a compost sample should be analysed for its nutrient content and bacterial population to confirm the activity inside the MBF.

Conclusions:

An active methane biofilter or properly named as a High-rate Methane Biofilter (HMBF) was installed at Hanna, AB. Many obstacles to the project were identified and addressed.

Preliminary results indicated that there are further obstacles that needs to be addressed.

The implementation of the system was a success. Additional data are being collected to determine the performance of the system. Based on the limited temperature data collected to date, it can be concluded that there is biological activity inside the MBF.

Future Work:

Continuous monitoring and onsite modifications of the system

Collection and analysis of data to determine the performance of the syetem

Outputs and deliverables:

S.S. Gunsekera, J.P.A. Hettiaratchi, E.M. Bartholameuz, E. Irvine. 2017. Design and Operation of Actively-Aerated Field-Scale Methane Biofilters, CSCE15th International Conference on Environmental Engineering, Abstract Accepted

Appendix 1: DBM Devon Project

Next Page



DESIGN BASED MEMORANDUM

METHANE BIOFILTERS FOR THE TREATMENT OF CASING GAS AT OIL WELL SITES

University of Calgary in Collaboration with Devon Canada Inc.

Background

Methane is a key greenhouse gas (GHG), and atmospheric methane emissions in Alberta are associated with a variety of industry sectors including sanitary landfills, wood waste landfills, feedlot operations, and the oil and gas sector. When the quantities released at individual locations are relatively small, this methane cannot be used as an energy source; therefore, environmentally acceptable methods are needed for their control. Recent research has shown that methanotrophic bacteria are capable of converting methane to carbon dioxide without producing toxic by-products. To utilize this capability of methanotrophic bacteria, Dr. Hettiaratchi's research team at the University of Calgary (UofC) has conducted extensive research to develop methane biofiltration (MBF) technology,

Currently, activities are being carried out to determine technical and commercial feasibility of the technology and to develop an accepted monitoring protocol in collaboration with Climate Change Emissions Management Corporation (CCEMC) and other industrial partners. The goal of the overall project is to develop a complete MBF technology package to allow its large-scale implementation throughout Alberta, which would contribute to a significant reduction of GHG emissions in Alberta.

One of the main GHG sources in Alberta is the oil and gas industry. Although fugitive emissions intensity from the upstream oil and gas sector has decreased by nearly 30% since efforts to reduce flaring and venting emissions expanded in 2000, fugitive emissions from this sector still account for 7.4% of Canada's total emissions in 2013, with venting accounting for the majority (56%) of these fugitive emissions (*Technical Report on Canada's Upstream Oil and Gas Industry*, Clearstone Engineering Ltd., 2014). Casing gas venting is one of the major sources of methane emissions associated with oil production.

Casing gas is generally emitted through a pressure relief valve in the assembly. This relief mechanism releases methane intermittently at high flow rates. On the other hand, MBFs are generally designed to handle steady flows, of relatively low pressures. Two of the main factors that affect the MBF efficiency are the residence time for methane in MBF and inlet source strength. An increased residence time improves system efficiency, as it provides more time for bacteria within the MBF media to metabolize methane. Due to this residence time requirement, the intermittent flow of casing gas at high flow rates is not ideal for MBF operation and the flow should be regulated before being directed to a MBF. Since the flow characteristics of vented casing gas represent a worst case scenario for MBF operation, successful adaptation of this technology to this application can provide a proof of concept for a wide variety of less complicated applications within the oil and gas industry.

UofC, in partnership with Devon Canada Corporation (Devon), intends to implement a MBF system at one of the oil well sites operated by Devon. This MBF is specifically designed to control casing gas emissions. This document details the work completed to date, work yet to be completed, and project milestones.

Goals and Objectives

The goal of the project is to develop an MBF system that can be applied to treat methane emissions associated with casing gas.

The project has three main objectives:

1. Design/Develop/Implement a MBF system and assess the technical feasibility of the technology
2. Assess the economic feasibility of the MBF system
3. Determine MBF efficiency and develop a monitoring protocol to quantify greenhouse gas (GHG) reductions associated with the technology

The following research questions will be answered at the completion of the project:

1. Methane oxidation efficiency is both a function of flow pattern and overall flow rates. What will be the impact of flow pattern and overall flow rate on efficiency of the MBF system?
2. An actively aerated MBF system is expected to have higher methane oxidation efficiency than a passively aerated system. What is the increase in efficiency?
3. The MBF efficiency monitoring protocols are not fully developed. A correlation between temperature and methane oxidation efficiency is to be developed in order to establish a new MBF performance monitoring protocol. What will be the effectiveness of such a protocol?

To answer the research questions and to evaluate if the objectives of the project are met, the following parameters will be measured:

- Temperature inside the MBF (frequency: once every 12 hrs, logged)
- Pressure inside the gravel layer (frequency: once every 12 hrs, logged)
- Inlet and exit gas concentrations for CH₄, CO₂, O₂, and N₂ (1-4 month periods)
- Inlet and exit flow rates (1-4 month periods)

The goals and objectives, as well as research questions, are part of a larger MBF project being conducted by UofC, which consists of research and analysis, laboratory work, and fieldwork.

The main *tasks* of the current project are:

1. Design a flow regulation system that can regulate casing gas without affecting either the existing operation of the well or the MBF operation
2. Design an actively aerated MBF
3. Select a suitable well site for the installation of the MBF
4. Construct the flow regulation system
5. Construct and install the MBF system
6. Continuously monitor and gather data from the system
7. Evaluate the system performance and develop protocols to quantify emissions reductions

Project Implementation

This section highlights the details related to project implementation, how each milestone is achieved, work already completed, work to be completed, and who will be responsible for undertaking the tasks.

Milestone 1: Design of the MBF system, including the flow regulation system

MBF system has two main components:

MBF Vessel and Accessories

The MBF design is the responsibility of UofC. MBF media will be selected based on laboratory tests and material availability. MBF vessel, equipment to be installed, sensors, and other components will be selected based on research requirements and the safety and operational requirements of Devon. All MBF components will comply with applicable codes and standards specified for use in the oil and gas industry, and these required codes and standards (including the equipment and potential suppliers) will be provided by Devon.

Flow Regulation System

Flow regulation/delivery system design will be completed by Devon and/or contractors. The flow regulation will be conducted according to the UofC specified flow rates and parameters that match the designed MBF system.

Milestone 2: Selecting a suitable well site for the installation of the MBF

This task is to be carried out by Devon with consultation and input from UofC.

Devon has selected four potential candidates for the installation of the MBF. Two are single well sites and the other two are multi-well pads. It is recommended to utilize one of the two multi-well pads as the flow of excess casing gas is considered more reliable. Both of the pads are considered easy access from main roads and would be suitable for regular visits to monitor and gather data.

Location	Monthly Vent (E3m³)	Average Daily vent (m³/d)	Comments
07-26-63-8W4 PAD	5.69	183.5	3-well pad, steady production, just off of hwy 55
13B-05-064-07W4M	4.46	143.9	Single well, steady production, fairly easy access
05B-23-063-08W4M	2.85	91.9	Single well, steady production, just off highway 55
10-01-63-6W4 PAD	2.32	74.8	2-well pad, steady production, access off of hwy 43

Based on the above details the well # 110-01-63-6W4 PAD is selected as the most suited for the considered application.

Milestone 3: Installation of the system

Installation of the flow regulation system

The installation of the flow regulation system is the responsibility of Devon at the selected well site.

Construction of the MBF

The MBF will be constructed at UofC Labs according to the specifications agreed upon with Devon.

Installation of MBF

Once constructed, the MBF will be transported and installed at the selected well site. The compost used as MBF media will be added to the MBF on site. The MBF system will be installed by both Devon and the UofC. UofC team will complete required training that satisfies Devon conditions to enter and execute work at Devon facilities. The standard training for UofC team for field work includes; Standard First Aid, H₂S training, CSTS and Generic WHMIS.

Milestone 4: Monitoring/Operation

MBF Operation

Once installed, the MBF will not require regular operation/maintenance. Devon will implement any standard emergency practices at well sites, as required. The MBF can be disconnected at any time, if necessary.

Real time data collection

Real time temperature and pressure inside the MBF will be logged using a data logger. The data logging system will not require maintenance. The UofC will replace any equipment, as necessary, and will inform Devon of all maintenance activities. The data will be downloaded during regular visits to the well site by the UofC team.

Data collection site visits

The UofC team will make regular visits to the MBF site. The frequency of site visits will change according to the research requirements and MBF status. The expected frequency of site visits is once every 4 weeks, for the first 4 months. Once the system is established, the frequency of site visits will be reduced to once every 4 months. During each visit, the inlet and outlet flow rates and inlet and outlet gas concentration will be measured. Devon will be notified in advance before a site visit, and a schedule of site visits will be agreed upon at the beginning of the project.

Milestone 5: Evaluation of the system performance

All system performance details will be duly communicated to Devon once evaluated by the UofC. The details that are communicated to Devon include; estimated methane oxidation efficiency, eliminated/released amounts of CH₄ by the MBF, summary of conclusions and any proposed modifications.

Year 1

Data collected from the system in the first year will be used to evaluate the system performance and methane oxidation efficiency.

Year 2

Additional data from the second year will be used to field verify the temperature vs. oxidation efficiency relationship.

Year 3

Long-term system performance trends will be identified. Cumulative data from the first three years of operation will be used to evaluate oxidation efficiency changes, if any, and develop MBF monitoring protocols.

MBFs can have long operational lifetimes, however, according to the UofC experience, the MBF media needs to be replaced every 3-5 years.

Milestone 6: Evaluation of the economic feasibility

The purpose of the Economic Feasibility is to assess the viability of using MBF as a potential casing gas treatment technology in comparison to other competing technologies (example; flaring). Therefore, only the costs associated with the design, construction and monitoring of MBF will be used in the feasibility assessment. The costs associated with site modifications and flow regulation will not be included. The site modifications and flow regulation would have to be conducted prior to application of any casing gas emission control technology and is not integral to assessing the economic feasibility of MBF.

The following costs are included in conducting the Economic Feasibility analysis:

1. Cost of Material for MBF; MBF vessel, MBF media and MBF piping system
2. Cost of standard monitoring system; inflow and in and out gas concentrations
3. MBF installation costs,
4. Cost of MBF performance monitoring.

The following costs are not included in the analysis:

1. Cost of installation and design of the flow regulations system
2. Cost of site modifications

The following expenses are considered research expenses and are not included in the analysis:

1. Cost of design of the MBF system
2. Cost of non-standard monitoring equipment such as temperature sensors, data loggers and pressure sensors
3. expenses occurred as a result of any specific field activity conducted for research purposes; monitoring and data collection site visits

The economic benefit is analyzed based on the financial benefit of carbon off-sets accrued from the application of the MBF system. The carbon off-sets will be calculated from avoided methane emissions, but subtracting the carbon dioxide production from methane oxidation.

Work Progress

Milestone 1 and 2, discussed above, have been partially completed (as noted in the table)

Milestone	Work Completed	Work to be done	Timeline
Milestone 1: Design of the MBF system, including the flow regulation	<ul style="list-style-type: none"> Detailed design of the MBF completed by UofC 	<ul style="list-style-type: none"> Modifications to the MBF detailed design based on Devon input Detailed design of the flow regulation system to be completed by Devon/contractors 	<ul style="list-style-type: none"> MBF modifications to be completed by UofC in consultation with Devon by <u>July 30, 2016</u> Final flow regulation design completed by Devon by <u>TBA</u>
Milestone 2: Select a suitable well site for the installation of the MBF	<ul style="list-style-type: none"> 4 potential well sites were analyzed by UofC and Devon 	<ul style="list-style-type: none"> Further Investigation to be carried out by Devon 	<ul style="list-style-type: none"> Site selection to be finalized by <u>July 30, 2016</u>
Work towards other milestones	<ul style="list-style-type: none"> UofC has initiated the selection of MBF media and monitoring equipment (sensors, meters, etc.) 	<ul style="list-style-type: none"> All relevant components has been selected by UofC 	

MBF Details

Design and safety details

MBF is designed in a cylindrical shape. A HDPE vessel is used as the MBF vessel. MBF has 4 main components, the bottom gravel layer and air/gas distribution system, the compost layer, sensor assembly and external piping.

Bottom Gravel layer and air/gas distribution system

Bottom gravel layer acts as a distribution layer for input gas. A distribution pipe system of PVC is connected to the main input line to properly distribute the air/methane mix. Bottom gravel layer consists of one PRV set at 10 psi, to avoid any excess pressure build up.

The compost layer

The compost layer consists of, industrial stabilized compost. The compost acts as the media where methanotrophic bacteria would grow in order to convert methane to carbon dioxide. The compost will be obtained from City of Calgary, East Calgary Landfill, since stabilized compost is not available near Bonnyville area. Copper grounding wires would be used ground the MBF Media. For installation purposes, this will be installed in line with the sensors. The grounding wires will be wither connected to the ground in the sensor assembly or as a separate ground.

Sensor Assembly

16 type T thermocouples will be used to measure temperature inside the compost layer, with one sensor measuring the atmospheric temperature and other 15 measuring temperature inside the compost. A data logger DT85 from Dycor technologies will be used to log the data from the sensors. A stand and mast package will be provided by the supplier for the data logger assembly. Sensors would be assembled in 3 different layers in 3 different depths. PVCs pipes would be used to ensure sensors are protected, although the sensors do not have any bare wires.

External piping

For external piping PVC would be used. Both the input and exit will be equipped with gas measuring ports with self-sealing quick connects. A bypass valve is used to separate the quick connect measuring ports and will only be opened when the reading are being taken.

MBF Equipment/Material selection UofC

UofC will select the equipment/material based on Devon specified requirements.

Equipment/Material	Specifications	Supplier/Potential Suppliers	Comments
MBF Vessel	HDPE, 4" thickness 60 °C maximum temperature	Flexahopper Plastics	
MBF Media	Industrial stabilized compost	City of Calgary	Compost samples are tested in UofC labs prior to field use
MBF Piping and Fittings	PVC Sch 80		
Temperature Sensors	Type K Thermocouples	Dycor Technologies	CSA CMG 60C 22 AWG LL83251 CM E129985 AWM II A/B80C 600v FT4 LL67097 ROHS (Subject to change)
Data loggers	16 Channel	Dycor Technologies	CSA Class 1 Div 2
Eagle 2 Gas Analyzer	Inbuilt IR Sensors	RKI Instruments	Mobile Equipment used during field visits.

			Intrinsically Safe, Class I; Groups A, B, C, D. Approvals: CSA / CE
GEM 5000 Gas Analyzer	Inbuilt IR Sensors	LandTec	Mobile Equipment used during field visits. ATEX : II 2G Ex ib IIA T1 Gb CSA: CLASS 2258 03 and CLASS 2258 83

Input Parameters for the MBF

Inlet CH₄ flowrate: 75 m³/day

Inlet Air flowrate: 425 m³/day

Total flowrate: 500 m³/day minimum

Inlet temperature: 5 °C – 30 °C

MBF site selection should be based on capability to provide these input parameters and the availability of space for installation of the MBF unit. Devon may consider other factors such as ease of access and expected life of the wellsite.

Project Closure and Modifications

After 3 years of operation, UofC and Devon will determine if it is necessary to continue the project.

Project modifications

Under following circumstances the project may need major modifications before the MBF reaches its expected life of 3 years.

- If the selected wellsite stops producing casing gas for over 6 months.
 - If this occurs before end of Year 1; the project should be moved to a new location
 - If this occurs after Year 1; UofC and Devon may mutually agree to discontinue

Continuation of project after 3 years

If the project is to be continued after the 3-year time period, a new agreement between UofC and Devon will be required before proceeding. In addition, MBF modifications and refilling of the MBF media will be conducted in agreement with Devon requirements.

Disposal of project Equipment and Material

- The MBF media can be disposed of at a local landfill
- The MBF itself can be reused at a different wellsite or will be disposed by UofC.

- Equipment related to flow regulation system will be disposed of by Devon as required

Project reporting

The project results and analysis will be reported to CCEMC as part of progress reports and final evaluation. Devon will be provided with project performance evaluation details and also any progress reports, if requested. Devon should inform UofC of any confidential information that needs to be withheld; such information will not be used by UofC for its analysis or reporting to other parties.

Appendix 2: Appendices for Bering Project

Appendix A

Gas Distribution System Design

Table 1: Flow Details

Total Flow Rate (m3/day)	240
Desired flow per branch (m3/day)	48
Desired flow per branch (m3/s)	0.000555556
Area of 2" Pipe	0.002038706

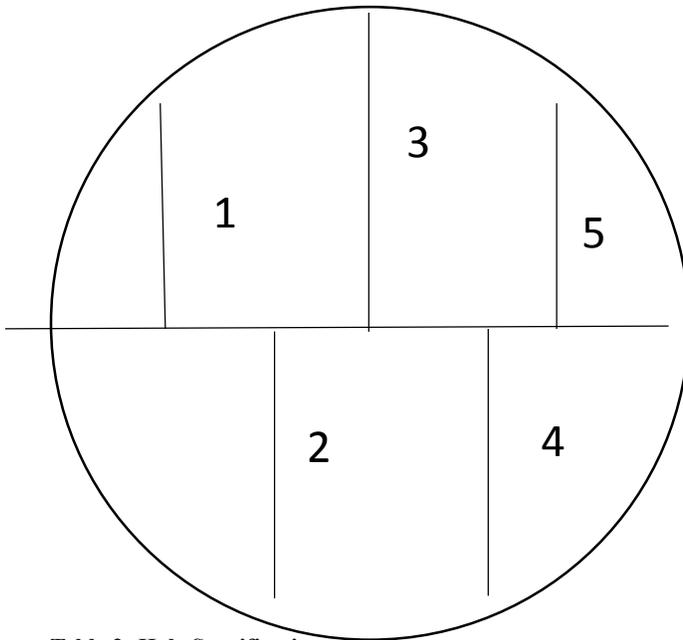


Table 2: Hole Specifications

Figure 1: Schematic of the Distribution system

BRANCH NO	1	2	3	4	5
Velocity	1.371198779	1.096959	0.822719	0.54848	0.27424
Area of holes(m2)	0.00040516	0.000506	0.000675	0.001013	0.002026
No. of Holes	9	10	15	12	16
Area of one hole	4.50178E-05	5.06E-05	4.5E-05	8.44E-05	0.000127
Diameter(m)	0.007572817	0.008032	0.007573	0.01037	0.0127
Diameter(inches)	0.298141801	0.316227	0.298142	0.408247	0.499999
Drill Bit to be used	19/64"	5/16"	19/64"	13/32"	1/2"

Compost Packing Method

$$\text{Average Diameter of Tank} = \frac{183+196}{2} = 189.5\text{cm} = 1.89\text{m}$$

$$\text{Volume of 10cm thick compost} = \frac{\pi}{4} * 1.89^2 * 0.1 = 0.28 \text{ m}^3$$

$$\text{Desired Density} = \frac{800\text{kg}}{\text{m}^3}$$

$$\text{Mass of compost to be added every 10cm} = \frac{800\text{kg}}{\text{m}^3} * 0.28\text{m}^3 = 224.4 \text{ kg}$$

$$\text{Average mass in one shovel} = 2.9\text{kg}$$

$$\text{No. of Shovels per 10cm} = \frac{224.4 \text{ kg}}{2.9 \text{ kg}} = 77$$

Appendix B



Figure 1: Initial Housing Construction



Figure 2: Spray Foam Insulation



Figure 3: Installation of Flowmeters



Figure 4: Pressure Testing



Figure 5: Gas distribution system



Figure 6: Outlet pipe re-entering the housing

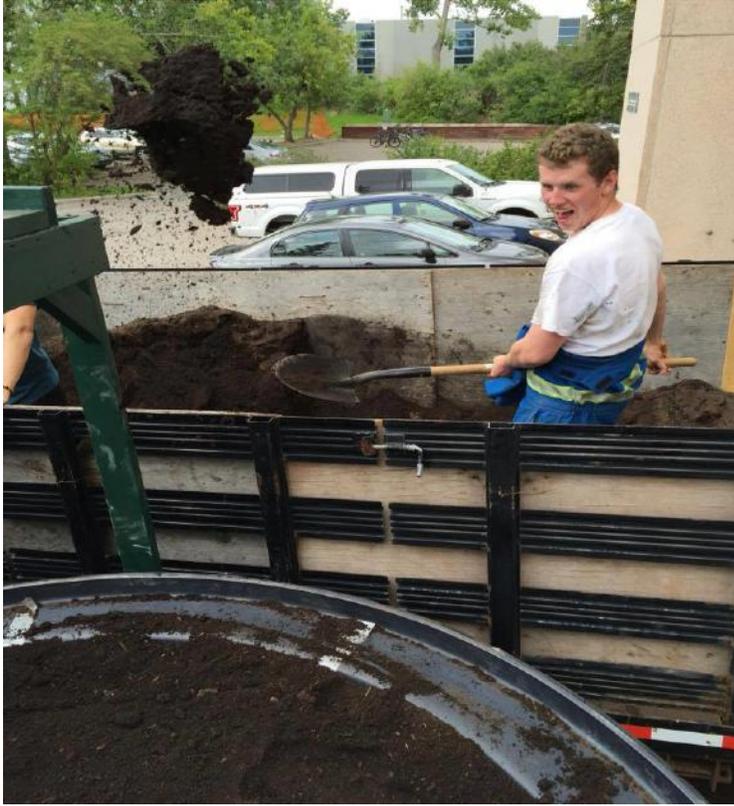


Figure 7: Shoveling compost into the MBF



Figure 8: Testing for uniform flow distribution



Figure 9: Packed MBF



Figure 10: Silicone and cover pipe insulation



Figure 11: Use of straps and a spreader bar to lift the unit



Figure 12: Transportation



Figure 13: Placing the unit in levelled ground



Figure 14: Installation of 100W 12V solar panels

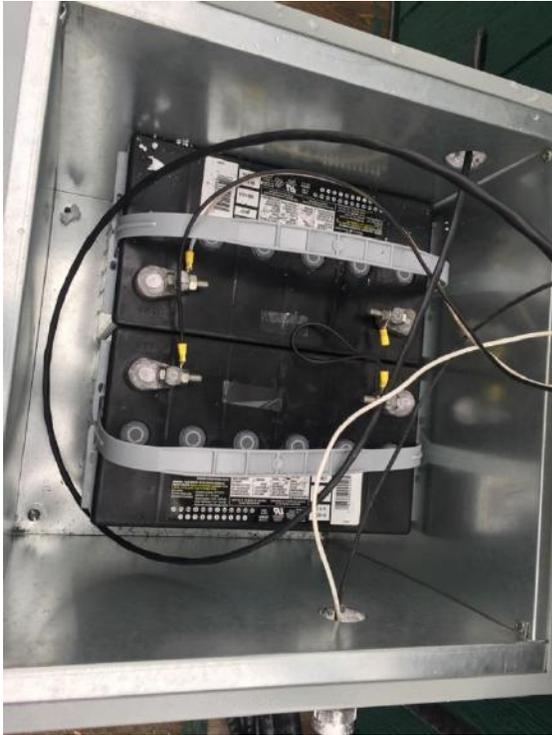


Figure 15: Two 12V 100 Ah AGM Batteries

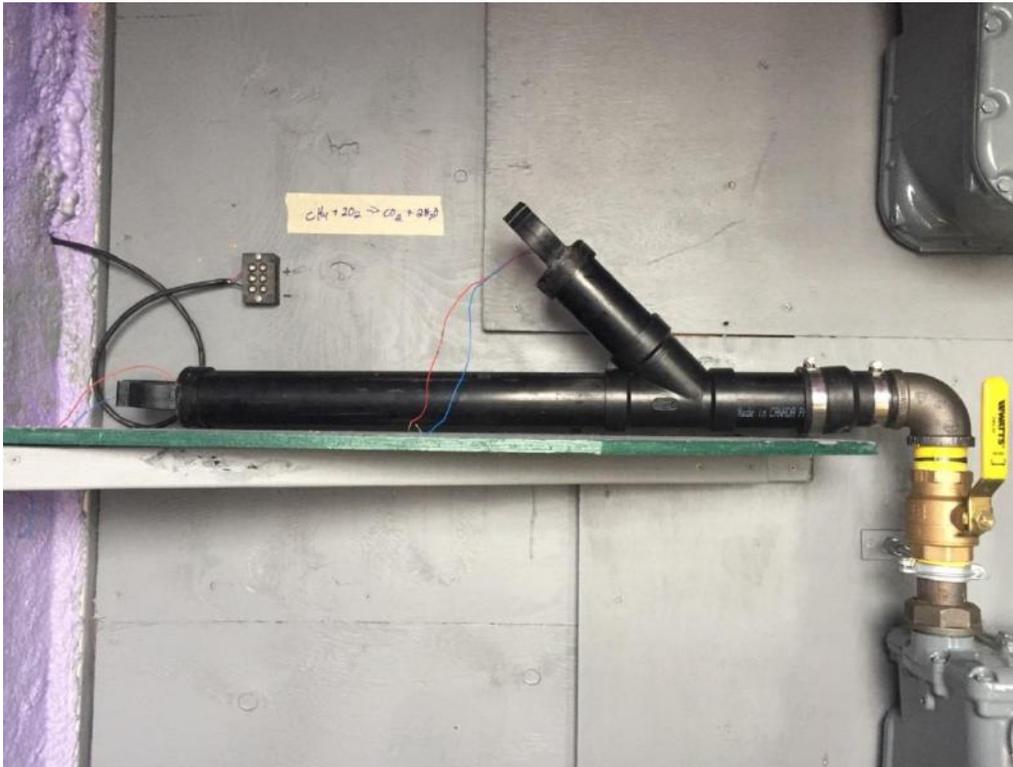


Figure 16: Connecting air blowers to the power supply

Appendix C

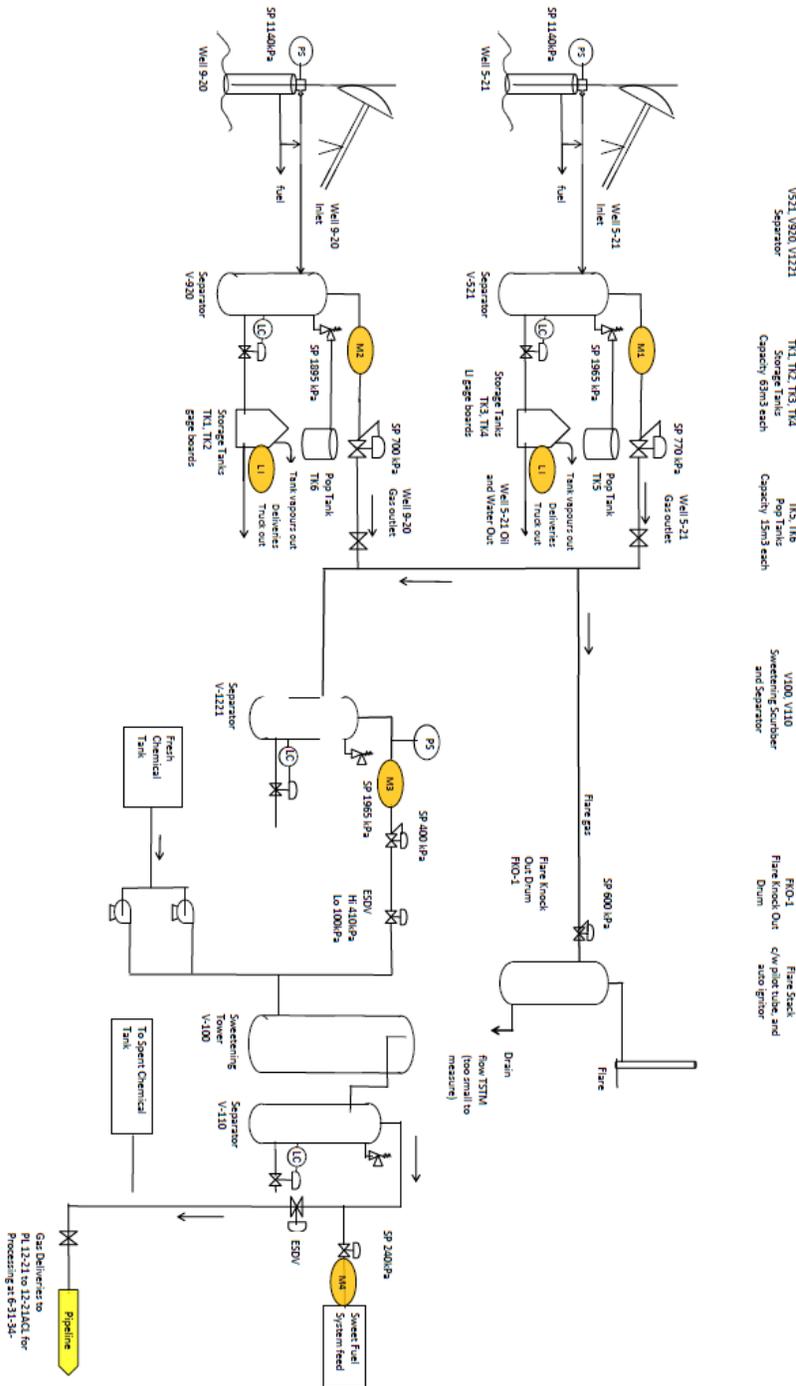
Materials and Equipment

Supplier	Number of parts	Part	Function on prototype
Home Depot	6	2'*6'*8'	Housing construction
Home Depot	20	2'*4'*8'	Housing construction
Home Depot	24	2'*4'*92.5' Studs	Housing construction
Home Depot	8	4*8 sheets of smart board	Housing construction
Home Depot	2	1/2'' *4*8 Plywood	Housing construction (roof)
Home Depot	2	3/4'' *4*8 plywood	Housing construction (allow for wall mount of PD meters)
Home Depot	1	32'' mandoor & door frame	Housing construction
Home Depot	1	Weiser exterior door lockset	Housing construction
Home Depot	1	30'' * 30'' sliding window	Housing construction
Home Depot	11	1*4*8 pressure treated	Housing construction (building trim)
Home Depot	3	Boxes of shingles	Housing construction
Home Depot	1	Box of 500 roofing nails	Housing construction
Home Depot	1	Large Box of #8 by 3' green wood screws.	Housing construction
Home Depot	2	Hunter green paint	Aesthetics for housing, stop spray foam on tank from deteriorating from UV light.
Home Depot	1	Grey paint	Aesthetics for the inside of the housing
Home Depot	3	Closed cell insulating tape 3/8'' * 3/4'' * 10'	Seal on tank
Home Depot	5	Copper insulation foam tubing	Seal on tank
Home Depot	2	9' length grade 70 chains	Lift system
Home Depot	4	Galvanized steel hooks (4400 lbs)	Lift system
Home Depot	8	Ratchet straps	Seal on tank
Rona	3	8' length 2'' ABS pipe	Piping system into tank and distribution system
Home Depot	2	2'' * 2'' rubbing coupling	At start of distribution system and at the exit line.
Home Depot	2	2'' * 1 1/2 '' rubbing coupling	To tie ABS to steel
Home Depot	1	2'' 90 degree fitting	ABS piping system inside housing.
Home Depot	5	2'' T fitting	Distribution system
Home Depot	7	2'' encaps	1 for air blower 6 for distribution system
Beyond Foam		Closed cell 2 lbs 2 inches of foam	Maintain temperature inside the bio filter and heat the housing
Josh the Gas fitter		Steel piping (1 1/2 inch and 1 inch pipe) and installation	Housing piping and gas monitoring
Barchard Engineering	3	PD meters AC 250, 2x AC 630	Meters to measure total flow and flow rate
Mouser Electronics	2	Blower (RL48 – 934175)	Blow air through the air line.

Prairiestorm Controls	1	24x24 12V Catadyne Heater	Heating for the housing
Prairiestorm Controls	2	12V 100 Ah AGM Battery	Power supply for the air blowers
Prairiestorm Controls	2	100W 12V Solar Panels	Power supply for the air blowers
Lakewood Systems	2	RX8K – 8 Channel Data Logger	Data logger for temperature sensors
Lakewood systems	8	TP10K20M Temperature Probe	Temperature sensor
Lakewood Systems	7	RXTP20 Temperature Probe	Temperature sensors

Appendix D

Flow Plan for the Site



V521, V920, V1221 Separator	T11, T12, T13, T14 Storage Tanks Capacity 63m3 each	T15, T16 Pop Tank Capacity 15m3 each	V100, V110 Sweetening Scrubber and Separator	FKO-1 Fire Knock Out Drum	Fire Stack 4" w pilot tube, and auto ignitor
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Bering Exploration Company ULC
 Sullivon Lake 12-21-34.13W4M
 Mill Well Battery 12-21-34-13W4
 EPD Well Battery 12-21-34-13W4
 EdLawrence TR24-002 Rev. 3 2014-09-24

Gas Delivered to
 PL 12-21 to 12-21AC for
 Processing at 6-31-38-

9.8 Progress Report: Methane Biofiltration by Methanotrophic Bacteria Using Biologically Stable Filter Materials

Project Name: *Methane biofiltration by methanotrophic bacteria using biologically stable filter materials*

Project Conducted By: Helen La

Reporting Period: *Jan-Dec 2016*

Summary:

Biofilters are a cost-effective method for the treatment of methane emissions through the oxidation activity of methanotrophs. Common materials for biofilters include compost, soil, and green waste due to their inherent physical and bio-chemical properties. Amongst these, compost and green waste inevitably biodegrade, which affects their long-term performance. This study investigates the use of biologically-stable materials including lava rock and biochar as alternative biofilter materials. An unreplicated 2^3 factorial design was implemented to develop a first-order relationship of the medium mixture, nitrogen content (i.e. urea-free), and moisture level to methane oxidation activities. Nitrogen was identified as the sole significant factor controlling methane oxidation capacity but quadratic effects may be important due to the detection of curvature. At peak activities *Methylobacter* and *Methylomicrobium* are the most dominant methanotrophs present in all treatments. Results indicate lava rock may be a promising biofilter material and can maintain high methane oxidation capacities.

Progress of Work:

Background:

Methane (CH_4) is a potent greenhouse gas (GHG) with a global warming potential of 34 over a 100 year time horizon as compared to CO_2 (IPCC 2013). In particular, CH_4 emissions from landfills contribute the largest GHG emission from the waste sector and contribute approximately 500 to 800 $\text{Mt-CO}_2\text{e}\cdot\text{yr}^{-1}$ (Bogner et al. 2007). When the CH_4 mixing ratio of landfill gas (LFG) is greater than 20-25% and is generated at a minimum rate of 10-15 $\text{m}^3\cdot\text{hr}^{-1}$, combustion methods are economically feasible; however, during a majority of the lifetime of a landfill the LFG production is low and the quality is poor, requiring alternative treatment methods (Haubrichs and Widmann 2006).

The use of methanotrophs in mitigating CH_4 emissions in traditional landfill soil covers has been established. Numerous studies have demonstrated the application of organically-labile materials such as compost for high CH_4 oxidation capacities but that would eventually taper off to a lower level of performance (Huber-Humer et al. 2011). There is some evidence that non-labile materials may provide better CH_4 oxidation efficiencies than their labile counterparts (Nikiema and Heitz 2009). In this study, the performance of CH_4 oxidation activities was compared between mixtures of biochar and lava rock. Biochar is an organic biomass that has undergone thermochemical conversions through pyrolysis, gasification, or by hydrothermal carbonization in an O_2 -limited environment. The high-temperature treatment creates a biologically-stable material (Zhang et al. 2015). Of particular interest in the use of biochar as a methanotrophic growth medium is the adsorption capacity for CH_4 , high water-holding capacity (WHC), and the potential to provide enhanced aeration, porosity, and surface area (Reddy et al. 2014). Lava rock—typically used in landscaping—was chosen as a potential bulking agent due to its inherent porous nature. The other factors tested include the WHC of the mixture and nitrogen amendments with a urea-free fertilizer. Previous batch experiments using a urea-based fertilizer (data not shown) demonstrated lack of CH_4 oxidation activity with increased nutrient additions and $\text{NH}_3(\text{g})$ could be detected daily during air flushing. $\text{NH}_3(\text{g})$ can act as a competitive inhibitor of methane monooxygenase or can result in the gradual accumulation of toxic oxidation byproducts of hydroxylamine and nitrite (Bedard and Knowles 1989). The objective of this study is to determine the CH_4 oxidation capacity of a biologically-stable medium in the form of lava rock and biochar that has been supplemented with nutrients and water and to determine whether a direction of optimum can be identified using a 2^3 factorial statistical design

Materials and methods:

Media Material

Two biologically-stable medium were studied in an unreplicated 2³ factorial design: 3-20 mm diameter lava rock (Burnco, Calgary, Alberta) and wood-based biochar (Diacarbon, Burnaby, British Columbia). The biochar feedstock is primarily sawdust that has undergone fast pyrolysis at 550°C with a residence time of 20-30 minutes. The overall size and morphology of the resulting biochar is similar to the untreated sawdust and ranges from 0.5 to 1 cm slivers. Compost acquired from East Calgary Landfill (ECL), in Calgary, Alberta in July 2015 and sieved through a no. 8 sieve (2.38 mm), was used as a starter material for methanotrophs.

Physical and Chemical Characterization Testing

Moisture content (ASTM D2216-10 2010), WHC (ASTM D2980-04 2010), and bulk density were determined for the ECL compost, biochar, and lava rock. The bulk density was determined by weighing a 300 mL filled beaker of the corresponding medium. Although the bulk density was not performed according to a standardized method, an error of <1% was confirmed by measuring the mass of water. ALS environmental laboratories analyzed the compost samples for total and bioavailable nitrogen and phosphorus content.

Batch Oxidation Experiments

Three factors were studied in a 2³ unreplicated factorial design to determine their potential interactive effects on methane oxidation rate (MOR) in batch oxidation experiments:

- mixture ratio (by volume) of lava rock and biochar (9:1 lava rock - biochar and 100% biochar);
- water content of the mixture (20% and 60% of WHC); and
- nutrient level (80 ppm-N and 401 ppm-N, or 0.2x and 1x the bioavailable nitrogen-level).

To test for curvature, three center-points were included. Direct comparison between treatment combinations were performed on a volumetric basis due to the large mass difference between the lava rock and biochar. Previous batch experiments with a single medium type have used a ratio of 10 g of medium or 1 to 1.5 cm of overall depth in 250-mL amber glass bottles. A diameter to depth ratio for 10 g of compost that would occupy a 250-mL amber bottle was calculated to determine an appropriate depth of medium for the 1-L amber bottles used in this study—which corresponded to 2.5 cm. Compost was added at 10% (v/v) into each bottle in order to provide a source of starter bacteria. To attain the target water content for the treatment combination, a predetermined amount of distilled water was added to each batch after considering the moisture content and the WHC of the medium mixture. All bottles and caps were thoroughly cleaned and autoclaved at 230°C prior to use.

Nutrients were added to obtain a nitrogen concentration of 80 mg-N/kg compost (dw) or 401 mg-N/kg compost (dw) for the low and high level, respectively, in 2.5 cm of compost (or ~166 mL by volume) after compensating for the amount of bioavailable nitrogen level in the 10% (v/v) of compost that is added into each of the batches. For a volume of 166 mL, 80 mg-N/kg compost (dw) and 401.5 mg-N/kg compost (dw) is equivalent to 38.3 mg-N/L of medium and 191.5 mg-N/L of medium, respectively. A urea-free fertilizer (Plant-Prod™) at a N:P:K ratio of 18:6:24 with pH buffer (4% bicarbonate) was used as nutrient. As the phosphorus in the fertilizer is actually in the form of P₂O₅ (phosphorus oxide), the actual elemental nitrogen to phosphorus ratio is 18:2.6. The nitrogen in the fertilizer is in two forms: nitrate (11.8%) and ammoniacal nitrogen (6.2%).

Following air flushing, each amber bottle was resealed by lining the rim with Teflon® tape, enclosed with a polypropylene cap and Teflon®/silicone liner and septum, and bound with electrical tape as an additional sealant. To serve as a control, a blank bottle was similarly prepared but without the addition of medium, water, or nutrients in order monitor CH₄ leakage. The bottles were placed in an incubator-shaker (G24, New Brunswick Scientific Co, Inc.) and set at ~30°C and shaken at the lowest speed setting in

order to ensure that the CH₄ gas was well-mixed and to reduce the opportunity of it separating from the air phase due to differential density. A photo of the batch experimental setup is provided in Figure 1.



Figure 1. Batch experimental set-up of the amber jars used for the 2³ factorial design.

A CH₄ headspace mixing ratio of approximately 5% (v/v) was prepared by withdrawing ~50 mL of air from the headspace and replacing an equivalent volume with CH₄ gas (99.9% purity, Praxair, Calgary, Alberta). Gas samples were withdrawn from each bottle through the septum using a 5-mL luer lock syringe and non-coring needle fitted with a two-way stainless steel stop-cock (Model 1005 SL SYR, Hamilton®, Reno, Nevada). A fresh layer of silicone was immediately applied to the septum in order to prevent gas leakage in between measurements.

Gas Chromatography Analysis

Following headspace sampling, each gas sample was immediately injected into a Varian CP4900 micro gas chromatograph (Varian Canada Inc.) equipped with a thermal conductivity detector (TCD). Gas concentrations were analyzed on “Galaxie Chromatography Data System” (Version 1.9.3.2, Varian Inc.). The micro GC is outfitted with two columns including a 10 m long MolSieve (MMS) and a PoraPLOT U (PPU), respectively, with the following specifications: injector temperature of 110°C and 100°C respectively; an 80°C oven temperature for both columns; and the carrier gas pressure is set at 29 psi and 40 psi, respectively. Helium is the carrier gas. A minimum of three gas measurements were taken over the course of 6.5 ± 0.5 hours per day in order to calculate the CH₄ oxidation rate each day. Prior to making measurements, the GC was calibrated using two ultra-high purity CH₄ certified standards at 1 and 80% CH₄ (v/v) (Praxair, Calgary, Alberta), respectively.

Data Analysis

Periodic gas sampling from each treatment combination was conducted until the CH₄ mixing ratio had depleted to <1% (v/v). A plot of CH₄ concentration (in units of $\mu\text{mol CH}_4 \cdot \text{mL}^{-1}$ of medium) with time was prepared to obtain the overall CH₄ oxidation rate for the respective incubation period. The 5% of injected CH₄, generally, depleted within 4 to 24 hours. For the treatment combinations that required more than 7 hours and less than 24 hours to deplete, it was not possible to acquire an accurate overall oxidation rate (i.e. the zero-order decay rate constant) and therefore, the trends depicted in this paper are meant to represent the overall oxidation behavior for each treatment combination rather than to provide accurate oxidation assessments.

The CH₄ oxidation potentials between treatment combinations were compared by evaluating the area of each curve. To smoothen the curves, the data were processed using a 5-point moving average and the area was then assessed using the discrete method.

DNA Extraction and Illumina Sequencing of 16S rRNA Gene

Approximately 1.5 ± 0.5 g of medium was obtained from each batch for further microbiological assay, up to 6 times, over the course of the experimental observation period of up to 100 days. DNA was extracted

from 0.2-0.8 g of the medium mixture using the FastDNA SPIN Kit for Soil (MP Biomedicals) following the manufacturer's instruction with some modification. Specifically, an additional purification step using 5.5 M guanidine thiocyanate (GTC) was employed in the washing step (Knief et al. 2003) due to the difficulty of obtaining high purity DNA from the medium mixture. The DNA concentration was determined by using a Qubit Fluorometer with a Quant-iT™ dsDNA HS Assay Kit (Invitrogen) and the purity of DNA was determined by using Nanovalve Plus™ (GE Healthcare).

The hypervariable V4 region of the 16S rRNA gene were amplified according to the Illumina protocol (Illumina reference guide Part # 15044223 Rev. B). The Illumina MiSeq system (Illumina, Inc.) was used to sequence 16S rRNA gene amplicon using standard protocols. Up to 96 samples were multiplexed on a single run. Communities were analyzed using the QIIME software. Operational taxonomic units (OTUs) were clustered at 97% identity using UCLUST, and taxonomic identifications obtained via BLAST against the Silva v. 108 reference database (Caporaso et al. 2010).

Results and Discussion:

Physical and Chemical Characterization

Table 1 provides the physical properties of biochar, lava rock, and compost. The manufacturer treats the biochar with a small amount of water following pyrolysis in order to reduce the amount of dust produced. For consistency, only one batch of biochar was used for these set of experiments. The ECL compost had dropped in moisture content from a previous value of 36.6% when it was freshly obtained from the landfill to 30.8% at the time of the experiments. The compost displayed a WHC of 153% (dw) and is less than half the amount of the biochar. As neither the biochar nor the lava rock is a significant source of nutrients for the methanotrophs (Zhang et al. 2015), the compost was analyzed for both bioavailable and total nitrogen (401.5 mg/kg and 13,750 mg/kg, respectively) and phosphorus (609.5 mg/kg and 2275 mg/kg, respectively) at ALS environmental laboratories (Calgary, Alberta).

Table 1. Physical properties of biochar, lava rock, and compost.

Medium	Bulk density (g/mL, ww)	Moisture Content (% ww)	Water holding capacity (% dw)
Compost	0.67 ± 0.005	30.8 ± 3.1	153 ± 7
Lava rock	0.80 ± 0.005	0.1 ± 0.03	12 ± 3
Biochar	0.21 ± 0.005	6.0 ± 0.06	330 ± 12

The ratio between total nitrogen and total phosphorus is approximately 6:1 in the ECL compost. As a result, the urea-free fertilizer with an actual elemental N:P ratio of 18:2.6 was chosen for nutrient matching. In addition, this fertilizer contains bicarbonate at 4% (w/w) to serve as a pH buffer and copper at 0.05% (w/w), which is a critical micronutrient for the methanotrophs (Dedysh and Dunfield 2014).

Batch Oxidation Experiments

Figure 2 provides the MORs over time for the 8 treatment combinations. The CH₄ oxidation activities reached a peak value of up to ~3 μmol·mL⁻¹·hr⁻¹ at an initial CH₄ mixing ratio of 5% (v/v) in all treatment combinations; however, the area of each peak is wider (i.e. CH₄ oxidation activities persisted longer) in the treatment combinations at 401 ppm-N—the high-level nutrient treatments. Following a period of marginal oxidation activity, at steady state, all of the treatment combinations, with the exception of treatment combination *ac* (90% lava rock, 10% biochar, 20%WHC, and 401 ppm) began displaying a resurgence in CH₄ oxidation activities. The CH₄ oxidation profiles for the center-point replicates also display similar patterns of a resurgence in CH₄ oxidation activity following a decline in peak activity (Figure 3). However, unlike the other treatment combinations, the center-points maintained oxidation rates of ~0.5 μmol·mL⁻¹·hr⁻¹ following the initial peak—and, therefore, displayed a higher level of CH₄ oxidation activity than the other treatment combinations at steady state.

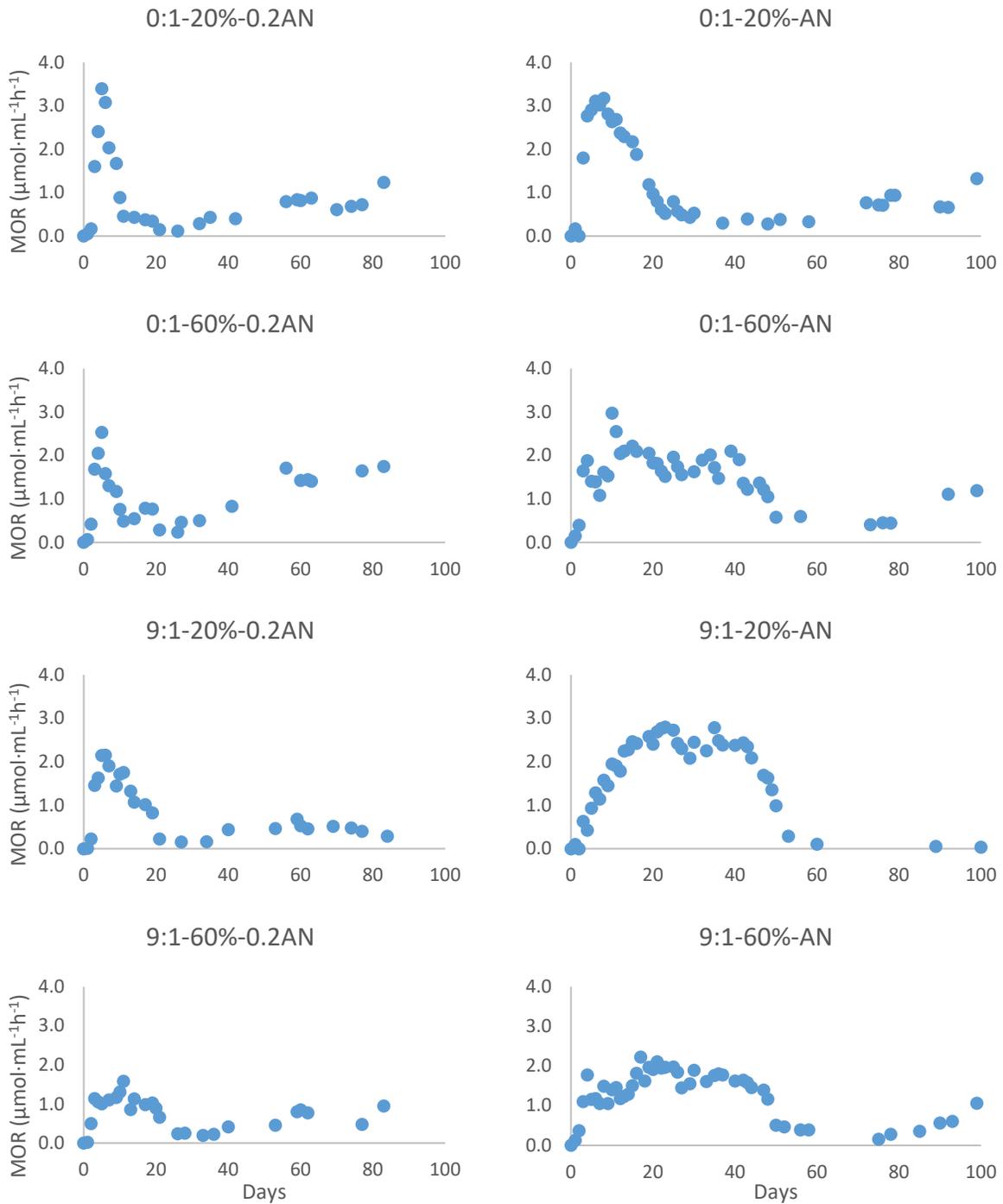


Figure 2. Methane oxidation rate profiles for the assigned treatment combination from the 2³ factorial experimental design. Treatment combinations are labelled as their mixture ratio (lava rock:biochar), the % WHC, and the bioavailable nitrogen content (0.2AN = 80 ppm and AN = 401 ppm) found in ECL compost.

The resurgence in CH₄ oxidation activity may be attributed to the release of nutrients previously locked up in biomass following endogenous decay and cell lysing. Along the same line, during marginal oxidation

activities at steady state, following peak oxidation, the existing population of methanotrophs go into a period of stasis as a result of the depletion of available nutrients. Further testing will be required to confirm the release of nutrients by analyzing for the nitrogen content in these samples. Additionally, the recent resurgence in CH₄ oxidation activity coincides with the visible loss of moisture in all of the treatment combinations. Daily air flushing of the samples may have increased the evaporation rate from the samples—especially since the batches were run for up to 100 days. The decline in CH₄ oxidation observed in the center-points on ~ Day 20 may have been a result of the mixture becoming over-saturated in moisture given that the oxidation of CH₄ produces water. Since the center-points are comprised of 45% lava rock and 55% biochar, the amount of water added to reach the 40% WHC of the mixture likely had a negative effect on MORs as lava rock does not retain water. Therefore, as the water was allowed to evaporate from the system, a resurgence in CH₄ oxidation activities was observed. This may have also been the case for the treatment combinations at the high level water addition (i.e. 60% WHC). However, both treatment combination (1) (100% biochar, 20%WHC, and 80 ppm) and c (100% biochar, 20%WHC, and 401 ppm) displayed a revival in CH₄ oxidation activity even though both contained minimal water addition; therefore, it is more likely that the revival in activity was due to the release of nutrients back into the system. A second rationale for the resurgence may be attributed to the transformation of methanotrophs that depend on the presence of mineral nitrogen to methanotrophs that can fix N₂. Auman et al. (2001) found N₂-fixing capabilities are widespread amongst methanotrophs after detecting the presence of *nifH* gene fragments (which encodes the iron protein of nitrogenase) and acetylene reduction capabilities in both Type I and Type II strains. A survey of the 16 genomes of *Methylobacterium* and *Methylobacter* (the major genera detected in our treatments, see below) presently available in the Integrated Microbial genomes database (Markowitz 2014) indicates that 12 of these have *nifH* genes. However, whether the particular methanotroph strains active in our batch experiments are capable of fixing N₂ is beyond the scope of this study. A third potential possibility for the fluctuation in oxidation activities may be a result of grazers preying on the methanotrophs. Further tests will be required to determine whether predators are in the system.

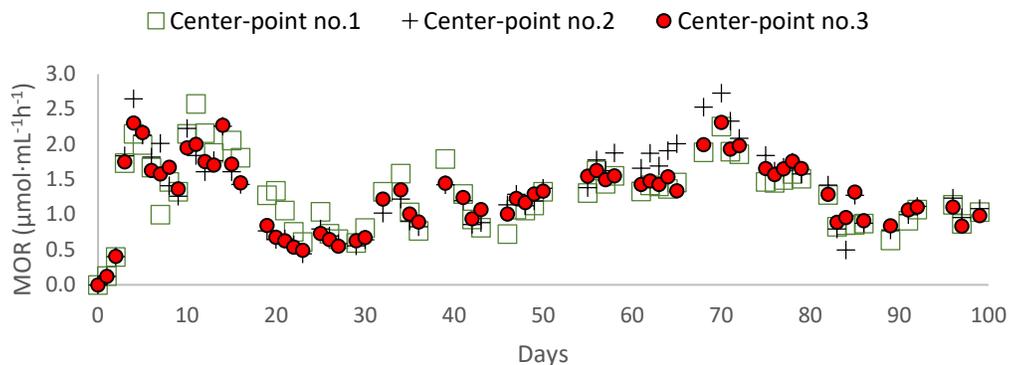


Figure 3. Center-point profile replicates for methane oxidation.

The integrated area of each peak provides the total CH₄ oxidation for each treatment combination and is presented in Table 2. Overall, the 1st and 2nd highest overall oxidation activity was observed with treatment combination *ac* (90% lava rock, 10% biochar, 20%WHC, and 401 ppm) and *bc* (100% biochar, 60%WHC, and 401 ppm) at 2381.68 μmol·mL⁻¹ and 1949.31 μmol·mL⁻¹, respectively. In other words, lower levels of water are preferred for a primarily lava rock system and more water is preferred in a 100% biochar system. Lava rock has a minimal WHC of 12% (dw basis) as compared to biochar with a WHC of 330% (dw basis)—making lava rock more susceptible to variations in moisture content. Under lower level nitrogen conditions and regardless of the media mixture combination, CH₄ oxidation activities were higher at lower levels of water addition (i.e. 20% WHC). Conversely, under higher levels of nitrogen addition, the 100% biochar mixture oxidized nearly two times as much CH₄ under the higher level moisture conditions (i.e. 60% WHC) than at the lower level moisture conditions (i.e. 20% WHC). This observation is consistent with the higher retention capacity of biochar for water and its ability to buffer against varying moisture settings.

Moisture content is an important abiotic factor in the regulation of methanotrophic activity in CH₄ biofiltration and biocover systems and serves to facilitate the transport of nutrients and gaseous components from the environment to the methanotrophic microorganisms. Under very low water contents, bacteria such as methanotrophs suffer from osmotic stress, which limits their activity (Bender and Conrad, 1995). This suggests that an optimum moisture content exists for a particular CH₄ biofilter medium. Too much moisture may result in a diffusion-limited system for CH₄ transfer into the biofilm. In fact, molecular diffusion of CH₄ in the liquid phase is 10⁴ times slower as compared to the air phase (Whalen et al. 1990). This can be better appreciated by the fact that the Ostwald solubility coefficient of CH₄ is only about 3% (v/v) at 17°C. Therefore, the moisture content of a biocover material needs to be optimized in order to provide sufficient moisture for methanotrophic activity but still allow adequate O₂ and CH₄ diffusion.

Table 2. Overall methane oxidation activity per treatment combination based on the unreplicated 2³ factorial design on mixture ratio, by volume, of lava rock: biochar; % water-holding capacity; and nitrogen content. All treatment combinations were inoculated with 10% (v/v) compost. Three center-points were included to test for curvature.

Treatment combination	Factor A Mixture (lava rock:biochar)	Factor B % WHC	Factor C Nitrogen (ppm)	Methane oxidation (μmol·mL ⁻¹)
(1)	0:1	20	80	397.97
<i>a</i>	9:1	20	80	593.14
<i>b</i>	0:1	60	80	297.32
<i>ab</i>	9:1	60	80	531.20
<i>c</i>	0:1	20	401	1015.53
<i>ac</i>	9:1	20	401	2381.68
<i>bc</i>	0:1	60	401	1949.31
<i>abc</i>	9:1	60	401	1774.35
Center-point 1	4.5:5.5	40	241	810.38
Center-point 2	4.5:5.5	40	241	747.15
Center-point 3	4.5:5.5	40	241	738.99

As a full ANOVA statistical analysis cannot be performed on an unreplicated factorial experimental design, due to the lack of an error term, the center-points were used as a source of error. The ANOVA indicates that the nitrogen-level is the only factor that is significant ($F(1,3) = 38.54$, $p < 0.05$). However, significant curvature is detected in the ANOVA ($F(1,3) = 177.11$, $p < 0.05$), and the underlying assumption of linearity of the 2³ factorial analysis does not hold. An important note to make here is that the CH₄ oxidation activity reported in Table 2, and also used in the ANOVA, is based on the 1st peak only. As result, there are likely second-order effects and the current experimental design must be augmented by the central composite model in order to map out the surface of the response area and identify an appropriate treatment combination that can be tested under pilot-scale conditions through laboratory-scale column studies.

Microbial Community at Peak Methane Oxidation Activity

DNA extraction and Illumina MiSeq sequencing of 16S rRNA gene amplicons was performed for all treatment combinations and including the center-point replicates at peak methane oxidation activity (Figure 2). A total of 759,393 paired-reads were collected from the Illumina sequencing runs. And a total of 1,367 OTUs were identified for 20 samples. At the peak of CH₄ oxidation activity (i.e. Day 6 for the low-level nutrient treatment combinations; Day 12 and Day 30 for the high-level nutrient treatment combinations; and Day 12 for the center-points), only gammaproteobacterial methanotrophs (i.e. Type I methanotrophs) were detected from all of the treatment combinations. In general, at peak activity all treatment combinations were dominated by *Methylobacter* followed by *Methylomicrobium*. For instance, in treatment combination *ac* (90% lava rock, 10% biochar, 20%WHC, and 401 ppm), which showed the highest CH₄ oxidation activity (Table 2), the relative abundances of *Methylobacter* and *Methylomicrobium* (relative to the entire bacterial community) on Day 12 and Day 30 ranged from 12.5 – 12.4% and 2.5 – 5.3%, respectively. Conversely, treatment combination *b* (100% biochar, 60%WHC, and 80 ppm), which showed the lowest CH₄ oxidation activity, non-methanotrophic bacteria including *Flavobacterium*,

Bdellovibrio, and *Pseudomonas* predominated the microbial community (5.1 – 7.9%) followed by *Methylobacter* and *Methylomicrobium* (4.9 and 3.3%, respectively). The predominant methanotroph species detected were not greatly affected by the experimental treatments (N, packing materials, water). This suggests that, at least in the time frames considered, the observed differences in CH₄ oxidation across the treatments were not connected to shifts in the methanotrophic community, but rather to physical factors like diffusion. Across longer incubation times the indigenous methanotrophic communities may change, however.

Conclusions:

This study investigated the influence of using lava rock and biochar mixtures, nitrogen content, and water content (based on the WHC of the mixture) on the CH₄ oxidation activity of methanotrophs in batch experiments. A 2³ factorial design identified nitrogen-level as the only significant factor; however, significant curvature was detected based on the three center-point replicates and, therefore, a first-order model could not be developed. A central composite design will be implemented to augment the first-order model from this study in order to better understand the response surface and to identify optimal conditions to support CH₄ oxidation. Over the course of the experimental period, which ranged from 84 to 100 days, all treatment combinations displayed a period of peak activity followed by a period of marginal, steady state, activity. The peak period lasted longer for all of the higher-level nutrient treatment combinations. *Methylobacter* and *Methylomicrobium* were the dominant methanotrophic genus at peak CH₄ oxidation activities regardless of treatment combination. It would be interesting to observe for potential methanotrophic community changes further along during the experimental period where bioavailable nitrogen may have been limiting in the system. A resurgence in CH₄ oxidation activity was observed for all treatment combinations with the exception of treatment combination *ac* (90% lava rock, 10% biochar, 20%WHC, and 401 ppm) after a period of time and is believed to be due to the subsequent release of bioavailable nitrogen following endogenous decay of the methanotrophic biomass, improved diffusion of CH₄ as the water content is lost from the system, or the subsequent die-off of grazers. Surprisingly, this study provided evidence that lava rock may be a suitable biocover material and can outperform biochar in CH₄ removal efficiency.

Future Work:

In the next phase of this research, column experiments will be implemented to determine whether lava rock and biochar packing materials can maintain high oxidation rates that are comparable to compost. A 1:1 mixture of compost and the inert packing medium will be tested first with a step-wise increase in CH₄ inlet flow rate. Subsequently, a predetermined amount of compost will be removed and replaced with the inert packing medium where the effects of nutrients and water addition on oxidation rates will be examined.

Outputs and deliverables:

Peer-Reviewed Conference Proceeding:

La, H. (primary presenter), Hettiaratchi, J.P.A., Achari, G., *Effect of nutrients on methanotrophic oxidation activity in batch experimental studies*, in: CSCE, 4th International Environmental Specialty Conference, Canadian Society for Civil Engineering, London, Ontario, 2016, pp. 1-9 (ENV 610).

Bartholameuz, E.M., Hettiaratchi, J.P.A., **La, H.** (primary presenter), *Analysis of reaction kinetic parameters in enzyme catalysed aerobic waste degradation*, in: CSCE, 4th International Environmental Specialty Conference, Canadian Society for Civil Engineering, London, Ontario, 2016, pp. 1-9 (ENV 623).

La, H. (primary presenter) Hettiaratchi, J. P. A., Achari, G., Dunfield, F. D., Kim, J., *Investigation of biologically stable methane biofilter medium for alternative landfill covers*, in: 2017 Geotechnical Frontiers Conference, Life Cycle Assessment & Sustainable Engineering, Orlando, Florida, USA, 2017, Accepted.

Poster Presentation:

La, H., Methane oxidation by methanotrophs in biologically-stable materials, oral presentation at: Canadian Prairies and Northern Section Annual Conference and General Meeting, Air and Waste Management Association, Calgary, Alberta, 2016.

References:

ASTM D2216-10. 2010. Standard Test Methods for Laboratory Determination of Water Content of Soil and Rock by Mass. ASTM International, West Conshohocken, PA.

ASTM D2980-04. 2010. Standard Test Method for Volume Mass, Moisture-Holding Capacity, and Porosity of Saturated Peat Materials. ASTM International, West Conshohocken, PA.

Auman, A.J., Speake, C.C., and Lidstrom, M.E. 2001. nifH sequences and nitrogen fixation in type I and type II methanotrophs. *Applied and Environmental Microbiology* **67**(9): 4009-+. doi: 10.1128/aem.67.9.4009-4016.2001.

Bedard, C., and Knowles, R. 1989. Physiology, biochemistry, and specific inhibitors of CH₄, NH₄⁺, and CO oxidation by methanotrophs and nitrifiers. *Microbiological Reviews* **53**(1): 68-84.

Bogner, J., M. Abdelrafie Ahmed, C. Diaz, A. Faaij, Q. Gao, S. Hashimoto, K. Mareckova, R. Pipatti, and Zhang, T. 2007. Waste Management, In *Climate Change 2007: Mitigation. In Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Edited by B. Metz and O.R. Davidson and P.R. Bosch and R. Dave and L.A. Meyer*, Cambridge, UK and New York, NY, USA.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Tumbaugh, P.J., Walters, W.A., Widmann, J., Yatsunencko, T., Zaneveld, J., and Knight, R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**(5): 335-336. doi: 10.1038/nmeth.f.303.

Dedysh, S.N., and Dunfield, P.F. 2014. Cultivation of methanotrophs. *In Springer Protocols Handbooks: Hydrocarbon and Lipid Microbiology Protocols. Edited by T.J. McGenity*. Springer-Verlag, Berlin Heidelberg.

Haubrichs, R., and Widmann, R. 2006. Evaluation of aerated biofilter systems for microbial methane oxidation of poor landfill gas. *Waste Management* **26**(4): 408-416. doi: <http://dx.doi.org/10.1016/j.wasman.2005.11.008>.

Huber-Humer, M., Tintner, J., Böhm, K., and Lechner, P. 2011. Scrutinizing compost properties and their impact on methane oxidation efficiency. *Waste Management* **31**(5): 871-883. doi: <http://dx.doi.org/10.1016/j.wasman.2010.09.023>.

Illumina reference guide Part # 15044223 Rev. B. 16S Metagenomic Sequencing Library Preparation. *In Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System*. Illumina. p. 28.

IPCC. 2013. Climate Change 2013: The Physical Science Basis. *In Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Edited by T.F. Stocker and D. Qin and G. Plattner and M.M.B. Tignor and S.K. Allen and J. Boschung and A. Nauels and Y. Xia and V. Bex and P.M. Midgley*, Cambridge, UK and New York, NY, USA. p. 1535.

Knief, C., Lipski, A., and Dunfield, P.F. 2003. Diversity and activity of methanotrophic bacteria in different upland soils. *Applied and Environmental Microbiology* **69**(11): 6703-6714. doi: 10.1128/aem.69.11.6703-6714.2003.

Markowitz, V.M. 2014. IMG/M: Integrated Microbial Genomes & Microbiomes. U.S. Department of Energy (DOE)-Joint Genome Institute.

Nikiema, J., and Heitz, M. 2009. The influence of the gas flow rate during methane biofiltration on an inorganic packing material. *The Canadian Journal of Chemical Engineering* **87**(1): 136-142. doi: 10.1002/cjce.20131.

Reddy, K.R., Yargicoglu, E.N., Yue, D.B., and Yaghoubi, P. 2014. Enhanced Microbial Methane Oxidation in Landfill Cover Soil Amended with Biochar. *Journal of Geotechnical and Geoenvironmental Engineering* **140**(9). doi: 10.1061/(asce)gt.1943-5606.0001148.

Whalen, S.C., Reeburgh, W.S., and Sandbeck, K.A. 1990. Rapid methane oxidation in a landfill cover soil. *Applied and Environmental Microbiology* **56**(11): 3405-3411.

Zhang, H., Voroney, R.P., and Price, G.W. 2015. Effects of temperature and processing conditions on biochar chemical properties and their influence on soil C and N transformations. *Soil Biology & Biochemistry* **83**: 19-28. doi: 10.1016/j.soilbio.2015.01.006.

9.9 Summary: International Workshop on Greenhouse Gas Emission Reductions
Using Biological Method



Summary Report

International Workshop on Greenhouse Gas Emission Reductions Using Biological Methods – 2015

August 27 – 28, 2015 8:30 am – 4:30 pm

Introduction

Although a number of technologies are available for mitigation of greenhouse gas (GHG) emissions, their selection and field implementation in a cost-efficient manner is still challenging. Recent laboratory and field research has shown that application of biological methods may provide an economical solution to this challenge. Such bio-based methods include methanotrophy; the conversion of methane into carbon dioxide by means of methane-oxidizing bacteria. This method is utilized in methane biofilters (MBFs) and landfill biocaps to address emissions from low volume point sources and area sources, respectively. In addition, GHG emissions associated with waste could be controlled by the application of landfill bioreactor/biocell technology. Since methane is a GHG with a global warming potential of 25 (compared to a global warming potential of 1 for carbon dioxide), methane bio-oxidation using methanotrophy may provide industries and municipalities with an excellent opportunity to reduce their GHG emissions in a cost effective manner. The current challenge is to ensure that the knowledge gained from laboratory- and field-based research is utilized in conducting large scale projects to control GHG emissions associated with solid waste disposal, oil and gas production, and agricultural operations. Since GHG emissions and climate change are global issues, a coordinated international effort is necessary to benefit from fundamental and applied research, and the wide-scale application of biological methods.

In response to this situation, the workshop on *Greenhouse Gas (GHG) Emission Reductions Using Biological Methods* was held to gather a select group of key experts and stakeholders who can evaluate, advise, and direct the next stages of field implementation of biological process based technologies to control GHG emissions from municipal and industrial sources. Leading researchers from universities across the world as well as industry professionals such as consultants and policy makers participated in the workshop, which provided a common platform to share knowledge and bridge the gap between university research and industry applications. The workshop was sponsored by CCEMC, MITACS and University of Calgary (international grants program and CEERE).

The Workshop

The workshop was conducted over two days with the focus of achieving the following objectives.

- Day 1 - Session 1A & B: To assess the viability of field implementation of methane biofilter (MBF) projects in terms of technical feasibility, and to identify barriers and solutions.
- Day 2 - Session 2: To assess the current status of knowledge of evapotranspirative (ET) and landfill bio-covers (LBCs).
- Day 2 - Session 3: To assess the GHG mitigation efforts and the potential application of landfill biocells in various parts of the world.

Session I opened with a presentation on bio-GHG program of CCEMC by Dr. Susan Wood-Bohn, followed by presentations focused on fundamentals of methanotrophy and MBF technology. Session 1B concluded with a breakout session in which participants discussed questions around fundamental scientific knowledge of methanotrophy and MBF, and potential methods to apply the scientific knowledge and to optimize MBF system design and operation.

Day One of the workshop continued with presentations on application of methanotrophy in field scale to control point-source low gas volumes in various industry sectors, and on lessons learned from field

application of MBFs thus far. Day One concluded with another breakout session focusing on strategies to promote large-scale field application of methane oxidation technologies. The questions, intended to stimulate group discussions, focused on the advantages of the technology over other competing strategies as well as the barriers against scale-up adaptations. During the discussions, participants shared the experiences and challenges they faced during their research as well as technology deployment. Solutions provided mainly explored the linkages between further required laboratory research and field-scale adaptations and their associated priorities. The topics discussed in each group were then brought to the wider group, and further expanded upon. Summary of the breakout session discussions are available at http://ucalgary.ca/mbf/files/mbf/2015-overview-of-breakout-discussion_v1_aug-27-2015.pdf

The Day Two consisted of two sessions and a number of presentations and two panel discussions on ET/biocover and landfill biocell technology. The Session Two presentations were on ET and landfill biocover projects in Quebec, Alberta and British Columbia, as well as experiences in United States and Australia. These were followed by a panel discussion focusing on limitations of the technologies and potential solutions to address these limitations. The Session Three included a number of presentations on GHG mitigation strategies at municipal, state and national level in various countries. The focus of some of the presentations and the panel discussion was on the experiences of international participants in implementing bioreactor/biocell technologies in their own jurisdictions. All presentations are available on the workshop website at <http://ucalgary.ca/mbf/workshop-2015>

Impact of the workshop

The workshop gave an opportunity to the participants to discuss a topic that has received less attention so far; bio-based technologies to mitigate GHG emissions. The structure of the workshop was such that all participants (about 50) were able to contribute to the discussions. Considering the focus of the workshop was narrow, it was possible to conduct in-depth discussions and knowledge sharing. This allowed raising key questions on bio-based methods and elucidating answers that could form the next phases of research and development, as well as full-scale deployment.

Breakout debriefs and panel discussions were excellent wrap-up to all of the presentations and discussions. One of the key issues identified by the breakout discussion groups was the necessity of developing a protocol that facilitates a more systematic application, monitoring and optimization of field-scale MBFs. The discussions on MBFs and LBCs, however, were more beneficial for attendees who were exposed to a relatively newer application of methanotrophy in which they had limited experience compared to former and current team members of the University of Calgary researchers. Nevertheless, the breakout sessions clearly pointed out to the need to promote MBF technology as a mitigation technique with a high potential.

Overall, the workshop reminded the attendees to conduct future research and development work in a coordinated manner; whether the work is done in North America or Asia. The need for the development of an international research group or a center was noted by a number of participants. Further feedback from individual participants are included at; <http://wcm.ucalgary.ca/mbf/workshop-2015/participants-comments>

A number of graduate students and junior researchers actively participated in the workshop. Key aspects of the workshop organization were assigned to several graduate students and junior researchers funded by CCEMC, MITACS and CEERE. During the two days of the workshop, these junior researchers had access to experienced researchers and practitioners in the field of GHG emission reductions using biological methods, and had the opportunity to learn from, and integrate with, these personnel. The workshop provided them a unique opportunity to network with industry professionals as well as leading professors from the academic world in a familiar and interactive setting. The workshop also enabled them

to gain a comprehensive understanding towards the state-of-the-art of GHG emission estimations, technology applications for GHG emission reductions from all viewpoints. Furthermore, they gained the opportunity to hear from experts on years' worth of research work on MBFs, ET and biocovers, and landfill biocells, and witness how far the technology has progressed, which thereby could give them a better idea of the grand scope of the research they are involved in. The representation of experts from across the world enlightened the students of the challenges faced in other parts of the world. Being an integral part of the workshop organizing committee, the students also gained first hand experience in organizing a global event and developed valuable soft skills.

9.10 Thesis: Investigating the Inhibitory Effect of Acidic pHs on Methane
Biofiltration Technology

UNIVERSITY OF CALGARY

Investigating the Inhibitory Effect of Acidic pHs on Methane Biofiltration Technology

by

Sonya Barzgar

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF Master of Science

GRADUATE PROGRAM IN CIVIL ENGINEERING

CALGARY, ALBERTA

February, 2017

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Abstract

This research was focused on assessing the effect of pH on methane oxidation by methanotrophs in compost biofilters. The batch experiments were run to study the dependency of maximum methane oxidation rate (V_{\max}) on pH. In addition, the impact of moisture content on CH_4 oxidation at different pH values was evaluated. The results from batch experiments confirmed that pH 4.5 is the lowest pH in which methanotrophs are still able to oxidize methane. Laboratory column experiments were performed to investigate the behaviour of compost biofilters operating at different pHs. The results confirmed that columns operated at pH 4.5 were found to oxidize methane at a rate of $53 \text{ g/m}^2/\text{d}$ compared to $146 \text{ g/m}^2/\text{d}$ in the columns operated at pH 7.5 (neutral). Observing no oxidation activity for columns operated at pH 2.5 leads to the fact that too acidic condition was suspected to be the cause for inhibiting methanotrophs ability to oxidize methane. Also, DNA sequencing analysis on the samples from column experiments indicated that the more acidic environment tends to inhibit the growth and activity of methanotrophs type I while being ineffective to the growth of methanotrophs type II. Biofilter columns operated at pH 2.5 contained only 2% methanotrophs type I, compared to 55% of the total microbial population in columns operated at pH 7.5. This study has revealed that the MOB population changes in the biofilter with acidification compromised its capacity to oxidize methane demonstrating that compost biofilter cannot operate efficiently under acidic conditions.

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I would also like to extend my thanks to my colleague, Helen La, for her insightful ideas and invaluable friendship.

Many thanks to Roshan Khadka for helping me in doing microbiology analysis.

Finally, I express my love and gratitude to my parents, Soheila and Hassan, and my little sister, Pariya, for providing me with unfailing support and continuous encouragement throughout my years of study, writing this thesis and my life in general.

Dedication

I dedicate this thesis to my mother, Soheila, who has been a source of encouragement and inspiration to me throughout my life. I would not be who I am today without her love and support. I am truly thankful for having her in my life, who has always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve.

Table of Contents

Abstract	ii
Acknowledgements	iii
Dedication	iv
Table of Contents	v
List of Tables.....	vii
List of Figures and Illustrations	viii
List of Symbols, Abbreviations and Nomenclature	x
Chapter 1: INTRODUCTION.....	1
1.1 Background.....	1
1.2 Scope and Objectives.....	3
Chapter 2: LITERATURE REVIEW.....	5
2.1 Greenhouse Gases.....	5
2.2 Methane as a Greenhouse Gas	5
2.3 Methane Elimination Methods.....	8
2.3.1 Flaring and Gas Collection.....	9
2.3.2 Biological Treatment	9
2.4 Methane Biofiltration Technology.....	10
2.4.1 Biofilter Design.....	10
2.4.2 Methane Biofilters Configuration	11
2.4.2.1 Flow Direction: Up-flow vs. Down-flow	11
2.4.2.2 Setting: Closed vs. Open.....	12
2.4.2.3 Aeration Mode: Active vs. Passive.....	12
2.5 Methanotrophic Bacteria.....	14
2.6 Factors Affecting Methane Bio-oxidation	16
2.6.1 Moisture Content	17
2.6.2 pH	18
2.6.3 Nutrients	19
2.6.4 Temperature.....	19
2.6.5 O ₂ Supply.....	20
2.6.6 Filter Bed	20
2.7 Hydrogen Sulfide (H ₂ S).....	22
2.7.1 Source	22
2.7.2 Toxicity.....	22
2.7.3 Effect on Methanotrophs Activity	23
2.8 Effect of Acidic pHs on Methane Oxidation	24
2.9 Batch Experiments	25
2.9.1 Reaction Kinetics.....	26
2.9.1.1 Michaelis-Menten Reaction Kinetics	26
2.10 Column Experiments	28

Chapter 3: MATERIALS AND METHODS	30
3.1 Materials	30
3.1.1 Compost Characteristics	30
3.1.1.1 Organic Carbon Content.....	31
3.1.1.2 Water Holding Capacity	32
3.1.1.3 Moisture Content	32
3.1.1.4 Bulk Density and Particle Density	33
3.1.1.5 Porosity	34
3.2 Batch Experiments	34
3.2.1 Pre-incubation Experiments	35
3.2.2 Incubation Experiments.....	36
3.2.2.1 V_{\max} Calculation	36
3.2.3 Experimental Design	37
3.2.3.1 OFAT Design	37
3.2.3.2 Central Composite Design (CCD).....	38
3.3 Column Experiments	41
3.3.1 Column Design	41
3.3.2 Experimental Design.....	43
3.3.3 Methane Removal Efficiency	44
3.3.4 Empty Bed Residence Time (EBRT) and True Residence Time (τ).....	45
3.4 Microbiological Analysis.....	46
Chapter 4: EXPERIMENTAL RESULTS AND DISCUSSION	48
4.1 Batch Experiments.....	48
4.1.1 Pre-incubation Experiments.....	48
4.1.2 OFAT Batch Experiments	49
4.1.3 Central Composite Design (CCD) Batch Experiments.....	52
4.2 Statistical Analysis for CCD Batch Experiments	55
4.2.1 Analysis of Variance (ANOVA)	55
4.2.2 Model Summary	60
4.2.3 Response Surface.....	62
4.3 Column Experiments	65
4.3.1 Methane Removal Efficiency at Different pHs	65
4.3.2 Gas Concentration Profiles.....	68
4.3.2.1 Concentration Profiles for the First Stage	68
4.3.2.2 Concentration Profiles for the Second Stage.....	70
4.4 Microbiological Analysis.....	73
Chapter 5: CONCLUSIONS.....	76
5.1 Conclusions.....	76
5.2 Recommendations for future research	78
REFERENCES	79
Appendix A: V_{\max} plots for OFAT batch experiments at different pHs	89
Appendix B: V_{\max} plots for CCD batch experiments at different pH and MC	97

List of Tables

Table 2-1 Atmospheric concentration and GWP of the most common GHGs.....	6
Table 2-2 Sources and atmospheric budgets of CH ₄ (Tg(CH ₄) yr ⁻¹)	7
Table 2-3 Optimum water content for some filter beds for methane elimination	17
Table 3-1 Compost characteristics used for the experiments.	31
Table 3-2 OFAT experimental design	38
Table 3-3 Coded and un-coded values of independent variables in Central Composite Design..	40
Table 3-4 Experimental design for column experiments.....	44
Table 4-1 V _{max} values from different methods for OFAT batch experiments	51
Table 4-2 V _{max} values from different methods for CCD batch experiments	55
Table 4-3 ANOVA table for Central Composite Design.....	58
Table 4-4 Model summary for CCD batch experiments.....	60
Table 4-5 The values of the coefficients for different parameters in the CCD model.....	61
Table 4-6 Methanotrophs community in the biofilter columns	74

List of Figures and Illustrations

Figure 2-1 Schematic representation of a lab-scale active MBF set-up (Du Plessis et al, 2003).	13
Figure 2-2 Schematic of a passive MBF for landfill gas emission control (Scheutz et al, 2009).	14
Figure 2-3 Pathways for the oxidation of methane and assimilation of formaldehyde. Abbreviations: CytC, cytochrome <i>c</i> ; FADH, formaldehyde dehydrogenase; FDH, formate dehydrogenase (Hanson and Hanson, 1996).	15
Figure 2-4 Graphical representation of Michaelis-Menten kinetic equations.	27
Figure 3-1 Photograph of pre-incubation experiments	35
Figure 3-2 Photograph of incubation experiments.	36
Figure 3-3 Location of different points in Central Composite Design (CCD) with two factors ..	40
Figure 3-4 Schematic diagram of laboratory biofilter columns.	42
Figure 3-5 Photograph of biofilter columns.	43
Figure 4-1 Methane oxidation rate vs time for pre-incubation experiments	49
Figure 4-2 Michaelis-Menten standard and linearized graphs and V_{max} values for the OFAT experiment at pH=8.5.	50
Figure 4-3 Maximum methane oxidation rate (V_{max}) vs pH for OFAT batch experiments.	52
Figure 4-4 Michaelis-Menten standard and linearized graphs and V_{max} values for the CCD experiment at pH=5.1 and MC=34%	53
Figure 4-5 Plot of main effects of MC and pH on V_{max} for CCD batch experiments	59
Figure 4-6 Plot of the interaction effect of MC and pH on V_{max} values for the CCD batch experiments	59
Figure 4-7 The response surface plot of V_{max} vs pH and MC	63
Figure 4-8 The contour plot of V_{max} vs pH and MC (%) (area only)	64
Figure 4-9 The contour plot of V_{max} vs pH and MC (%) (area and contour lines)	64
Figure 4-10 CH ₄ oxidation rate in g/m ² /day over time for 6 columns operated at different pHs.	67
Figure 4-11 CH ₄ removal efficiency (%) over time for 6 columns operated at different pHs	68

Figure 4-12 Gas Concentration Profile for day 25 (first stage) for 6 columns at steady state condition 69

Figure 4-13 Gas Concentration Profile for day 56 (second stage) for 6 columns at steady state condition 72

Figure 4-14 Bar chart plot of microbial population in the biofilter columns..... 75

List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
θ	Porosity
τ	True residence time
ρ_b	Dry bulk density
ρ_s	Particle density
C	Concentration
$C_{m,in}$	CH ₄ concentration in column inlet point
$C_{m,out}$	CH ₄ concentration in column outlet point
°C	Celcius
η	Efficiency
CH ₄	Methane
CO ₂	Carbon dioxide
H ₂ S	Hydrogen sulfide
N ₂ O	Nitrous Oxide
O ₃	Ozone
NH ₃	Ammonia
H ₂ O	Water
hr	hours
kg	kilograms
L	Litres
K_m	Half-saturation constant
m	meters
mg	milligrams
min	minute
mol	moles
μmol	micromoles
ppb	Parts per billion
O ₂	Oxygen
P	Pressure
Q	Flow rate

V_{\max}	Maximum CH_4 oxidation rate
dw	Dry weight
V_t	Total volume
V_w	Volume of water

Abbreviation	Definition
EBRT	Empty bed residence time
EPS	Exopolymeric substances
GHG	Greenhouse gas
GWP	Global warming potential
IPCC	Intergovernmental Panel on Climate Change
MBF	Methane Biofilters
MC	Moisture content
MMO	Methane monooxygenase

Chapter 1: INTRODUCTION

1.1 Background

Global warming refers to the recent and ongoing rise in global average temperature near Earth's surface. Certain greenhouse gases (GHGs) such as carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), and water vapour (H₂O) absorb most of the long-wave radiations of the Earth's surface. Increasing concentrations of the greenhouse gases raises the temperature of the first two layers of the atmosphere as well as the Earth's surface (Cubasch et al, 2013). Earth's average temperature has risen by 1.5°F over the past century, and is expected to rise another 0.5 to 8.6°F over the next hundred years (EPA, 2010).

The effect of methane on global warming is described by using an indicator, known as Global Warming Potential (GWP), a quantified measure of the globally averaged relative radiative forcing impacts of a particular greenhouse gas. Methane is a strong greenhouse gas with a global warming potential of 28-34 related to carbon dioxide on 100-year time horizon, and 85 over a time period of 20 years (IPCC, 2013; Myhre et al, 2013; Caulton et al, 2014).

Through fermentation processes, CH₄ is naturally produced under low O₂ conditions by methanogenic bacteria (Conrad, 1996). In addition to natural emissions, anthropogenic, industrial, and biogenic sources emit a significant amount of methane to the atmosphere. Anthropogenic CH₄ emissions including petroleum systems, landfills, wastewater treatment plants, rice paddy agriculture, and livestock farms account for more than 50% of total CH₄ budget. Since industrial time, the atmospheric methane concentration has increased from 722 ± 25 ppb in year 1750 to 1812 ± 22 ppb in 2013 (Myhre et al, 2013).

Effective treatment technologies are needed to mitigate CH₄ emissions from anthropogenic sources in order to reduce its impact on the global warming phenomenon. Flaring and gas combustion for energy production are two common mitigation technologies which have proven to be efficient in reducing CH₄ concentrations in the atmosphere. Flaring is a good option, but it requires CH₄ concentrations higher than 20% (v/v). Also, flaring produces some toxic by-products such as sulfur dioxide (SO₂) and carbon monoxide (CO), which are easily released to the atmosphere (Johnson and Coderre, 2012). Combustion is another option which is used for producing electricity and hot water, but can only be applied when the concentrations of CH₄ are higher than 30% (v/v) (Nikiema et al, 2007).

Biological treatment is a cost-effective technology for mitigating CH₄ emissions when the concentrations are lower than 7% (v/v). Methane biofiltration offers the simplest and most cost-effective implementation and operation with a potential of treating gas contains sour gas (H₂S) without producing any toxic by-pollutants (Lebrero et al, 2016). Methane biofilters (MBFs) consist of a layer of granular material that can sustain methanotrophic bacteria which are responsible for methane bio-oxidation. Methanotrophic bacteria, so called methanotrophs, use CH₄ as their carbon and energy source, and convert it to CO₂, water, and biomass which are all less harmful than CH₄ (Mancebo et al, 2016). The mechanism is affected by a variety of factors including CH₄ concentrations and flow rates, O₂ content, temperature, moisture content, pH, and types of the granular media used (Nikiema et al, 2007).

In addition to CH₄, other gases such as ammonia (NH₃) and hydrogen sulfide (H₂S) are produced in landfill, livestock facilities, petroleum refining, waste water treatment plants, and also in the treatment of "sour" natural gas and other fuels (Yang and Allen, 1994; Kim et al, 2012; Syed et al, 2006). Hydrogen sulfide is a very toxic air pollutant and could affect the methane oxidation

by changing methanotrophs behaviour in the system (Cáceres et al, 2014). Depending on its concentration, H₂S may have both positive and negative effects on the CH₄ oxidation efficiency. At low contents, H₂S may provide the sufficient nutrients for methanotrophs growth in the biofilm, whereas at high concentrations, it can inhibit the bio-oxidation of CH₄ due to its toxicity and changing the pH of system (Caceres et al, 2014; Yu et al, 2009).

In the presence of water and air, hydrogen sulfide oxidizes to sulfuric acid resulting in pH reduction which can inhibit methanotrophs growth and activity (Nikiema et al, 2007; Pratt et al, 2012). According to Hanson and Hanson (1996), methanotrophs are able to metabolise methane in a pH range of 4-9. So, abrupt variations in pH can inhibit their growth.

In fact, little research has been done to investigate the effect of other gases such as H₂S when completely mixed with methane in different proportions depending on the source of emissions (Melse and Van der Werf, 2005; Caceres et al, 2014). Therefore, a comprehensive study is needed to investigate the effect of pH reduction on methane oxidation as a result of H₂S presence.

1.2 Scope and Objectives

The goal of this work is to determine the effect of pH on methane oxidation by methanotrophs. With the aim of finding the inhibitory effect of low pHs, different sets of batch experiments were performed by changing the pH and moisture content and investigating the methane oxidation at those conditions. Also, column experiments were conducted in addition to batch experiments to adequately simulate the field conditions. In fact, compost column experiments allow more thorough analyses of effect of low pHs on methane oxidation efficiency. In order to address the general research questions regarding the effect of pH on methane oxidation, the following questions are investigated further:

- What is the minimum value of pH in which methanotrophs can still oxidize methane?
- How important is the role of moisture content in methane oxidation in the presence of sulfuric acid?
- Will very low pHs have an inhibitory effect on methane oxidation?
- How is the methane biofilter performance affected by variations in pH?

To answer the above-mentioned research questions, the following objectives need to be met;

- Determine the minimum pH value in which methane oxidation still occurs.
- Identify the effect of moisture content on methane oxidation when sulfuric acid is present in the media.
- Determine whether there is an inhibitory effect on methane oxidation at very low pHs.
- Evaluate the performance of methane biofilters at different pH values.

Chapter 2: LITERATURE REVIEW

2.1 Greenhouse Gases

Atmospheric trace gases, such as carbon dioxide, methane, ozone, nitrous oxide (N₂O), and several others are responsible for global warming problem. These gases are known as greenhouse gases (GHGs). Due to the human activities, especially after the industrial revolution, the concentration of these gases in the atmosphere have been increased, which raises the global temperature continuously (Etheridge et al, 1998).

2.2 Methane as a Greenhouse Gas

Although, methane has the second largest radiative forcing of the long-lived greenhouse gases after CO₂ (Forster et al, 2007), it is a more powerful greenhouse gas than CO₂. On the 100-year time horizon, methane (CH₄) has a global warming potential of 34 times higher than that of carbon dioxide (CO₂) (IPCC, 2013). Since 1750, the global atmospheric concentration of CH₄ has increased from the value of about 722 to 1834 parts per billion (ppb), and was 1803 ppb in 2011. During the past 800,000 years, methane concentrations substantially exceed the highest concentrations recorded in ice cores (IPCC, 2013). The atmospheric concentration and GWP of different greenhouse gases are shown in Table 2-1.

Table 2-1: Atmospheric concentration and GWP of the most common GHGs

Gas	Pre-1750 atmospheric concentration	Recent atmospheric concentration	GWP (100-yr time horizon)	Atmospheric lifetime (years)	Increased radiative forcing (W/m ²)
Concentrations in parts per million (ppm)					
Carbon dioxide (CO ₂)	280	399.5	1	100~300	1.94
Concentrations in parts per billion (ppb)					
Methane (CH ₄)	722	1834	34	12.4	0.50
Nitrous oxide (N ₂ O)	270	328	265	121	0.20
Tropospheric Ozone (O ₃)	237	337	n.a.	Hours-days	0.40

Source: IPCC, 2014.

Among, non-CO₂ GHGS, there is a much more interest in reducing the methane emissions, due to its higher GWP and shorter residence time (Hogan et al, 1991). The reason for an increase in atmospheric CH₄ concentrations was not fully understood yet. One of the reasons could be a lack of information on some sources and sinks of methane, which makes the prediction of future concentrations problematic (Dlugokencky et al, 1998). Table 2-2 presents the major sources of methane emissions. Although the major contributors to the global CH₄ budget likely have been identified, most of them are quite uncertain quantitatively because of the difficulty in assessing emission rates of highly variable biospheric sources (IPCC, 2013).

Table 2-2: Sources and atmospheric budgets of CH₄ (Tg(CH₄) yr⁻¹)

References	Hein et al, 1997	Houweling et al, 2000	Olivier et al, 2005	Wuebbles and Hayhoe, 2002	Scheehle et al, 2002	J. Wang et al, 2004	Mikaloff Fletcher et al, 2004	Chen and Prinn, 2006
Base year	1983-1989		2000		1990	1994	1999	1996-2001
Natural Sources		222		145		200	260	168
Wetlands	231	163		100		176	231	145
Termites		20		20		20	29	23
Ocean		15		4				
Hydrates				5		4		
Geological Sources		4		14				
Wild animals		15						
Wildfires		5		2				
Anthropogenic Sources	361		320	358	264	307	350	428
Energy					74	77		
Coal mining	32		34	46			30	48
Gas, oil industry	68		64	60			52	36
Landfills & waste	43		66	61	69	49	35	
Ruminants	92		80	81	76	83	91	189
Rice agriculture	83		39	60	31	57	54	112
Biomass burning	43		50	14	41	88	43	
C3 vegetation			27					
C4 vegetation			9					
Total sources	592			503		507	610	596

Source: IPCC, 2013.

Anthropogenic emissions account for 50–65% of the global CH₄ budget of ~395–427 Tera grams of carbon per year (Tg C/y) (526–569 Tg CH₄) (Kirschke et al, 2013; Ciais et al, 2014). Landfills and oil and gas production are two of the most significant sources of anthropogenic CH₄ emissions. Following the anaerobic degradation of organic fraction of solid waste, an equal amount of methane and carbon dioxide are produced in landfills (Pokhrel, 2006). Every year, thousand tons of these gases escape directly to the atmosphere, which causes an increase in global temperature.

Although landfills are contributed to only a small percentage (3.4% for Canada) of the total methane emissions from all sectors, they generally constitute the most important sources of anthropogenic CH₄ (Nikiema et al, 2007). For example, in Canada and the United States, around 25% and 34%, respectively, of the total methane emissions are directly related to landfill installations (Environment Canada, 2014; EPA, 2006).

Over the last two decades, researchers have focused on understanding fundamental processes controlling CH₄ oxidation in landfill settings. Laboratory experimental designs have evolved from simple batch experiments for determining CH₄ oxidation rates and short-term responses to environmental factors to more advanced column set-ups that more closely resemble the dynamic behaviour in landfill settings and thereby allow long-term performance to be studied. More recently, researchers found out that improving landfill covers by adding organic-rich materials such as sludge and composts will increase CH₄ oxidation (Scheutz et al, 2009).

2.3 Methane Elimination Methods

Over the past 35 years, global anthropogenic methane emissions have exceeded those from natural sources (IPCC, 2013). So, a new academic research, updated policies, and of course, design and operational changes is needed to reduce landfill methane emissions (Huber-Humer et al, 2008).

2.3.1 Flaring and Gas Collection

Various technologies such as flaring and power generation are used to control the CH₄ emissions issued from landfills (Nikiema et al, 2007). Methane capturing and complete destruction in thermal (flares) systems for energy recovery can be a desirable option, but there should be sufficient quantities of CH₄ available for economic energy recovery (Hettiaratchi et al, 2011; Huber-Humer et al, 2008). Flaring requires a minimum CH₄ concentration of 5–15 % (v/v), and power generation requires a continuous CH₄ concentration of 40–60 % (v/v) (Haubrichs and Widmann, 2006; Menard et al, 2012).

Furthermore, gas collection systems are not 100% efficient, since a considerable amount of emissions may also escape preferentially from the installed landfill equipment. A German reporter documented that the gas utilization and/or flaring systems are leaking considerable methane to the atmosphere. These systems are only economically feasible when methane concentrations are high (Huber-Humer et al, 2008).

2.3.2 Biological Treatment

Bio-oxidation of methane is an existing technology, when methane is present at concentrations below its inferior explosive limit 5% (v/v), and is not feasible to use it as a fuel, due to the low concentrations, or its combustion in flares is inefficient (Scheutz et al, 2009; Veillette et al, 2012). Unlike technologies, such as incineration for energy recovery, biological treatment does not produce marketable by-products such as nitrous oxide (N₂O), dioxin and other highly toxic contaminants (Jaffrin et al, 2003).

2.4 Methane Biofiltration Technology

Biofiltration is a well known cost-effective technology for reducing low volume point source emissions of CH₄ (Hettiaratchi et al, 2011). Unlike conventional mitigation technologies, methane mitigation using soil biofilters offers a clean technology with no net production of N₂O or any toxic by-products (Syed et al, 2016). Methane biofilters harbor methanotrophs living in a porous medium to oxidize CH₄ and convert it to water vapor, CO₂, and new biomass. These products are much less harmful for the environment than the initial substrate (Pratt et al, 2012).

Annually, at least 10–25% of the total CH₄ emitted from landfills is oxidized by microorganisms (Chanton and Liptay, 2000; Stralis-Pavese et al, 2006). Furthermore, biofiltration produces lesser CO₂ in comparison with regular chemical oxidation processes. Also, since biofiltration is often being performed at normal atmospheric pressure and temperature, it has a lower ranges operational costs than traditional technologies (Ottengraf, 1986).

In the first reaction of methane oxidation, methane mono-oxygenase (MMO) enzyme is responsible for CH₄ oxidation to methanol. In a second reaction, methanol is converted to formaldehyde by a methanol dehydrogenase. Then, the carbon from CH₄ can be assimilated into biomass by RuMP or serine pathway, depending on the microorganism, giving rise to the classification of methanotrophs type I and II, respectively (Semrau et al, 2011; Lee et al, 2012). In this process, CO₂ is produced as a by-product of the energy transduction during the oxidation of CH₄ (Trotsenko and Murrel, 2008).

2.4.1 Biofilter Design

Biofilters for methane mitigation are used for filtering air for odour or organic contaminants. Biofilters are operated as self-contained, fixed-bed reactors containing a packing material to support and sustain a population of methane oxidizers which are called methanotrophic

bacteria (Scheutz et al, 2009). Contact between the microorganisms and the polluting CH₄ occurs in the biofilm which is immobilized on the filter bed (Nikiema et al, 2007).

In biofilters, a humidified airstream is forced through a porous packed bed on which a microbial community is immobilized (Delhomenie and Heitz, 2005). A liquid feed flows in either a co/counter-current direction with the contaminated airstream, and provides enough moisture for biofilter. Once the gas dissolves into the liquid phase, it becomes available to the immobilised microbial community (Gerrity, 2015). A steady supply of gas is required in biofilters and they require either an active or passive gas collection system to feed the filter (Huber-Humer et al, 2008).

2.4.2 Methane Biofilter Configuration

Several biofilters have been tested in laboratory and field experiments with different designs, media and gas flow regimes. Depending on their application purposes, biofilters can be operated with different aeration, flow orientation, and setting (Huber-Humer et al, 2008).

2.4.2.1 Flow Direction: Up-flow vs. Down-flow

Typically, biofilters are configured either in an up-flow or a down-flow mode. When the biofilters are operated as an open bed system, the flow of the polluted gas in the bed proceeds upwards, which allows for the diffusive ingress of atmospheric O₂ from the ambient air into the bed (passive ventilation) (Scheutz et al, 2009). Up-flow operation may come with disadvantage of difficulty in controlling the operational parameters, such as the temperature and moisture levels (Gebert et al, 2003). Moreover, O₂ transfer to the bed's lowest layers is a very important limiting factor for the overall performance (Scheutz and Kjeldsen, 2004; Nikiema et al, 2007). However, up-flow operation has the advantage of easier leachate removal and prevention of CH₄ accumulation in the headspace.

In contrast, the down-flow operation is proved to be favourable regarding uniform material, moisture distribution, and drainage handling. In case of down-flow operation, moisture can more easily be provided through further additional of water at the top portion (Quigley et al, 2004).

2.4.2.2 Setting: Close vs. Open

The methane biofilters are operated as either open or fully contained beds (close). Open biofilters are more commonly operated in landfills, and since they require large surface area, they are installed outside (Gerrity, 2015). Open biofilters with ascending gas flows are exposed to climate conditions, and passive feed is driven solely by the pressure gradient between the landfill and the atmosphere (Straka et al, 1999; Dever et al, 2005).

On the other hand, closed biofilter which can employ either ascending/descending gas flows, are usually installed indoors. Fully contained systems allow better control of operational parameters such as temperature, moisture content, and inlet gas fluxes (Mudliar et al, 2010; Nikiema et al, 2007). The majority of biofilters, as used in lab-scale experiments, are closed systems. In this kind of biofilters, air is supplied by a forced ventilation system (Huber-Humer et al, 2008).

2.4.2.3 Aeration Mode: Active vs. Passive

Biofilters are operated as either passively aerated or actively aerated systems. Actively aerated systems are usually operated at constant temperature and water content, in which gas is supplied to the biofilter at a controlled flow rate (Schuetz et al, 2009). Air is supplied to the biofilter either through a separate feed line or can be combined with methane flow (Haubrichs and Widmann, 2006; Streese and Stegmann, 2003). Figure 2-1 shows a schematic diagram of actively aerated biofilters.

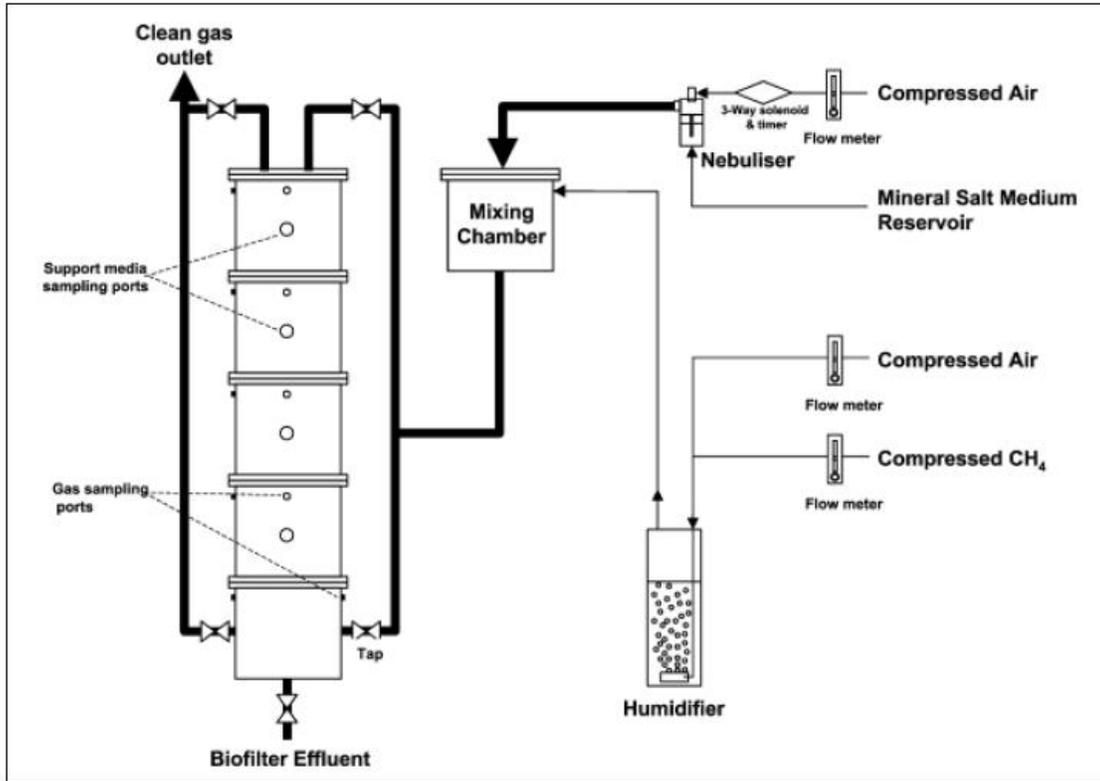


Figure 2-1: Schematic representation of a lab-scale active MBF set-up (Du Plessis et al, 2003)

Nevertheless, in passive systems, a pressure gradient between the biofilter and ambient air controls the gas flow and drives it to the biofilter (Straka et al, 1999; Dever et al, 2005). When these pressure gradients are low, diffusion plays an important role, in which O₂ is supplied to the media through the atmospheric air (Gebert and Groengroeft, 2006). A schematic diagram of passive landfill MBF is shown in Figure 2-2.

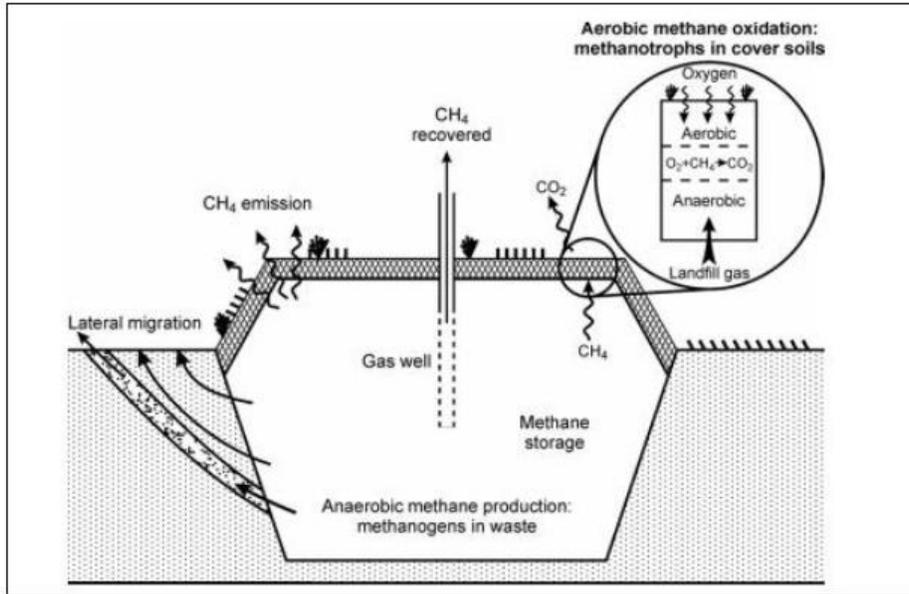


Figure 2-2: Schematic of a passive MBF for landfill gas emission control (Scheutz et al, 2009)

2.5 Methanotrophic Bacteria

Although there are some nitrifying bacteria that use methane, it is believed that the most microbial methane uptake in landfills is accomplished by methanotrophs, which are a subset of a physiological group of bacteria known as methylotrophs (Conrad, 1996). These obligate aerobes which are known as methane oxidizers or methanotrophs have the ability to utilize methane as a carbon and energy source and incorporate its carbon into biomass (Hanson and Hanson, 1996; Huber-Humer et al, 2008). Methanotrophs are naturally occurring in many ecosystems containing methane and are particularly abundant in landfill cover soils and biofilters. Also, there exists a plenty of them at the interface of aerobic and anaerobic regions of wetlands, rice paddies and peat bogs (Krumholz et al, 1995; Sundh et al, 1995).

There are three basic steps in methane oxidation by methanotrophs. Figure 2-3 presents the complete pathway for methane oxidation to CO₂. The first step is the oxidation of methane to methanol utilizing the enzyme methane monooxygenases (MMOs) (Hanson and Hanson, 1996;

Auman and Lidstrom, 2002). The next steps are transformation of methanol into formaldehyde (CHOH) and the subsequent oxidation of formaldehyde to formate (CHOOH) (Scheutz et al, 2009). In a dissimilatory pathway, the latter compound is being oxidized to CO₂ which leads to the synthesis of cell components, necessary for the growth of methanotrophs (Hanson and Hanson, 1996).

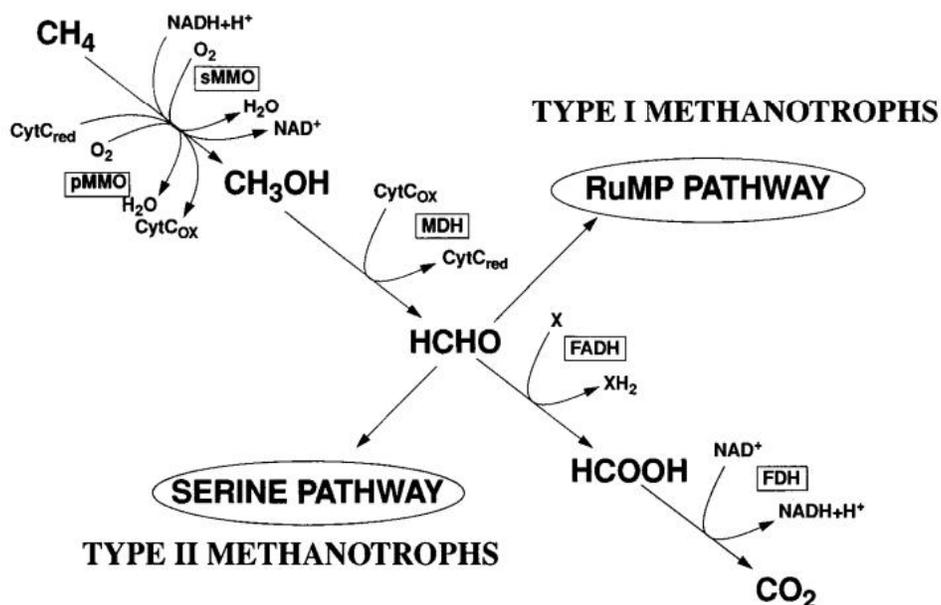


Figure 2-3: Pathways for the oxidation of methane and assimilation of formaldehyde.

Abbreviations: CytC, cytochrome c; FADH, formaldehyde dehydrogenase; FDH, formate dehydrogenase (Hanson and Hanson, 1996).

Methanotrophs are generally classified into type I and type II based on their different characteristics. Some of their differences are: (1) different methane consumption rates (Bogner et al, 1997); (2) their tolerance to temperature and moisture content vary (3) their oxygen requirements vary; (4) some have the ability to co-metabolize non-methane organic substrates; and (5) their different propensities to produce exopolymeric substances (EPS) (Malashenko et al, 2001; Scheutz et al, 2009; Hanson and Hanson, 1996; Bowman et al, 1993).

Type I included the genera *Methylococcus*, *Methylomicrobium*, *Methylobacter* and *Methylomonas* and formed the family *Methylococcaceae*. Type II included the genera *Methylosinus* and *Methylocystis* (Scheutz et al, 2009). Type I methanotrophic bacteria are responsible for oxidation of atmospheric methane, due to their high affection to methane which enables them to metabolize methane even at very low concentrations (Pokhrel, 2006). In contrast, type II methanotrophic bacteria have low affection to methane and therefore they are able to grow only at high concentrations of methane such as landfills and wetlands (Henckel et al, 2000).

Type I growth rate is high only under favorable conditions. Although type II grow very slowly, they are able to tolerate the extreme environmental condition for longer periods (Hanson and Hanson, 1996). Type II methanotrophs can live in very low concentrations of oxygen and nitrogen. Moreover, they can survive in natural soils under all unexpected conditions, as they are able to protect themselves against heat and desiccation (Mancinelli, 1995; Pokhrel, 2006).

2.6 Factors Affecting Methane Bio-oxidation

There are several factors affecting methanotrophs growth which are responsible for methane oxidation. Changes in these conditions may either enhance or reduce the methane oxidation. Some of these factors are:

- Moisture Content
- pH
- Nutrients
- Temperature
- O₂ Supply
- Filter Bed

2.6.1 Moisture Content

Moisture content is one of the key factors for methane oxidation as it is considered as a transport medium for residual compounds removal and also nutrient supply (Borjesson et al, 1998; Nikiema et al, 2007). The optimum filter bed water content is dependent on both the gas flow rate and the type of media (soil, compost or other material employed) (Christophersen et al, 2000). In soils, we can reach the optimal CH₄ oxidation rate in moisture content values between 13 and 15.5 % wt/wt, on a dry basis (Boeckx and Van Cleemput, 1996; Stein and Hettiaratchi, 2001). However, there exists a case for biofiltration in landfill cover soil, in which optimum moisture content was 25–30 % wt/wt on a dry basis (Giani et al, 2002). Table 2-3 shows the optimum moisture content for different filter beds. For composts, optimal bed moisture lies between 25% and 50% wt/wt (Humer and Lechner, 1999).

Table 2-3: Optimum water content for some filter beds for methane elimination

Filter bed	Water content: % wt/wt	Authors
Compost	25-50	(Humer and Lechner, 1999)
Landfill cover soil	13-30	(Boeckx and Van Cleemput, 1996; Park et al, 2002; Stein and Hettiaratchi, 2001; Visvanathan et al, 1999; Giani et al, 2002)
Meadow soil	30-50	(Mingxing and Jing, 2002)
Woodland soil	18-33	(Mingxing and Jing, 2002)
Various soils	11-35	(Bender and Conrad, 1995; Christophersen et al, 2000)

High moisture content limits the methane oxidation rate by acting as a barrier for CH₄ and O₂ flow (Humer and Lechner, 1999; Park et al, 2002). Since the molecular diffusion in water is about 10⁴ times slower than in the air, when the volume of water significantly exceeds the voids volume in soil, the air- filled voids lose their interconnection and the gases have to diffuse in the liquid phase (Cabral et al, 2004; Whalen and Reeburgh, 1996). Therefore, this phenomenon slows down the gaseous transport in soil, and reduces the CH₄ oxidation. On the other hand, low moisture content acts as a rate-limiting factor by creating microbial water stress resulting from desiccation (Scheutz et al, 2009).

2.6.2 pH

The optimal pH values for methane oxidation which promotes the growth of methanotrophic bacteria is found to be neutral (Nikiema et al, 2007). According to Hanson and Hanson (1996), methanotrophs are able to metabolise methane in a wide pH range of 4-9. However, an optimum activity and growth rate takes place within a pH range of 5.5-8.5 (Figueroa, 1993). Amaral et al. (1998) reported a methanotrophs activity in forest soils at pH range of 4.5-7.5, and he recorded the optimal pH near neutral values. Similarly, observations by Hutsch et al. (1994) indicate that a significant methane uptake in soil was happened at pH 7.5, and a minimum methane uptake was reported at pH 4.1 (Haththotuwa, 2005). Although methanotrophs can live in pH values around 4, abrupt variations in the pH can inhibit their growth. When the pH of the soil was changed by around 2 units, a significant inhibition was observed (Bender and Conrad, 1995).

It is important to note that the biofilter pH value is mainly dependent on the properties of the material used in the filter bed. Loamy materials are usually less prone to acidification, due to higher buffer capacity, however sandy substrates can undergo pH values as low as 4.5 (Scheutz et al, 2009).

The experiments carried out by Hilger et al. (2000) recorded a drop in pH near the top of soil columns, owing to the oxidation of the landfill gas to methanol and formic acid. It has been reported that at the bottom layers of columns, a rise in pH levels was observed due to reduction reactions caused by reduced pore spaces and low O₂ concentrations. In fact, at low oxygen regions, type II methanotrophs produce NH₄ which causes an increase in pH values. Addition of lime to the columns could be a good solution for that (Nikiema et al, 2007).

2.6.3 Nutrients

Another significant factor for the success of CH₄ biofiltration is the existence of nutrients. There are some nutrients such as nitrogen, phosphorus, and ammonia which are necessary for the growth of microorganisms (Trotsenko and Khmelenina, 2002). These nutrients must exist in the biofilter either in a bioavailable form or can be added as a solution to humidify the filter bed (Nikiema et al, 2005).

2.6.4 Temperature

When methane oxidation occurs, 880 kJ per mole of heat is generated, and a larger portion of this energy is transferred to both the filtering material and to the mixture of gases inside the biofilter (Nikiema et al, 2007). As a result of heat generation, a temperature gradient is created in the biofilter between its lower and upper surfaces (Humer and Lechner, 1999; Nikiema et al, 2005). The input gas flow rate, the conversion, the type of filtering material are the key parameters controlling the significance of this thermal gradient (Nikiema et al, 2007).

To test the influence of temperature on methane biofiltration, experiments were conducted with common filter materials, such as soil and compost. Generally, the optimal temperature for methane oxidation is found to be between 29 and 30°C for compost, and between 25 and 36°C for

soil (Dammann et al, 1999; Mor et al, 2006), although CH₄ oxidation can occur down to 1–2 °C (Prieme and Christensen, 1997).

Omel'chenko et al. (1993) investigated the growth of isolated methanotrophs in acid soils, and reported their optimum growth at temperatures of 10°C or lower. Type I methanotrophic bacteria can adapt to low temperatures, indicating that temperature could determine which of the two main types of methanotrophs will predominate in a given environmental system (Borjesson et al, 2004). Therefore, type I methanotrophs are more dominant at 10°C than at 20°C, and they have a lower temperature optimum than methanotrophs type II (Gebert et al, 2003).

2.6.5 O₂ Supply

Methanotrophic bacteria are able to grow even at very low O₂ concentrations and achieve optimum CH₄ conversion rates. Wilshusen et al. (2004) reported that in pure methanotrophic cultures, O₂ concentrations ranging from 0.45 to 20% provide maximum CH₄ oxidation rates in both type I and II bacteria. Furthermore, experiments carried out on paddy fields concluded that optimum CH₄ oxidation is achievable at O₂ mixing ratios greater than 1 to 3%, however, a rapid decrease was observed at lower levels. Similarly, Czepiel et al. (1996) showed that CH₄ oxidation dropped significantly at O₂ mixing ratios below 3%. For biofilter material, Gebert et al. (2003) found that CH₄ oxidation only started at O₂ concentrations above 1.7–2.6 %. The maximum CH₄ oxidation rates can be reached at O₂ concentrations of approximately 9% (Scheutz et al, 2009).

2.6.6 Filter Bed

To design a biofilter, a suitable packed bed film material should be used to maintain the optimum conditions for bacteria growth. Generally, a good filter material must have; (1) high specific surface area, (2) high porosity, (3) sufficient water holding capacity, (4) availability of

enough nutrients, (5) resistance towards microbial degradation, and (6) sufficient homogeneity to prevent preferential flow (Scheutz et al, 2009).

Various media which have been used in biofilters are compost including composted wastes, wood chips, bark mulch and peat; inorganic materials such as glass beads, bottom ash or porous clay pellets; as well as mixtures of organic and inert materials (Wilshusen et al, 2004; Dever et al, 2005; Scheutz et al, 2009). Among them, compost and soil are two media that used commonly in methane biofilters. One of the main advantages of using soil is that it is cheap and readily available (Syed et al, 2006). Compost is the most widely used filter bed material. It is very rich in nutrients and has good water holding capacity as well as good air permeability. Furthermore, it has the advantage of having a substantial and diverse indigenous microflora (Pokhrel et al, 2011).

Compost has been used as the filter bed media for a while because it has the specific characteristics required for bacteria growth which are availability of intrinsic nutrients, a high specific surface area, high porosity, good moisture retention capacity, structural integrity and biological stability (Delhomenie and Heitz, 2005). Recently, researchers have focused on the effect of the physical characteristics of compost-based materials on the performance of methane biofilters. According to Humer and Lechner (1999), media containing high porosity enhance CH₄ oxidation rates. Although small particle sizes provide large specific surface areas, they create resistance to gas flow. In contrast, favorable gas flow occurs with large particle sizes, but reduces the number of potential sites for microbial activity (Delhomenie et al, 2002).

Pedersen et al. (2011) distinguished seven different compost materials based on the different parameters such as water content, loss of ignition, pH, total organic carbon, total nitrogen, ammonium-nitrogen, nitrate-nitrogen, carbon/nitrogen (C/N) ratio, total phosphorus, sulfate, and copper. This study revealed a relationship between low C/N ratios and high CH₄ oxidation rates

leading to the fact that C/N ratio could be good representative of the CH₄ removal capacity of granular media (Mancebo and Hettiaratchi, 2015).

2.7 Hydrogen Sulfide (H₂S)

Different sources of gaseous emissions containing low concentrations of CH₄ are emitted to the atmosphere, and these emissions also contain different proportions of other gases such as ammonia (NH₃) and hydrogen sulfide (H₂S), depending on the source (Ying et al, 2012; Melse and Van der Werf, 2005; Lopez et al, 2013).

2.7.1 Source

Considerable amounts of H₂S are produced in closed or abandoned landfills, livestock facilities, petroleum refining, animal houses, some sections of waste water treatment plants, and also in the treatment of "sour" natural gas and other fuels (Yang and Allen, 1994; Kim et al, 2005; Syed et al, 2006).

CH₄ and CO₂ are produced in landfills as well as some trace components such as volatile organic compounds and reduced sulfur compounds including H₂S, methyl mercaptan, dimethyl sulfide and dimethyl disulfide (Scheutz et al, 2009; Kim et al, 2005). In fact, H₂S accounts for more than 90 % of the mass concentration of all sulfur gases in landfill gas (LFG), and it is considered as the main source of odor at landfill sites with attribution to 4.47–10.92 % of total odor concentrations (Kim et al, 2005; Li et al, 2013).

2.7.2 Toxicity

In addition to its unpleasant odor, hydrogen sulfide (H₂S) is a highly toxic air pollutant (Roth, 1993). Through the inhalation, reacting with enzymes in the bloodstream, hydrogen sulfide inhibits cellular respiration which is leading to some healthy problems such as pulmonary

paralysis, sudden collapse, or even death. Even very low concentrations of H₂S cause irritation to mucous membranes and may also cause headaches, dizziness, and nausea. Exposure to higher concentrations (200-300 ppm) may result in respiratory arrest leading to coma and unconsciousness. Exposures to concentrations greater than 700 ppm could cause death in 30 minutes (MSDS, 1996; Syed et al, 2006).

2.7.3 Effect on Methanotrophs Activity

In fact, little research has been done to investigate the effect could cause in the presence of other gases like H₂S when completely mixed with methane in different proportions depending on the source of emission (Melse and Van der Werf, 2005; Caceres et al, 2014). Gaseous H₂S could affect the methanotrophs activity by changing their capability to oxidize methane (Caceres et al, 2014). It is known that H₂S may have both positive and negative effects on the CH₄ removal efficiency, depending on its concentration. At low concentrations, H₂S may provide the sufficient nutrients for methanotrophs growth in the biofilm, whereas at high concentrations it may be inhibitory to the bio-oxidation of CH₄ due to its toxicity and also reducing the pH of system (Caceres et al, 2014; Yu et al, 2009).

In the presence of water and air, hydrogen sulfide oxidizes to sulfuric acid resulting in the pH reduction which inhibits methanotrophs growth and activity (Nikiema et al, 2007; Pratt et al, 2012). Furthermore, it has been reported that reduced sulfur compounds such as methanethiol and CS₂ have a significant inhibitory effect on the bio-oxidation of CH₄ in landfills, in which methanotrophs type I have been more affected than type II (Borjesson, 2001; Lee et al, 2012; Caceres et al, 2014).

Moreover, H₂S can change methanotrophs capacity to oxidize methane due to its high solubility in water at neutral pH, which is found to be 3.85 g per kg of water at 1 atm and 20°C

(Caceres et al, 2014). H₂S binds to metal ions, which are cofactors of enzyme proteins in microbial cells, and inhibit the enzyme protein activities. It can also reduce essential disulphide bonds of cellular proteins, resulting in destruction of proteins' three-dimensional structures and, consequently, their activities (Yu et al, 2009).

Yu et al. (2009) tested the effect of different concentrations of H₂S on methane oxidation in a liquid medium containing Nitrate Mineral Salts (NMS) and soil. He observed an increase in methane oxidation from 91.8 to 97.0% as a result of an increase in H₂S concentration from 0 to 0.033%. However, with a further increase in H₂S concentration, from 0.033 to 0.66%, the CH₄ oxidation rate was dropped to 95.5%. Based on the statistical analysis, it has been recorded that there were no significant differences in methane oxidation rates when H₂S concentration increased from 0 to 0.066% (Yu et al, 2009).

2.8 Effect of Acidic pHs on Methane Oxidation

Syed et al. (2016) investigated the CH₄-oxidising capacity of a reconstituted acidic soil biofilter operating at low pH (3.72) and 60% water holding capacity (WHC). In this study, the acidic soil biofilter achieved a maximum CH₄ removal rate of 30.3 g/m³/hr, and removal efficiency of 57%. Both types I and II MOB communities, along with some uncultured novel MOB strains or species in the biofilter column, were present.

Syed et al. (2016) also focused on determining the changes in pH and moisture content throughout the study period. Based on the experimental data, a fluctuation has been observed in moisture values on days 10 and 29 across the top, middle and bottom portions of the biofilter, but these moisture values had decreased by the end of the study period (day 90). However, during the whole experiment, the moisture content was more or less around the optimal range. Regarding the

pH, no significant change has been recorded during the study period and it remained at 3.8. However, there has been a decline from 3.72 to 3.25 at the base of the biofilter (Syed et al, 2014).

This study has indicated that a soil biofilter is able to oxidize methane efficiency if sufficient moisture levels are maintained, regardless of the soil acidity. Therefore, diverse native population of MOB (types I and II) had the ability to adapt to changing acidic conditions at high moisture values. However, there is no research to investigate their behavior at lower moisture values (Syed et al, 2016).

2.9 Batch Experiments

Batch experiments, also called incubation experiments, have been used mostly to determine the methane oxidation capacities of different media under different conditions. Some researchers (Boeckx and van Cleemput, 1996; Christophersen et al, 2000) used incubation experiments to assess the changes in methane oxidation rate at different moisture content and temperatures. Identifying the optimum moisture and temperature values in which methane oxidation rate is maximum was another objective of their research.

Barratt (1995) conducted batch experiments with different fresh porous materials to determine their maximum methane oxidation capacities and also to find the time required for the development of methanotrophic activities. Some researchers (Whalen et al, 1990; Boeckx and van Cleemput, 1996; Borjesson et al, 1998; Christoperesen et al, 2000) used soil collected from surface and different depths of the actual landfill final cover. Others (Wilshusen et al, 2004; Mancebo and Hettiaratchi, 2015; Pokhrel et al, 2011) also conducted experiments on compost and compost mixes to improve compost's already favorable conditions for bacteria to live in. Wilshusen et al. (2004) have found that compost has superior oxidation capacity compared to mineral soil.

2.9.1 Reaction Kinetics

Chemical and biochemical reaction kinetics are categorized as zero, first and second order with respect to substrate concentration. Zero order reaction is represented by a constant reaction regardless of the substrate concentration. In the first order reaction, the reaction rate is proportional to substrate concentration. Similarly, in second order reaction, the rate of reaction is proportional to the square of the substrate concentration (Leskovac, 2003).

2.9.1.1 Michaelis-Menten Reaction Kinetics

Some of the biochemical reaction kinetics do not follow the order of reaction. One of them is Michaelis-Menten reaction kinetics, also known as hyperbolic kinetics, which does not strictly follow order of reactions. In this reaction kinetic, reaction rate versus substrate concentration has the form of a rectangular hyperbola. Equation 2-1 presents the single substrate Michaelis-Menten reaction kinetics:

Equation 2-1:
$$\frac{V}{V_{max}} = \left(\frac{S}{K_m + S} \right)$$

Where:

V = Reaction rate

V_{max} = Maximum reaction rate which is obtained when all enzyme is bound in the enzyme substrate complex ($S \gg K$)

S = Substrate concentration

K_m = Michaelis Menten Constant (Substrate concentration at half of maximum reaction)

Since the plot of reaction rate vs substrate concentration is non-linear, it is difficult to estimate accurately the values of kinetic parameters V_{max} and K_m . Thus, the non-linear form of the

equation should be transformed into different linear forms to calculate kinetic parameters. Three most common linear plots of Michaelis-Menten reaction equations are:

1. Lineweaver-Burk (1935) Plot: This plot is the most widely used plot which is also known as “double reciprocal plot”. In this plot, the plotting parameters are $1/V$ and $1/S$.
2. Eadie (1942) and Hofstee (1952) plot: In this plot, V/S are plotted vs V , so the kinetic variables (V and S) are not separated, and the reaction rate exists in both sides.
3. Hanes (1932) Plot: This plot is considered as the most reliable plot among the three (Rudolph and Fromm, 1979). In this linearized plot, S/V are plotted against S , and S appears in both coordinates.

The graphical forms of these three plots are presented in Figure 2-4.

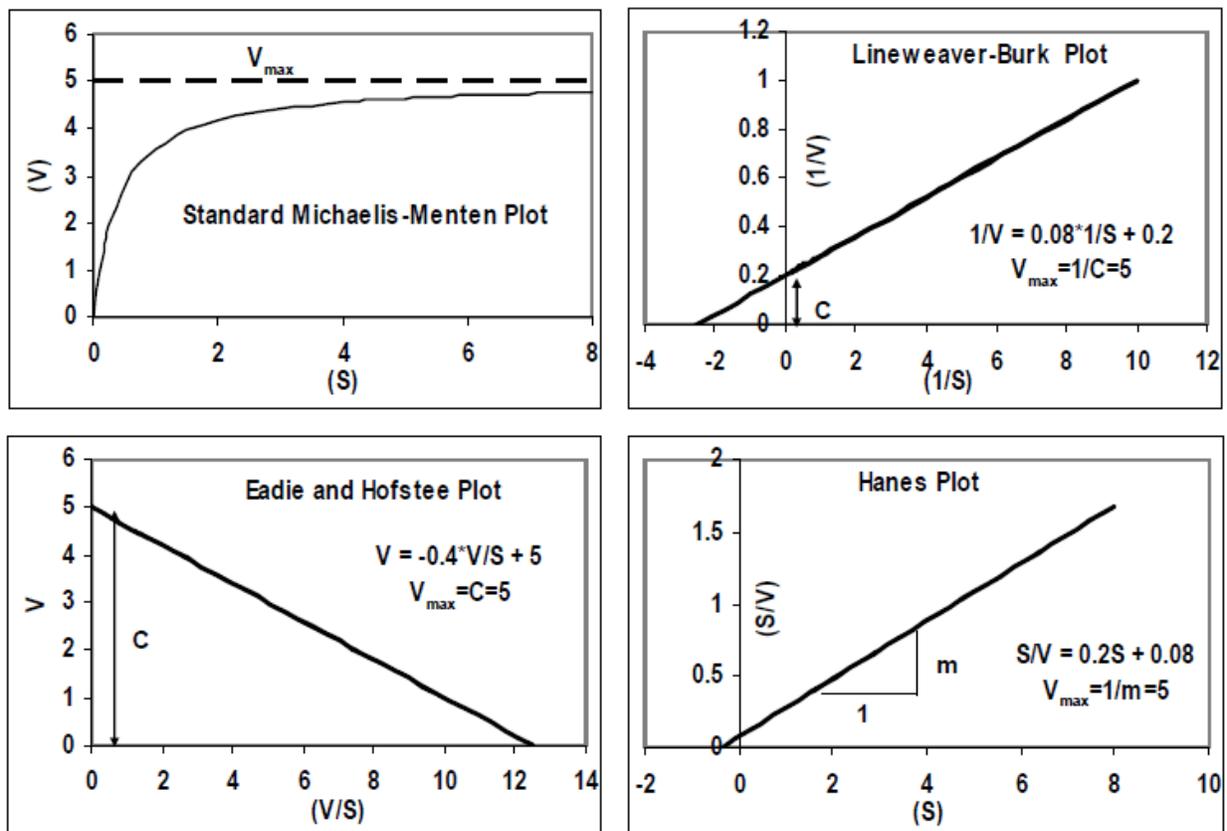


Figure 2-4: Graphical representation of Michaelis-Menten kinetic equations

2.10 Column Experiments

Column experiments are generally used to simulate landfill cover systems and predict methane oxidation rate and methane removal efficiency in the laboratory. In column experiments, the depth of the media mirrors original landfill conditions and the oxidation rates can be extrapolated in field landfill cover systems. These experiments have been conducted either at indoor (Kightley et al, 1995; De Visscher et al, 1999; Hilger et al, 1999); or outdoor environments (Visvanathan et al, 1999).

Several types of materials or material mixes have been used by researchers as the column media, such as actual landfill cover soil (De Visscher et al, 1999; Hilger et al, 2000; Pokhrel, 2006); compost (Figuerola, 1993; Fornés et al, 2003; Wilshusen et al, 2004), compost-soil (Pokhrel, 2006), compost-perlite and compost-sand (Mancebo, 2011). De Visscher et al. (1999) conducted column experiments with actual landfill final cover soil. Some researchers (Figuerola, 1993; Fornes et al, 2003; Wilshusen et al, 2004) used compost as their column media. Humer and Lechner (1999) and Cossu et al. (2003) conducted experiments with both soil and compost to compare their capacity to oxidize methane. The reported maximum methane oxidation rates were 240 g/m²/day (De Visscher et al, 1999) and 400 g/m²/day (Wilshusen et al, 2004) in soil and compost, respectively.

Most of the work performed to date investigating the factors that influence methane oxidation in compost have relied on batch experiments, in which compost is placed in bottles which are then injected with methane. But, batch experiments are not able to simulate the reduction in the areal extent of oxygen penetration caused by its advective displacement by methane and by its consumption due to microbial methane oxidation. For this study, compost column experiments have been used in addition to batch experiments to adequately simulate these mass transfer

limitations. Furthermore, compost column experiments allow more thorough analyses of the interaction between the many factors which influence CH₄ oxidation rates.

Chapter 3: MATERIAL AND METHODS

3.1 Overview

The overall goal of this study was to investigate the effect of pH reduction on methane microbial oxidation as a result of hydrogen sulfide oxidation when it is completely mixed with methane in different proportions depending on the source of emissions. To that end, laboratory batch experiments were carried out first to evaluate the effect of different MC and pH. Later on, column experiments were conducted taking into consideration results from previous batch experiments to investigate the inhibitory effect of acidic pHs on methane oxidation by methanotrophic bacteria.

3.2 Materials

The material used in this study is the leaf compost obtained from the City of Calgary East landfill. Compost was used in batch experiments and also in passively aerated biofilter column experiments. Methane (99% pure) was used to feed the columns. Sulfuric acid was used to change the pH of compost to the desired values.

3.2.1 Compost Characteristics

Physical parameters of the compost, such as average particle size, particle density, organic content, moisture content, and water holding capacity were determined according to commonly-used standard methods. These values are presented at Table 3-1. The standard methods to determine the compost characteristics including organic carbon content, water holding capacity, moisture content, bulk density and particle density as well as porosity are explained through sections 3.2.1.1 to 3.2.1.5.

Table 3-1: Compost characteristics used for the experiments

Parameter	Unit	Value
Particle Size	mm	< 2.35
Organic Content	%	38
Particle Density	g/cm ³	1.24
Bulk Density	g/cm ³	0.54
Porosity	-	0.56
Water Holding Capacity	%	68.2

3.2.1.1 Organic Carbon Content

Organic matter content is expressed as an estimate of the total organic carbon content. The loss-on-ignition method (LOI) is used for determination of the organic matter (Schumacher, 2002). 10 grams of dry samples were passed through at most a 10 mm screen in ceramic crucibles and heated in a furnace at a temperature of 440°C for 18 hours. Samples were then cooled and weighed, and the organic matter fraction was determined by measuring the difference between the initial and final mass divided by the initial mass (Schumacher, 2002; ASTM International, 2014). The organic fraction of the compost used in this study was measured at 38% on a dry weight basis. Organic content was calculated using Equation 3-1.

Equation 3-1:
$$\% OC = \frac{m_{dry} - m_{burned}}{m_{burned}} \times 100$$

Where:

m_{dry} = the mass of samples after drying in the oven at 110°C, in grams (g).

m_{burned} = the mass of samples after burning in the oven at 440°C, in grams (g).

OC = organic content in percentage of dry compost, in percentage (%).

3.2.1.2 Water Holding Capacity

The water content remaining in the compost after it has been saturated is called water holding capacity which is determined using simple funnel experiments (Klute, 1986). A filter paper was wetted, drained, folded and fitted into a funnel which has a volume of 500 cm³. First, total weight of the funnel and wet filter paper was measured. Then, funnels were filled up with compost at a fixed density up to the top. The funnel was clamped to a stand. The bottom tip of the funnel was connected to a tube which was connected at the other end to a burette filled with water and clamped at the same level. Water released from the burette slowly removes the air from the compost placed in the funnel. Once the compost became saturated, the funnel, filter paper, and the saturated sample were weighed. After saturation, a tube which supplies the water was disconnected from the funnel. A beaker was then placed at the bottom of each funnel to collect any drained water. To prevent the loss of moisture from the top surface, the funnels were covered during the experiments. When the water was drained for 4 to 5 hours, the weight of the funnels with the wet samples were determined and water holding capacity of the compost was calculated.

3.2.1.3 Moisture Content

Moisture content is determined gravimetrically by measuring the weight lost after heating at 105°C for 18 hours in accordance with ASTM standard (ASTM International, 2014). First, the wet mass of samples and crucibles were measured. Then, samples were introduced into an oven to be heated for 18 hours at a temperature of 105°C. Afterwards, the samples were weighed again and the moisture content is determined using Equation 3-2. Moisture content was expressed as percentage of wet compost weight.

Equation 3-2:
$$\% MC = \frac{m_{wet} - m_{dry}}{m_{wet}} \times 100$$

Where:

m_{wet} = the mass of samples before drying.

m_{dry} = the mass of samples after drying.

MC = moisture content in percentage of wet weight.

3.2.1.4 Bulk Density and Particle Density

Particle density is defined as the mass of particles divided by the volume occupied by particles. For particle density calculation, a known weight of the material was introduced into a graduated cylinder. A known volume of water was added to the cylinder then. The difference between the total volume and the volume of water represents the volume occupied by particles. Then particle density is calculated using Equation 3-3.

Equation 3-3:
$$\rho_s = \frac{W}{V_t - V_w}$$

Where:

W = material weight (g)

V_t = total volume (cm³)

V_w = water volume (cm³)

Bulk density is defined as the total mass of material divided by volume of the mixture. Since the compost is too porous and difficult to handle and achieve a proper measurement, a known weight of material was added to the column. The volume of mixture was measured then, thus bulk density is calculated with Equation 3-4.

Equation 3-4:
$$\rho_b = \frac{W_s}{V}$$

Where:

W_s = weight of solids (g)

V = volume of the mixture (cm³)

3.2.1.5 Porosity

In material characterization, porosity is defined as the ratio of void volume to total volume. It is very important to determine the material porosity in the columns, since porosity is one of the key parameters affecting gas flow through the material. Sufficient porosity is essential to ensure air diffusion, adequate retention time, as well as minimized pressure drop (Huber-Humer et al, 2008). According to the literature (Devinny et al, 1999), the porosity values of 30-80 % are recommended to ensure both gas plug flow and low pressure drop. In order to characterize the porosity of compost, we use Equation 3-5, shown below.

Equation 3-5:
$$\theta = 1 - \frac{\rho_b}{\rho_s}$$

Where:

θ = porosity

ρ_b = bulk density (g/cm³)

ρ_s = particle density (g/cm³)

3.2 Batch Experiments

Batch experiments were conducted to study the following:

- How different pH values affect the methane oxidation rate.
- Whether there is a negative effect on methanotrophs growth at acidic pH.
- The relationship between CH₄ oxidation rates and the pH values.
- Determine the impact of moisture content on CH₄ oxidation at different pH values.
- Determine the minimum pH value that bacteria can live in the presence of sulfuric acid.

3.2.1 Pre-incubation Experiments

The pre-incubation was done to ensure the biofilter material have enough methanotrophs. This was done by incubating the compost in 1000 ml airtight glass bottles under 5-6 % initial methane concentrations, as shown in Figure 3-1. For each pre-incubation experiment, approximately 100 grams of compost were placed in the bottle with lid and septa. The bottles were sealed then with using silicone. Moisture content was maintained at an optimum value of 35% (Humer and Lechner, 1999). A headspace methane concentration of approximately 5% was injected to the bottle with a needle syringe. To avoid the higher pressure inside the bottles, the same volume of air from the bottles was withdrawn using a needle-syringe prior to add the methane. Incubations were performed at a nominal temperature of 22°C. A headspace gas concentration was measured at different time intervals using Micro-Gas Chromatography until a steady state condition for oxidation was observed. At steady state conditions, the methanotrophic bacteria reach their maximum capacity to oxidize methane.



Figure 3-1: Photograph of pre-incubation experiments

3.2.2 Incubation Experiments

Two sets of batch experiments were done to achieve the study objectives. For these experiments, 250 ml air tight glass bottles were filled with pre-determined weight of compost at desired MC and pH values, as shown in Figure 3-2. The pre-determined amount of sulfuric acid was added to the bottles to maintain the different pHs at their desired values. The bottles were sealed with rubber septa and methane was injected using a needle syringe. Excessive pressure build-up inside the serum bottle was released by withdrawing the same volume of air from bottles. The headspace gas concentration was measured by removing 5 ml gas samples from the bottles. Concentration of CH₄ in the head space was determined using a portable micro gas chromatograph (GC). The gas composition was determined using the GALAXIE software package in communication with the GC.



Figure 3-2: Photograph of incubation experiments

3.2.2.1 V_{max} Calculation

The headspace gas concentrations were measured at different time intervals until the all methane in bottles is nearly consumed. The gas concentrations were measured at at-least 3 different

time intervals. Using the methane concentration in different time intervals, methane oxidation capacities of compost was calculated.

The goal of the batch experiments was to determine the maximum methane oxidation rate (V_{\max}) and develop relationships between V_{\max} , moisture content, and pH. To calculate the V_{\max} , the methane oxidation rate should be obtained for different initial concentrations of methane. So, varying quantities of CH_4 were supplied to the glass bottles headspace. The resulting CH_4 oxidation rates were used to calculate the maximum rate of CH_4 oxidation (V_{\max}) and the apparent saturation constant (K_m). 2%, 4.5%, 8%, and 10% were used as different initial concentrations of methane. The oxidation rates determined at different initial headspace concentrations were used to calculate V_{\max} by plotting oxidation rate with respect to initial headspace concentration. Since the direct plot of methane oxidation rate vs initial gas concentration is non-linear, V_{\max} values can be calculated either by linearizing the plots or using non-linear regression in some statistical software such as SPSS and MINITAB. There are three linearization methods (i.e. Eadie-Hofstee, Lineweaver-Burk, and Hanes) available. All fore methods were compared, and the best-fitted model was used for the analysis.

3.2.3 Experimental Design

Two sets of batch experiments were performed in this study which are explained below.

3.2.3.1 OFAT Design

The purpose of doing this set of batch experiments was to determine the relationship between V_{\max} and different pH values to see how the pH affects methane oxidation rate. Furthermore, determining the minimum pH in which methanotrophs can live was another objective of this experiments.

For this experiments, one factor at a time (OFAT) experimental design was used. Using different concentrations of sulfuric acid, various pHs were maintained in the bottles. Other factors such as temperature, nutrients content, methane concentration, oxygen concentration, and moisture content (30%) were kept constant during the experiments. In total, 9 experimental runs were performed with 9 different pHs. Table 3-2 presents the values of our independent variable, which is pH, as well as corresponding sulfuric acid concentrations in different runs.

Table 3-2: OFAT experimental design

Runs	pH	Sulfuric Acid Concentration ($\mu\text{mol/g}$)
1	8.2	0
2	7.7	5
3	7.3	10
4	6.7	20
5	6.2	30
6	5.5	90
7	4.5	200
8	3.5	270
9	2.5	360

3.2.3.2 Central Composite Design (CCD)

The goal of this set of batch experiments was to see the effect of moisture content on methane oxidation in the presence of sulfuric acid. Moisture content is the most important parameter affecting methanotrophs behaviour to oxidize methane, so this experiments were designed to determine its effect on V_{max} at different pH values. Moreover, investigating the interaction effect between moisture content and pH, as two independent variables, was another objective of this experiment.

Central Composite Design (CCD) was used to study the effect of different MC and pH values on V_{\max} . Along with changing the moisture content of compost, the pre-determined amount of sulfuric acid was added to the bottles to change the initial pH values. Other factors such as temperature, nutrients content, methane concentration, and oxygen concentration were kept constant during the experiments.

Central Composite Design (CCD) is commonly used to design response surface experiments. Central composite designs contain a factorial or fractional factorial design with center points, augmented with a group of star points which allow estimation of curvature. This design is commonly used to estimate first and second order terms efficiently and also to model a response variable with curvature (Wu et al, 2000).

In CCD, the distance from the center of the design space to a factorial point is ± 1 unit for each factor, however, the distance from the center of the design space to a star point is $|\alpha| > 1$. Depending on the number of factors, the precise value of α is different. Also, the number of center point runs is dependent on the type of design and also the number of factors involved in the design. The star points represent new low and high values for each factor in the design. The value of α is calculated using Equation 3-6.

Equation 3-6:
$$\alpha = [2^k]^{1/4}$$

Where k is the number of factors.

In central composite design with 2 factors, the value of α is $\sqrt{2}$ which is equal to 1.414. Figure 3-3 shows the coded values in this design. In total, 13 runs are needed in CCD with 2 independent variables which contains 4 factorial points, 4 star points and 5 center points.

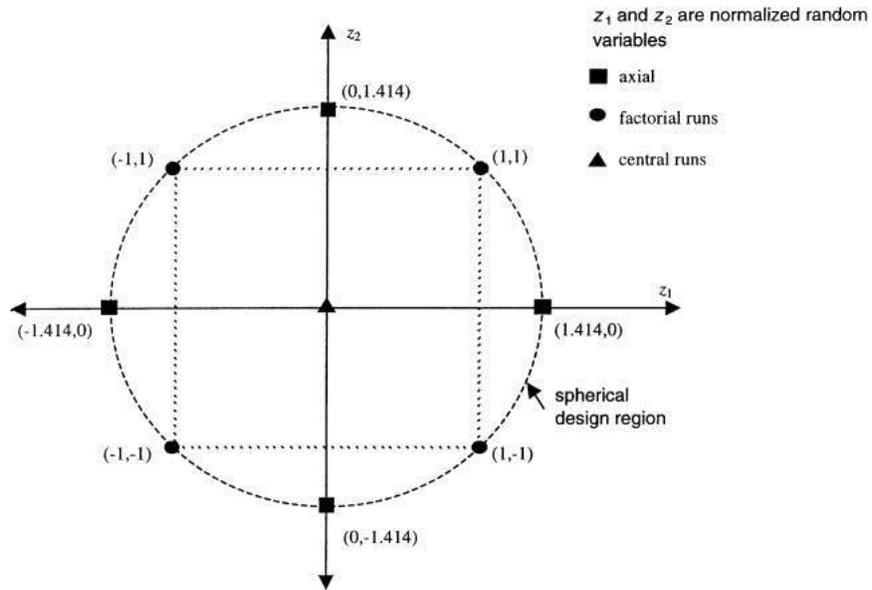


Figure 3-3: Location of different points in Central Composite Design (CCD) with two factors

Table 3-3 represents the coded and un-coded values of the independent variables which are pH and moisture content in the central composite design (CCD). Moisture content of 44% (in the range of optimum MC for compost), and pH of 6.5 were chosen as the central point of CCD.

Table 3-3: Coded and un-coded values of independent variables in Central Composite Design

Runs	MC (coded)	pH (coded)	MC (un-coded)	pH (un-coded)
1	-1	-1	34	5.1
2	-1	+1	34	7.9
3	+1	-1	54	5.1
4	+1	+1	54	7.9
5	0	-1.414	44	4.5
6	0	+1.414	44	8.5
7	-1.414	0	30	6.5
8	+1.414	0	58	6.5
9	0	0	44	6.5
10	0	0	44	6.5
11	0	0	44	6.5
12	0	0	44	6.5
13	0	0	44	6.5

3.3 Column Experiments

The column experiments have been used commonly to simulate landfill cover systems under controlled conditions (Kightley et al, 1995; Hilger et al, 2000; Visvanathan et al, 1999). In this study, column experiments were conducted to determine the methane oxidation rate as well as methane removal efficiency at different pHs in compost biofilters under controlled and normal laboratory conditions. The main objectives were to do the following:

- Study changes in methane oxidation rate with time.
- Determine pH changes in methane biofilters over time.
- Up-scale the results found from batch experiments.
- Assess the impact of acidic pH on methane oxidation efficiency.
- Determine the optimum pH for maximum methane oxidation rates.
- Determine the minimum pH without any inhibitory effect on methanotrophs growth.

3.3.1 Column Design

A schematic diagram of the compost columns used for this research is shown in Figure 3-4. Columns were made of 1.2 m long rigid plexiglass tube with an inner diameter of 14 cm and 0.635 cm thickness. The top and bottom of each column were capped with two plexiglass endcaps fitted with rubber O-rings. A perforated plate was placed at the bottom of the column to support packing media. The perforated plate covered with fine mesh was located over the 10 cm gravel layer to ensure even gas distribution across the column surface area. Each column had one inlet port at the bottom for methane supply, one inlet and one outlet for air supply at the top. Sampling ports were drilled along the height of the columns at 6 cm intervals. Ports were threaded for 1/8" NPT fittings and were fitted with 1/4" Swagelok adaptors. Water bubbles were also used at connections and joints to do the leakage test.

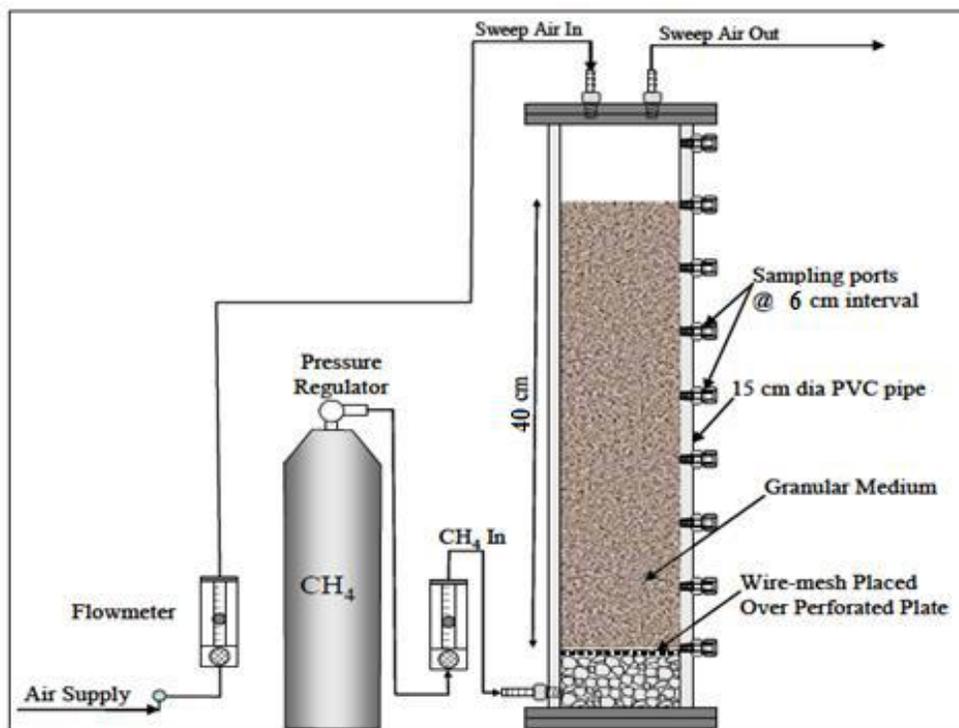


Figure 3-4: Schematic diagram of laboratory biofilter columns

The columns used in this work were filled with compost as a granular material. Compost was collected from the East City of Calgary, and sieved with a 2.5 mm screen to retain particles less than 2.5 mm in diameter. Compost was packed in the column in 10 cm increments up to a height of 40 cm to ensure uniform density. Air was supplied across the middle of each column through ports along the column height. Methane (99 % pure) was supplied from the bottom of columns. The inlet gas pressure was maintained at 2 psi. Flow meters were used to control methane and air flow rates during the experiments. The columns were located in the laboratory where the temperature is held at 20°C and supported in a steel structure for safety purposes as shown in Figure 3-5. Vertical gas concentration profile and headspace concentrations were monitored regularly. The CH₄ concentration at different points were determined using a portable micro gas chromatograph (GC) VARIAN CP 4900. The gas composition was determined using a GALAXIE software package in communication with the GC.

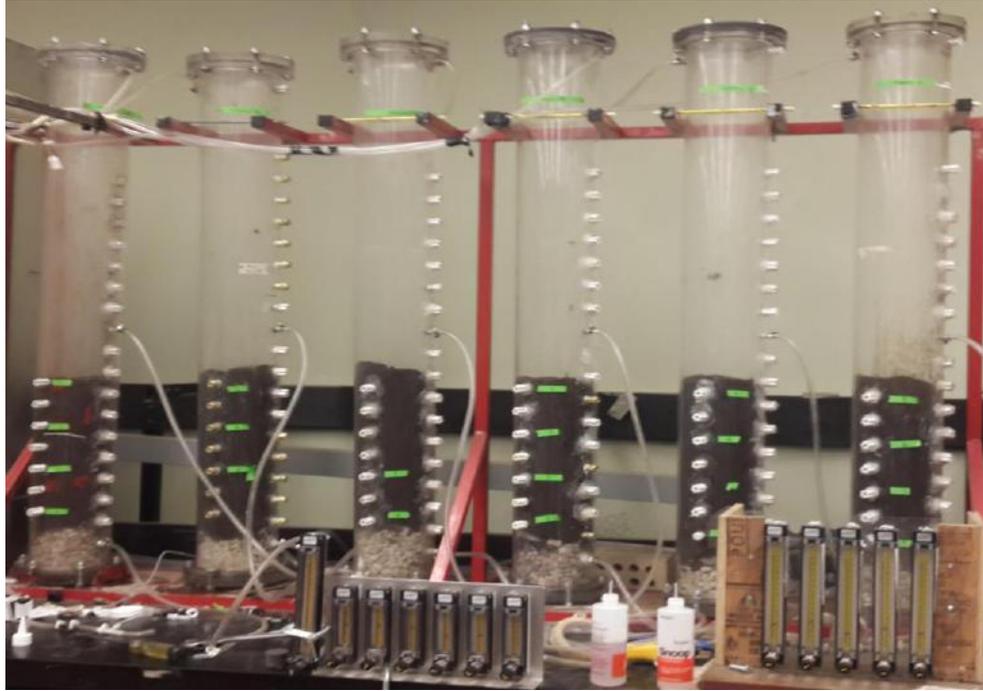


Figure 3-5: Photograph of biofilter columns

3.3.2 Experimental Design

In this experiments, methane was supplied from the bottom with a flow rate of 3 ml/min, which corresponds to a CH_4 flux of $180 \text{ g/m}^2/\text{day}$. Air was provided from the middle port to achieve passive aeration condition. In the first few days, all columns were received air with an identical flow rate of 20 ml/min. Since the oxygen concentration at the bottom of columns were low (less than 1%), the air flow rate was changed to 60 ml/min then. Columns were operated at 38% moisture content which was obtained from the batch experiments as the optimum value. Columns were operated for 25 days at compost original pH which was 8.5. Once maximum removal efficiencies were achieved in all 6 columns, and the methanorophic bacteria reached their maximum capability to oxidize methane, the columns were dismantled to run them at different pHs.

After dismantling, moisture content and pH were both measured for different layers in the columns to monitor their change over time. Then, the pre-determined concentrations of sulfuric acid were added to the compost to change its pH. In total, 6 columns were used at 3 different pHs with a replicate of each one. Table 3-4 shows the experimental design for column experiments.

Table 3-4: Experimental design for column experiments

Experimental Columns	MC	pH
1	38	7.5
2 (Replicate of column 1)	38	7.5
3	38	4.5
4 (Replicate of column 3)	38	4.5
5	38	2.5
6 (Replicate of column 5)	38	2.5

3.3.3 Methane Removal Efficiency

The methane removal efficiency was calculated with the Equation 3-7. The CH₄ concentration in the effluent was determined using a portable micro gas chromatograph (GC) VARIAN CP 4900. The outlet flow rates were measured using a mass balance for N₂.

Equation 3-7:
$$Removal\ Efficiency = \frac{Q_{in} \cdot C_{CH_4,in} - Q_{out} \cdot C_{CH_4,out}}{Q_{in} \cdot C_{CH_4,in}} \times 100$$

Where:

Q_{in} = inlet flow rate (m³/day)

Q_{out} = outlet flow rate (m³/day)

$C_{CH_4,in}$ = methane concentration at the inlet = 0.99%

$C_{CH_4,out}$ = methane concentration at the outlet (g/m³)

To calculate the outlet flow rate, a mass balance for N₂ were performed like the following:

Equation 3-8:
$$Q_{Air} \times C_{N2,in} = Q_{out} \times C_{N2,out}$$

So, the Q_{out} will be obtained using Equation 3-9:

Equation 3-9:
$$Q_{out} = \frac{Q_{Air} \times C_{N2,in}}{C_{N2,out}}$$

Where:

Q_{Air} = sweep air flow rate (m³/day)

$C_{N2,in}$ = nitrogen concentration at the inlet = 79%

$C_{N2,out}$ = nitrogen concentration at the outlet

3.4 Empty Bed Residence Time (EBRT) and True Residence Time (τ)

Empty Bed Residence Time (EBRT) and true residence time (τ) are two important factors involved in column experiments. EBRT is the time gas takes to flow through the whole bed volume and is calculated by dividing the reactor volume by gas flow rate with Equation 3-10.

Equation 3-10:
$$EBRT = \frac{V_f}{Q}$$

Where:

V_f = the total bed volume (ml)

Q = flow rate (ml/min)

$EBRT$ = Empty Bed Residence Time

In general, higher residence times ensure higher removal efficiencies (Limbri et al, 2013). By increasing the residence time from 5 to 20 minutes, an improve of 70% in CH₄ oxidation efficiency was observed (Sly et al, 1993). Although EBRT values for VOC removals are in the

order of seconds to minutes, higher values are suggested for CH₄ degradation (Nikiema et al, 2007; Limbri et al, 2013).

On the other hand, true residence time (τ) is calculated by multiplying the total granular medium bed volume by the porosity divided by the gas flow rate using the Equation 3-11.

Equation 3-11:
$$\tau = \frac{V_f \theta}{Q}$$

Where:

V_f = the total bed volume (ml)

Q = flow rate (ml/min)

θ = porosity

τ = True Residence Time

3.4 Microbiological Analysis

Microbiological analysis was performed including the DNA extraction and Illumina sequencing of 16S rRNA and *pmoA* genes in order to investigate the microbial population in column samples. DNA was extracted from 0.5 g of compost using the FastDNA SPIN Kit for Soil (MP Biomedicals) following the manufacturer's instruction with the following modification. Since it was difficult to get the high purity DNA from compost, additional purification steps using 5.5 M guanidine thiocyanate (GTC) were introduced in the washing step (Knief et al, 2003). DNA was eluted in Qiagen buffer EB (Qiagen). The DNA concentration was determined by using a Qubit Fluorometer with a Quant-iTTM dsDNA HS Assay Kit (Invitrogen) and the purity of DNA was determined by using Nanovalue PlusTM (GE Healthcare).

The hypervariable V4 region of the 16S rRNA gene were amplified according to the protocol(http://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparat

[ion.html](#)). Primers used for amplification were 341f (forward primer) and 785r (reverse primer). The Illumina MiSeq system (Illumina, Inc.) was used to sequence 16S rRNA gene amplicons using the standard illumine protocol (mentioned above). Communities were analyzed using QIIME software (Caporaso et al, 2010). This allowed the determination of ecological statistics and detection of individual species.

Chapter 4: EXPERIMENTAL RESULTS AND DISCUSSION

4.1 Batch Experiments

In this experiments, the influence of different pH and moisture contents on methane oxidation were evaluated by measuring maximum methane oxidation rate (V_{\max}). Using the V_{\max} values, the relationship between different factors and methane oxidation rates were obtained. The data were presented for pre-incubation experiments, OFAT experiments and also CCD batch experiments.

4.1.1 Pre-Incubation Experiments

In order to measure oxidation kinetic parameters, it is essential to make sure that microbial growth has reached a steady state condition. For this reason, in most V_{\max} studies, pre-incubation is necessary as the preliminary step. Pre-incubation is the process through which the media is microbially activated to ensure a steady state methanotrophic growth.

Pre-incubation experiments were performed using 7 big glass bottles. 1000 ml glass bottles were filled with 105 g of compost at 30% moisture content. 70 ml methane were added to the bottles to maintain 7.5% initial concentration of methane. CH_4 oxidation rate over time are presented in Figure 4-1. The CH_4 oxidation rates increase with time until reaching the steady state condition. Observing a constant oxidation rate with time is an indication of steady state condition. In this condition, the methanotrophic bacteria reach their maximum capacity to oxidize methane and we observe the maximum methane oxidation rate. Once the steady state condition has been reached, the samples were used for batch experiments.

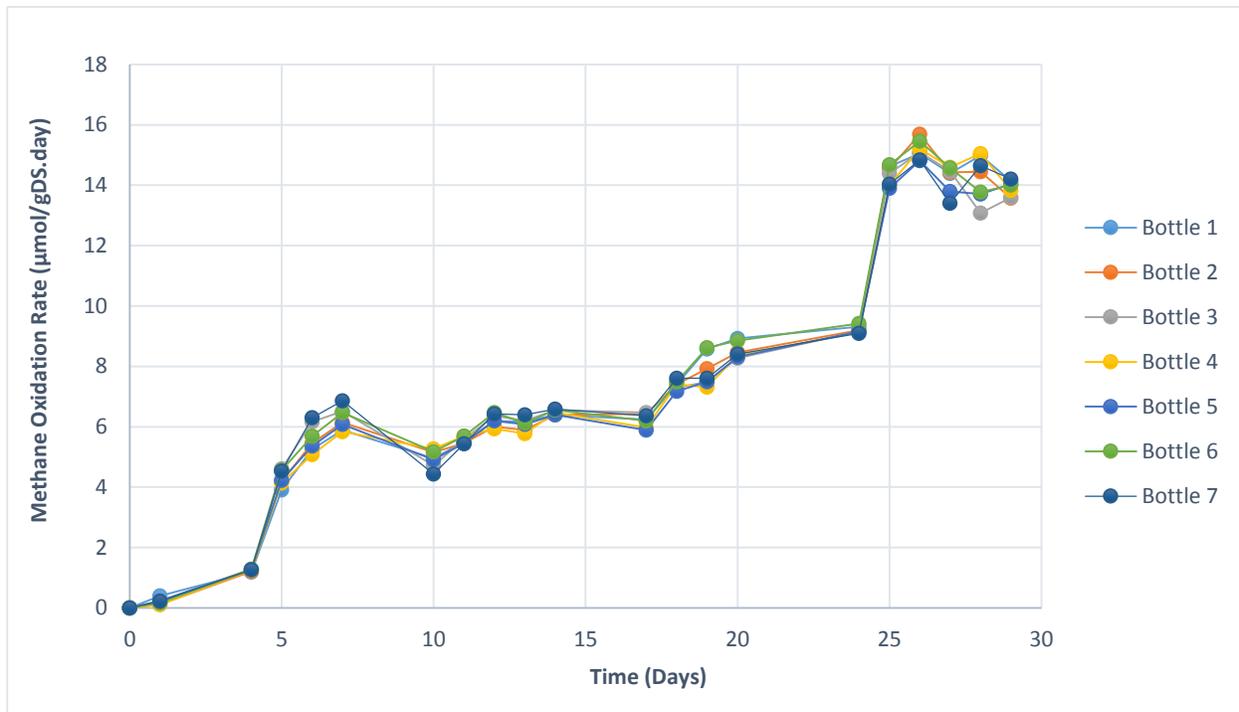


Figure 4-1: Methane oxidation rate vs time for pre-incubation experiments

4.1.2 OFAT Batch Experiments

This set of batch experiments were done by placing 15 grams of compost into 250 ml glass bottles at constant 30% moisture content and different pH values. V_{\max} values were calculated using 5 different methods namely; Hanes method, Lineweaver-Burk method, Eadie and Hofstee method, and non-linear regression using SPSS IBM STATISTICS software as well as EXCEL SOLVER function.

Figures 4-2 presents the Michaelis-Menten Kinetic standard and linearized plots for batch experiments at pH 8.2. V_{\max} and K_m values for each pH from different plots are presented in this figure. The plots from 4 different methods for batch experiments at other pH values can be found in appendix A.

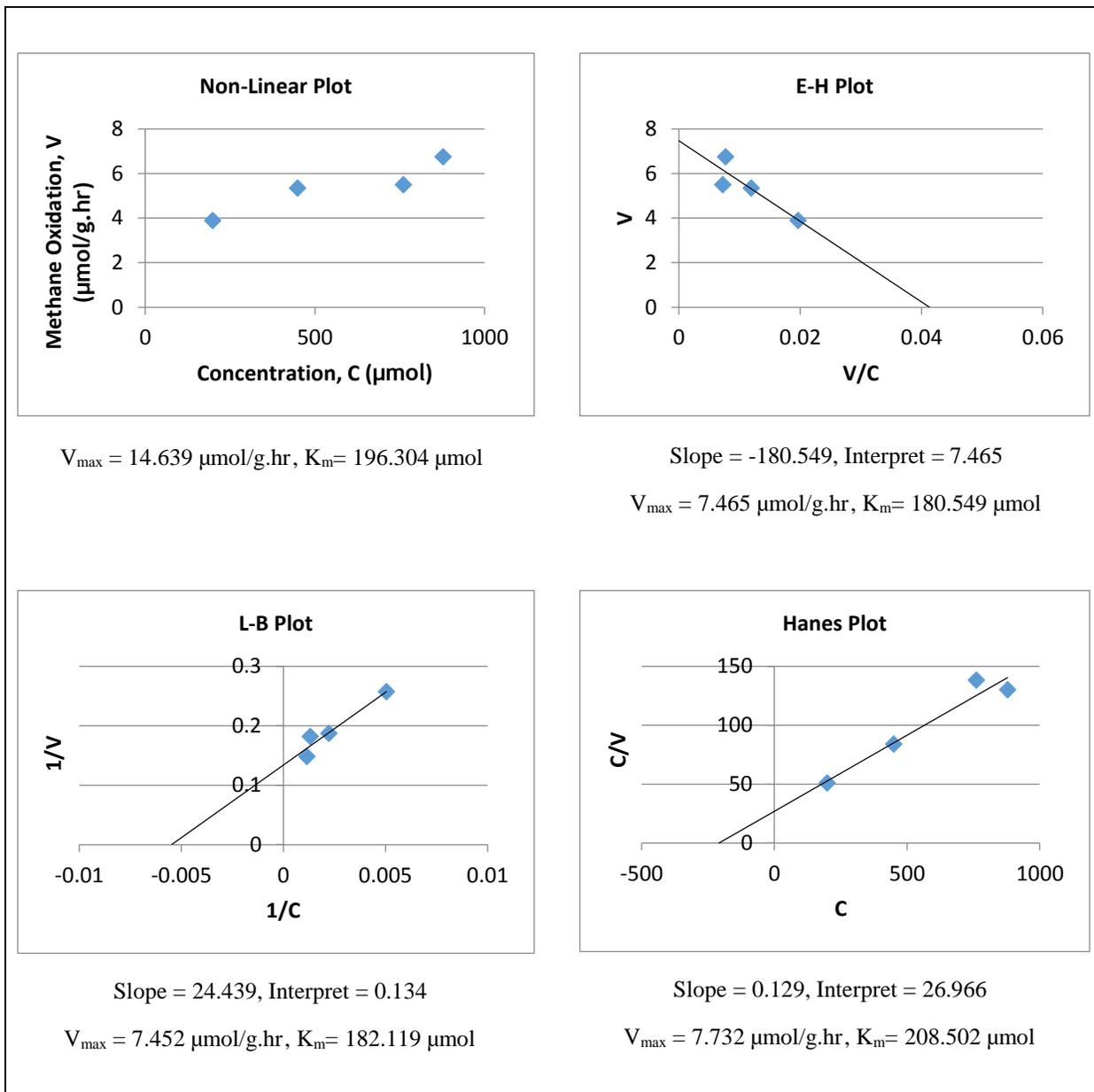


Figure 4-2: Michaelis-Menten standard and linearized graphs and V_{max} values for the OFAT experiment at pH=8.2

Table 4-1 presents the V_{max} values for different batches from 5 different methods. It is important to note that the linearized methods are all outdated and there is no need to linearize the data, and doing so will reduce the accuracy of our calculated V_{max} and K_m values. When we linearize the data, it assumes that the points follow a Gaussian distribution with equal SD at each

point. Once we break this assumption, the linearization no longer makes sense. Since non-linear estimates are better than linearized ones, we used the values we got from non-linear regression method. If the experimental data do not have any issues, the SOLVER and non-linear regression using SPSS will be comparable. As it is shown in Table 4-1, the experimental data do not have any issues, since the difference between V_{\max} values from non-linear regression using SPSS and EXCEL SOLVER are closed to zero.

Table 4-1: V_{\max} values from different methods for OFAT batch experiments

pH	Hanes V_{\max} ($\mu\text{mol/g. hr}$)	E-H V_{\max} ($\mu\text{mol/g. hr}$)	L-B V_{\max} ($\mu\text{mol/g. hr}$)	SPSS V_{\max} ($\mu\text{mol/g. hr}$)	Excel SOLVER V_{\max} ($\mu\text{mol/g. hr}$)	Difference between SPSS and SOLVER values
8.2	7.732	7.465	7.452	14.639	14.639	0.00%
7.7	12.323	7.826	7.938	17.278	17.278	0.00%
7.2	12.068	8.307	8.365	19.153	19.153	0.00%
6.7	13.062	8.604	8.4	15.021	15.022	0.00%
6.2	11.074	7.936	7.884	13.573	13.573	0.00%
5.5	9.545	6.456	6.617	12.111	12.112	-0.01%
4.5	3.943	3.805	3.787	3.885	3.885	-0.01%
3.5	4.531	2.115	10.905	1.281	1.280	0.02%
2.5	-12.804	-0.023	-1.259	0.97	0.97	0.00%

V_{\max} data against different pH values were plotted in Figure 4-3. From 9 different pH values, we can see that the highest oxidation occurs at pH 7.2. Therefore, it is concluded that neutral condition provides the favorable environment for methanotrophs growth. The experimental data show a significant methane uptake in pH range of 5.5-8.2. However, a sharp decrease in V_{\max} values was observed when we switch from pH 5.5 to pH 4.5. When we are in the acidic zone, the V_{\max} values decrease with a reduction in pH. Observing very low V_{\max} values for pHs below 4.5 indicates that there is no activity for methanotrophs below pH 4.5. Methanotrophic bacteria are not able to tolerate too acidic conditions, and they cannot survive in such an unfavorable condition.

Since the experimental data does not follow a linear trend, we can conclude that there is no linear relationship between V_{\max} and pH.

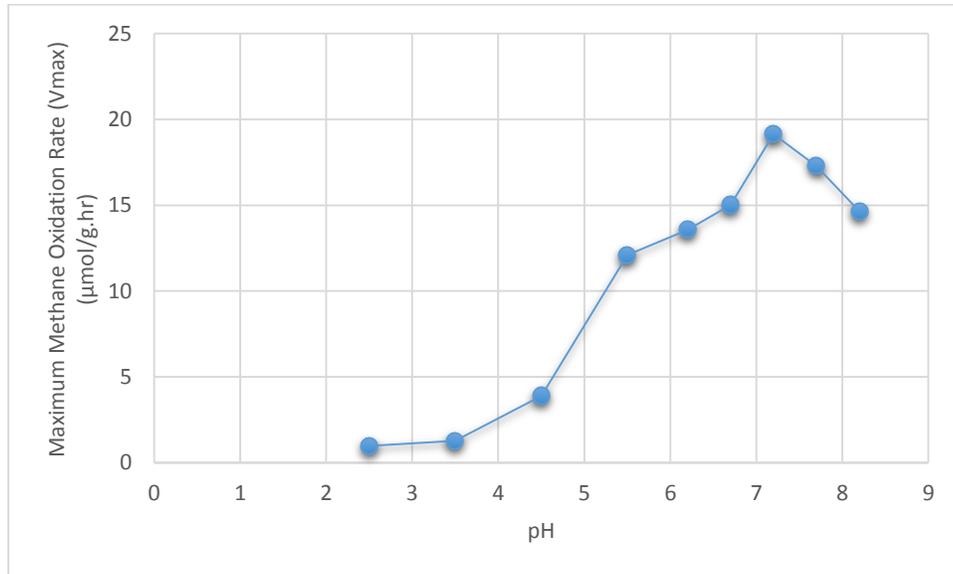


Figure 4-3: Maximum methane oxidation rate (V_{\max}) vs pH for OFAT batch experiments

4.1.3 Central Composite Design (CCD) Batch Experiments

In order to see the effect of moisture content on maximum methane oxidation rate (V_{\max}) in the presence of sulfuric acid, another set of batch experiments were conducted by changing MC as well as pH. Central composite design (CCD) were used for this set of experiments. 250 ml glass bottles were filled with 14 grams of compost at different moisture content and pH values. V_{\max} values were calculated using 5 different methods, same as the previous batch experiments. Afterwards, the values from non-linear regression in SPSS were used to do the statistical analysis.

Figures 4-4 presents the Michaelis-Menten Kinetic standard and linearized plots for batch experiments at 34% moisture content and pH 5.1. V_{\max} and K_m values from different plots are presented in this figure. The plots from 4 different methods for batch experiments at other pH and MC values can be found in appendix B.

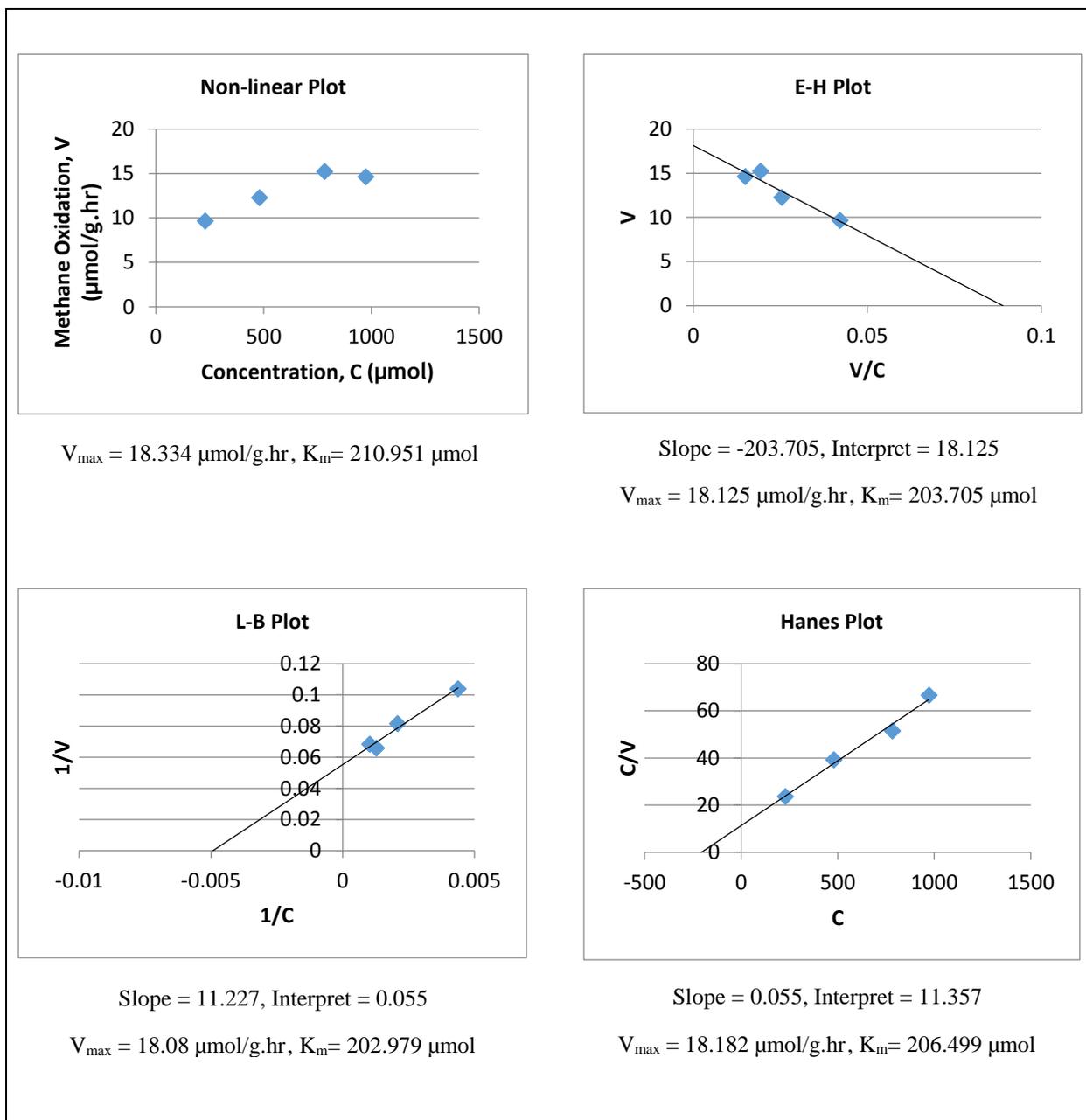


Figure 4-4: Michaelis-Menten standard and linearized graphs and V_{max} values for the CCD batch experiment at pH=5.1 and MC=34%

The V_{max} values from different methods for CCD batch experiments are shown in Table 4-2 for different combination of MC and pH values. Although we expected to see the lowest V_{max} at the lowest pH (from the previous set of batch experiments), the lowest V_{max} was happened at the

highest moisture content. So, at pH=4.5 and MC=44%, the V_{\max} value was 10.414 $\mu\text{mol/g.hr}$, which is much higher than 2.736 $\mu\text{mol/g.hr}$ (the V_{\max} value at pH=6.5 and MC=58%). This indicates that moisture content plays an important role in our experiments. The results from previous set of experiments indicated that the V_{\max} value was really low at pH=4.5 and MC=30% (3.885 $\mu\text{mol/g.hr}$), however, when sufficient moisture levels are maintained, methanotrophic bacteria are able to oxidize methane efficiently. Therefore, diverse native population of MOB (types I and II) have the ability to adapt to changing acidic conditions at high moisture values.

Also, Table 4-2 shows that at a constant pH, increasing the moisture content decreases the V_{\max} values significantly. The decrease in V_{\max} values which was observed as a results of increasing moisture content were from 18.334 $\mu\text{mol/g.hr}$ to 8.781 $\mu\text{mol/g.hr}$ by an increase in MC from 34% to 54%, from 18.296 $\mu\text{mol/g.hr}$ to 8.994 $\mu\text{mol/g.hr}$ by an increase in MC from 34% to 54%, and from 19.127 $\mu\text{mol/g.hr}$ to 2.736 $\mu\text{mol/g.hr}$ by an increase in MC from 44% to 58%, at pH 5.1, 7.9, and 6.5, respectively. This reduction in V_{\max} values due to an increase in moisture content can be explained by the fact that high moisture content limits the methane oxidation rate by acting as a barrier for CH_4 and O_2 flow. When the volume of water significantly exceeds the voids volume in soil, the air- filled voids lose their interconnection and the gases have to diffuse in the liquid phase (Since the molecular diffusion in water is 104 times higher than in air). Therefore, this phenomenon slows down the gaseous transport in compost leading to a reduction in CH_4 oxidation rate.

Moreover, Table 4-2 highlights that while we are within the pH range of 5-8, decreasing the pH at constant moisture content does not change the V_{\max} significantly. When we decrease the pH from 7.9 to 5.1 at both constant MC of 34% and 54%, the changes in V_{\max} values were really negligible (from 18.296 $\mu\text{mol/g.hr}$ to 18.334 $\mu\text{mol/g.hr}$ at MC=34% and from 8.994 $\mu\text{mol/g.hr}$ to

8.781 $\mu\text{mol/g}\cdot\text{hr}$ at MC=54%). However, decreasing the pH from 6.5 to 4.5 at MC=44% made a considerable reduction in V_{max} (from 19.127 $\mu\text{mol/g}\cdot\text{hr}$ to 10.414 $\mu\text{mol/g}\cdot\text{hr}$). Therefore, while we are beyond the pH range of 5-8, either decreasing or increasing the pH, causes a significant change in CH_4 oxidation rate.

Table 4-2: V_{max} values from different methods for CCD batch experiments

MC %	pH	E-H V_{max} ($\mu\text{mol/g}\cdot\text{hr}$)	L-B V_{max} ($\mu\text{mol/g}\cdot\text{hr}$)	Hanes V_{max} ($\mu\text{mol/g}\cdot\text{hr}$)	SPSS V_{max} ($\mu\text{mol/g}\cdot\text{hr}$)	Excel SOLVER V_{max} ($\mu\text{mol/g}\cdot\text{hr}$)	Difference between SPSS and SOLVER values
34	5.1	18.125	18.08	18.182	18.334	18.334	0.00%
34	7.9	17.953	17.932	18.45	18.296	18.296	0.00%
54	5.1	5.827	5.443	6.832	8.783	8.781	0.02%
54	7.9	7.083	8.079	7.851	8.994	8.994	0.01%
44	4.5	9.994	9.956	10.513	10.414	10.414	0.00%
44	8.5	19.573	19.565	19.988	20.346	20.346	0.00%
30	6.5	17.661	17.658	18.313	18.234	18.234	0.00%
58	6.5	2.589	2.6	2.806	2.736	2.736	-0.01%
44	6.5	17.4	17.219	19.294	18.648	18.648	0.00%
44	6.5	18.251	18.119	20.535	19.683	19.683	0.00%
44	6.5	20.182	20.06	20.433	20.46	20.460	0.00%
44	6.5	18.632	18.439	19.526	19.229	19.229	0.00%
44	6.5	16.913	16.791	18.096	17.598	17.598	0.00%

4.2 Statistical Analysis for CCD Batch Experiments

To interpret the results obtained from this experiments, statistical analysis in MINITAB 17.3 was performed. ANOVA table, main effect plots, interaction plots, contour and surface plots, as well as the best-fitted model are presented here.

4.2.1 Analysis of Variance (ANOVA)

As the discussion above implies, a combination of moisture content and pH control the methane oxidation in the experiments done. This leads to observing different maximum CH_4

oxidation rate in different runs of the experiments. Therefore, a two-way ANOVA is performed to evaluate the extent of the effects and interactions of these factors on the maximum methane oxidation rate (V_{\max}).

To analyze the effects of moisture content and pH, a two-way ANOVA has been performed using MINITAB 17.3. The two-way ANOVA compares the mean differences between groups that have been split on two independent variables. The primary purpose of a two-way ANOVA is to understand if there is an interaction between the two independent variables on the dependent variable (Montgomery, 2008). There are three sets of hypothesis with the two-way ANOVA.

The null hypotheses for each of the sets are given below:

1. The population means of the first factor are equal. This is like the one-way ANOVA for the row factor. (Null hypothesis: $H_0: \mu_1 = \mu_2 = \dots = \mu_k$, Alternative hypothesis: H_1 : Means are not all equal; Where k is the number of independent comparison groups)
2. The population means of the second factor are equal. This is like the one-way ANOVA for the column factor.
3. There is no interaction between the two factors. This is similar to performing a test for independence with contingency tables. (null hypothesis: $H_0 =$ no significant interaction between the two factors)

The results from the ANOVA analysis in the MINITAB software were interpreted using the significance values. MINITAB provides the p-values for the specified confidence level and expresses the importance of the parameter in terms of the significance. For main effects, our null hypothesis is like there is no effect from our independent variable on the response. If this p-value is lower than the confidence level used for the analysis, in this case 0.05 (95%), we conclude that the effect of this factor on our response variable is highly significant. On the other hand, if it is

higher than the confidence level, we confirm that there is no significant effect from the independent variable on the response.

Table 4-3 presents the ANOVA table results for Central Composite Design experiment. Looking at the significance value obtained for MC, it can be observed that the p-value from ANOVA table (0.000) is less than the significance level of 0.05. Therefore, the probability for the null hypothesis to be true is low enough and we can reject the null hypothesis and conclude that moisture values have a statistically significant effect on maximum CH₄ oxidation rate. This is understandable, as changing the moisture content directly changes the resulting V_{max}. The same happened for the pH values. The results reveal that the p-value for pH (0.047) is less than the 0.05, so the null hypothesis can be rejected. Therefore, at a confidence level of 0.05, the pH values affect the oxidation rate significantly.

Moreover, the significance value obtained for the square effect of MC (0.001) is lower than the confidence level, meaning that there is a significant effect from MC² on CH₄ oxidation. However, the significance value for the square effect of pH (0.072) is more than 0.05 and there is no sufficient data available to reject the null hypothesis. Thus, accepting the null hypothesis, we can conclude that the effect of pH² on CH₄ oxidation is not important. Furthermore, the table suggests that with a p-value of 0.954, the interaction between the moisture content and pH does not have a statistically significant effect on V_{max}. This means that simultaneous influence of these two parameters can be additive.

Table 4-3: ANOVA table for Central Composite Design

Source	Degree of	Sum of	Mean	F-Value	P-Value
Model	5	362.507	72.501	16.45	0.001
Linear	2	233.137	116.569	26.45	0.001
MC	1	207.616	207.616	47.12	0.000
pH	1	25.521	25.521	5.79	0.047
Square	2	129.354	64.677	14.68	0.003
MC*MC	1	120.057	120.057	27.25	0.001
pH*pH	1	19.826	19.826	4.5	0.072
2-Way Interaction	1	0.016	0.016	0.00	0.954
MC*pH	1	0.016	0.016	0.00	0.954
Error	7	30.845	4.406		
Lack-of-Fit	3	26.182	8.272	7.49	0.041
Pure Error	4	4.664	1.166		
Total	12	393.352			

Figure 4-5 shows the main effects plot for V_{\max} . Looking at the main effects plot, it can be observed that the maximum V_{\max} occurs at MC=38% and pH=7.2. In the left plot, after reaching the maximum point (MC=39%), increasing the MC decreases the V_{\max} . This result can be confirmed based on the V_{\max} values from Table 4-2. The similar trend happened for pH values. After pH=7.2, decreasing the pH causes a reduction in V_{\max} values. This V_{\max} reduction may happen due to the sulfuric acid presence. At lower pHs, higher concentrations of sulfuric acid are present which hinders the methanotrophs growth. Therefore, methanotrophs are affected by the inhibitory effect of low pHs and thus apparently they are not able to oxidize methane properly. It is important to note that while we move within the pH range of 6-8, the V_{\max} values are quite equal.

However, beyond that range ($6 < \text{pH} < 8$), the V_{max} values are fairly different and we can see a sharp decrease in V_{max} by decreasing the pH.

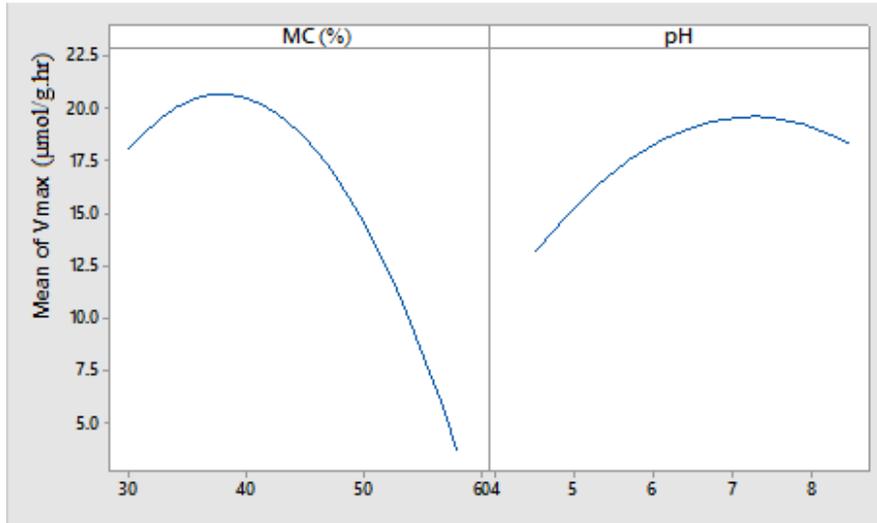


Figure 4-5: Plot of main effects of MC and pH on V_{max} values for the CCD batch experiments

Figure 4-6 presents the plot for interaction effect of MC and pH on V_{max} . Since the lines do not intersect each other, it is concluded that there is no interaction effect of MC and pH on CH_4 oxidation. These results are also approved based on the p-value for the interaction effect from Table 4-3.

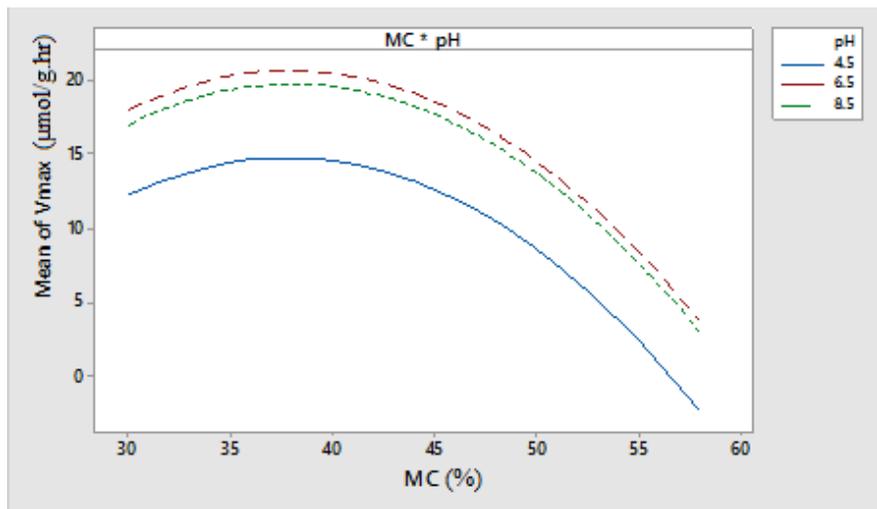


Figure 4-6: Plot of interaction effect of MC and pH on V_{max} values for the CCD batch experiments

4.2.2 Model Summary

In this part of statistical analysis, the results of the curvature model which fits the experimental data are presented. Table 4-4 indicates the different R^2 values for the CCD model. Since the value of R^2 is very close to 1 (0.9216), it is concluded that the model fits well the experimental data.

Table 4-4: Model summary for CCD batch experiments

R-sq	R-sq (adj)	R-sq (pred)
92.16	86.56	49.98

Table 4-5 shows the coded and un-coded values of coefficients in this model. In the coded form, the coded values of each factor should be used to find the V_{\max} value. However, in the un-coded form, the exact value of each parameter should be entered into the model to get the appropriate V_{\max} value.

The standard deviation of the estimate of a regression coefficient measures how precisely the model estimates the coefficient's unknown value. The standard error of the coefficient (SE Coef) is always positive. The standard error of the coefficient is used to measure the precision of the estimate of the coefficient. The smaller the standard error, the more precise the estimate. Dividing the coded value of coefficient by its standard error calculates a t-value. If the p-value associated with this t-statistic is less than our alpha level (0.05), we conclude that the coefficient is significantly different from zero. Looking at the SE Coef values for different parameters in Table 4-5, we can identify the accuracy of the estimation of coefficients in the model. The SE Coef values for constant, MC, and pH coefficients are 0.939, 1.04 and 1.05, respectively. Since these values are really small, it is concluded that the estimation of the coefficients for these three parameters is

accurate enough. In contrast, much higher values are observed for the standard error of the coefficients of MC^2 , pH^2 , and $MC*pH$ (1.58, 1.6 and 2.1, respectively). Therefore, there is no precise estimation of the coefficients for these three factors.

Furthermore, using the standard error of the coefficients, we can interpret the importance of each factor on the response variable. The standard error of the MC coefficient is smaller than that of $MC*pH$. Therefore, our model was able to estimate the coefficient for MC with greater precision. In fact, the t-value of the $MC*pH$ coefficient (0.06) is too small to declare statistical significance. The resulting p-value (0.954) is much greater than our confidence level (0.05), so that we cannot conclude this coefficient differs from zero. We can remove the $MC*pH$ variable from our regression model and continue the analysis. The same can happen for the $pH*pH$ coefficient, since the p-value is higher than the level of confidence. However, the p-values for other factors (constant, MC, pH, and MC^2) are much lower than the confidence level, so it can be concluded that the coefficients for these factors are different from zero. Thus, we should continue the analysis considering these factors.

Table 4-5: The values of the coefficients for different parameters in the CCD model

Term	Coded-Coef	Uncoded-Coef	SE Coef	T-Value	P-Value
Constant	19.124	-82.8	0.939	20.37	0.000
MC	-7.17	3.173	1.04	-6.86	0.000
pH	2.54	12.09	1.05	2.41	0.047
$MC*MC$	-8.27	-0.0422	1.58	-5.22	0.001
$pH*pH$	-3.39	-0.848	1.6	-2.12	0.072
$MC*pH$	0.12	0.0044	2.1	0.06	0.954

Putting the un-coded values of the coefficients in the model, we come up with Equation 4-1 as the model which gives the V_{max} values for different MC and pHs.

$$\text{Equation 4-1: } V_{max} = -82.8 + 3.173 MC + 12.09 pH - 0.0422 MC^2 - 0.848 pH^2 + 0.0044 MC \times pH$$

4.2.3 Response Surface

Response Surface Methodology (RSM) is used to lead the researcher rapidly and efficiently to the vicinity of the optimum region (Imandi et al, 2007). Prior to performing the experiments, the region is of course unknown, this is why choice of the experimental design method is of great importance. A design that produces an estimate with equal accuracy in all directions provides a more unbiased response surface.

Response surface methodology (RSM) was implemented to investigate the relationship between the fixed variables (moisture content and pH) and the response (V_{max}) for the analysis of the effect of pH on methane oxidation efficiency. The response surface plot sketched in Figure 4-7 provides a three-dimensional (3D) view of the response (V_{max}) surface versus various combinations of independent parameters (MC and pH) selected based on the Central Composite Design (CCD). This response plot shows the amount of V_{max} in each point, and also indicates the exact amount of V_{max} at around pH 7.2 and moisture content of 38% which is the highest amount of methane oxidation rate. The methane oxidation efficiency reduces as we move further from this point, implying that the response attributed to V_{max} reduces by either increasing or decreasing in any of the studied variables.

As evident from the graph, when operated at pH 4.5 and moisture content of 58%, the CCD batch experiments yield the lowest V_{max} value. This is due to the fact that too low pH and high

moisture both have an inhibitory effect on methane oxidation efficiency. However, when an optimum moisture is provided for the methanotrophic bacteria, they are still able to oxidize methane even at low pH. This can be confirmed by observing a relatively high V_{\max} value at pH 4.5 and MC 38% ($12.5 \mu\text{mol/g.hr}$). In contrast, Figure 4-7 highlights that very high moisture inhibits methane oxidation rate even at optimum pHs. For example, at MC of 58%, the V_{\max} values are not dependent on pH, and methane oxidation rates are too small at all different pHs. This indicates a very important role of moisture content in methane oxidation efficiency.

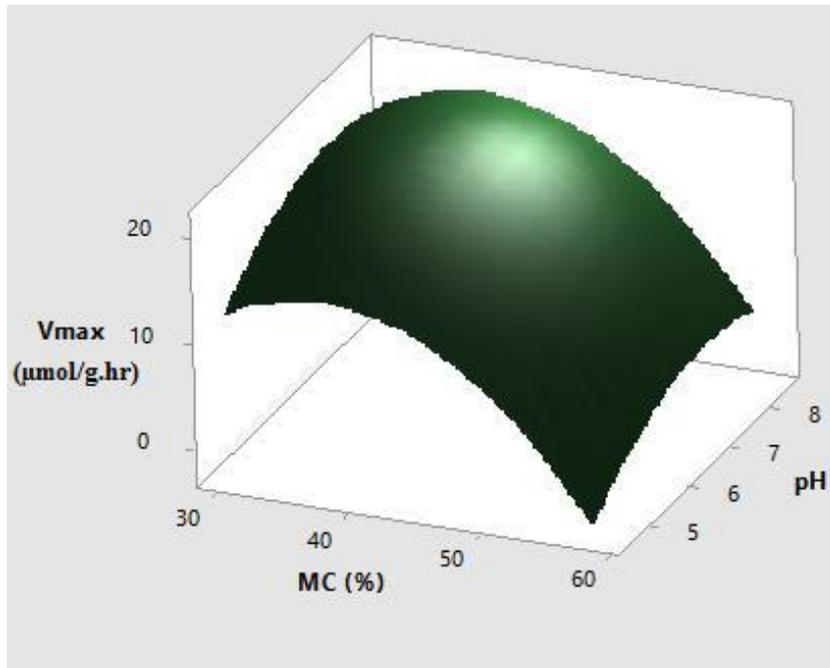


Figure 4-7: The response surface plot of V_{\max} vs pH and MC

The contour plots are also presented in Figures 4-8 and 4-9. As it can be shown in Figures 4-8 and 4-9, contour plots have a symmetrical shape approximately containing circular contours and they represent the V_{\max} responses on the MC-pH plane. The elliptical contours show the optimum point which is located at the center of the region and has the highest amount of maximum

methane oxidation rate (V_{\max}). The contour levels revealed a peak centered in the vicinity of 38% moisture and pH 7.2.

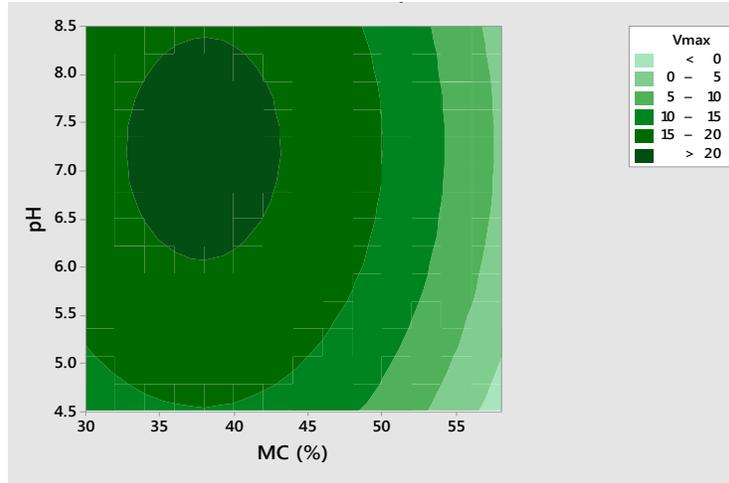


Figure 4-8: The contour plot of V_{\max} vs pH and MC (%) (area only)

Also, Figure 4-9 indicates that while we are located within the MC range of 33-43% (close to the optimum moisture), a constant V_{\max} of 20 $\mu\text{mol/g.hr}$ is observed. However, beyond the given range (33% <MC< 43%), methane oxidation rate drops suddenly.

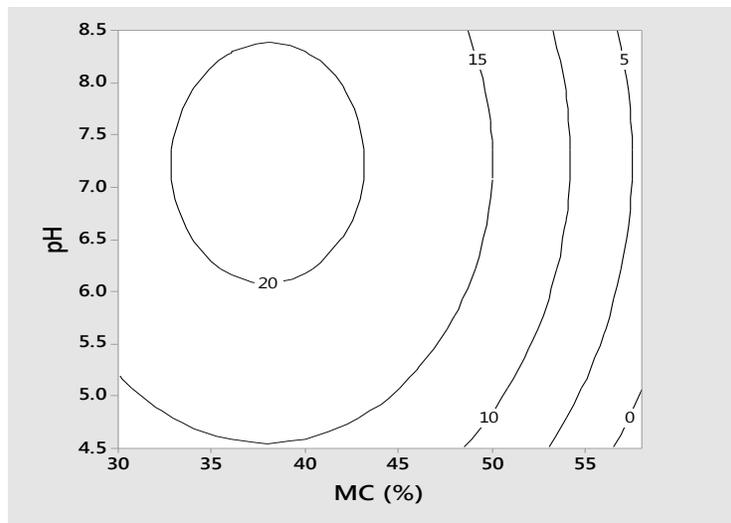


Figure 4-9: The contour plot of V_{\max} vs pH and MC (%) (area and contour lines)

4.3 Column Experiments

In this experiments, the influence of pH on methane oxidation efficiency was evaluated by measuring methane oxidation rate and removal efficiency. Using the methane oxidation rate in each column, the effect of different pH values on methane removal efficiency was obtained. Methane oxidation rates as well as removal efficiencies for columns before and after adding sulfuric acid were presented. Furthermore, vertical gas concentration profiles in each of the biofilter columns were presented at different days and stages.

Considering the oxidation results from the batch experiments, 3 different pHs (7.5, 4.5, and 2.5) with one replicate of each were used to pursue column experiments. From the CCD batch experiments, we know that the optimum MC for methane oxidation in compost medium is 38%, therefore, we used this value as the moisture content of 6 columns, according to the experimental design presented in Table 3-4. Biofilters were subjected to 99.99% pure methane at flow rate of 3 ml/min. Air was introduced at flow rates of 20 ml/min and followed by an increase to 60 ml/min in day 18. Over 56 days of operation, the overall performance of biofilter columns were discussed and compared. This comparison is based on the achieved removal rates and efficiencies.

4.3.1 Methane Removal Efficiency at Different pHs

Figure 4-10 and Figure 4-11 present the oxidation rate values in $\text{g/m}^2/\text{day}$ and oxidation efficiencies in % for 6 columns over time, respectively. Biofilter columns were operated at 3 different pHs as evident from Figure 4-10. The CH_4 oxidation rates increase with time until reaching the steady state condition in which the methanotrophic bacteria achieve their maximum capacity to oxidize methane and maximum methane oxidation rate happens. As shown in Figure 4-10, the maximum oxidation rate for 6 columns was achieved after 25 days of operation. The average maximum oxidation rate for 6 columns were $359.19 \text{ g CH}_4/\text{m}^3/\text{day}$ ($143.68 \text{ g/m}^2/\text{day}$) for

column one, 353.29 g CH₄/m³/day (141.32 g/m²/day) for column two, 356.07 g CH₄/m³/day (142.43 g/m²/day) for column three, 345.8 g CH₄/m³/day (138.32 g/m²/day) for column fore, 362.59 g CH₄/m³/day (145.04 g/m²/day) for column five, and 367.26 g CH₄/m³/day (146.9 g/m²/day) for column six. The maximum removal efficiencies for columns 1, 2, 3, 4, 5, and 6 at steady state conditions were 79%, 78%, 78%, 76%, 80%, and 81%, respectively. Since all 6 columns were operated under the same condition (without any acid and at compost initial pH) until the day 25, the CH₄ oxidation rates and removal efficiencies were pretty the same for all of them.

The second stage started with dismantling all six columns and adjusting their pH to the pre-determined values, according to the experimental design presented in Table 3-4. The performance of columns 1 and 2 was stable during the second stage, since they were still operated as the control columns without any sulfuric acid inside them (pH 7.5). They showed a constant oxidation rate which was the maximum rate after reaching the steady state condition (142.95 g/m²/day and 141.57 g/m²/day for columns 1 and 2, respectively). However, a steady decrease in performance of other 4 columns was observed during this stage, which is due to the pH changes.

As can be seen in Figure 4-10, methane oxidation rates for columns running at pH 4.5 decrease in the time period between days 25 to 47 after acid addition. After 47 days of operation, according to Figure 4-11, columns 3 and 4 reached the steady state condition with removal efficiencies of 29.5% and 30.7%, respectively. These values correspond to the average oxidation rates of 134.625 g /m³/day (53.85 g/m²/day), and 140.12 g /m³/day (56.05 g/m²/day), respectively. Therefore, considering the 30% removal efficiency at pH 4.5, it is concluded that methanotrophic bacteria are still able to oxidize methane at pH 4.5. In fact, we expected to see some CH₄ oxidation happening at pH 4.5, as we observed a considerable V_{max} value (3.885 μmol/g.hr) at pH 4.5 in batch experiments.

A similar trend was shown for columns 5 and 6 which were running at pH 2.5, however, they were unable to oxidize methane at the end of experiments. A sharp reduction in performance of these columns was observed in the period between days 25 to 39. After 14 days of operation at pH 2.5, no oxidation was observed for these two columns. This indicates that too acidic condition inhibited the methanotrophs growth, resulting in hindering the CH₄ oxidation. As it is shown in Figure 4-10, this low pH was not able to kill the methanotrophic bacteria very immediately and a zero oxidation rate was observed after 14 days of acid addition. This might be due to the high solubility of sulfuric acid in water. Some molecules of sulfuric acid solute in the moist parts of compost, so a considerable amount of H⁺ ions are not available for methanotrophic bacteria. Therefore, much more time is needed to see the inhibitory effect of low pH on methanotrophs activity. An increase in the pH of compost over time can be a good approve of our explanations.

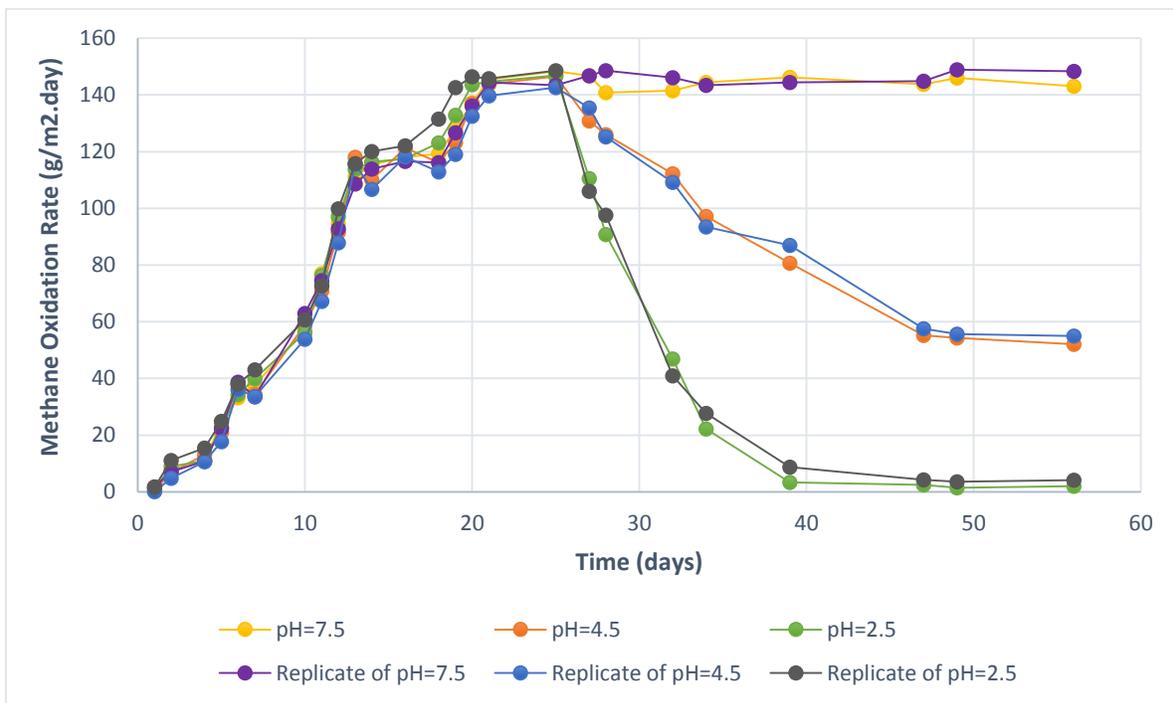


Figure 4-10: CH₄ oxidation rate in g/m²/day over time for 6 columns operated at different pHs

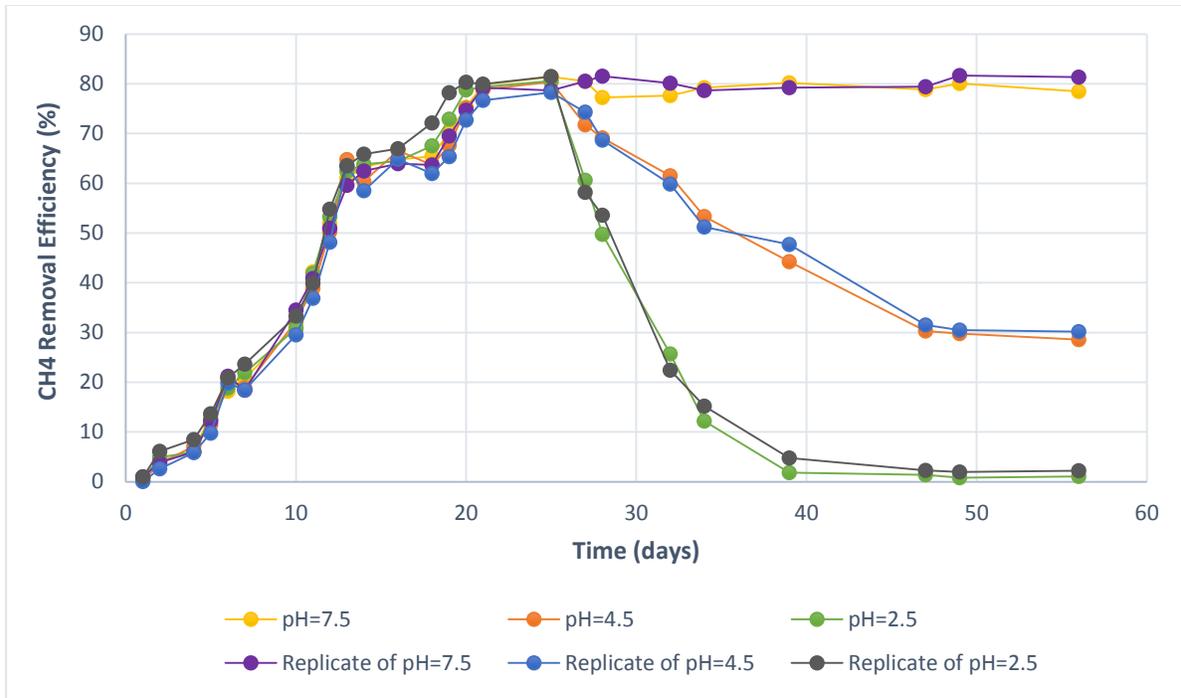


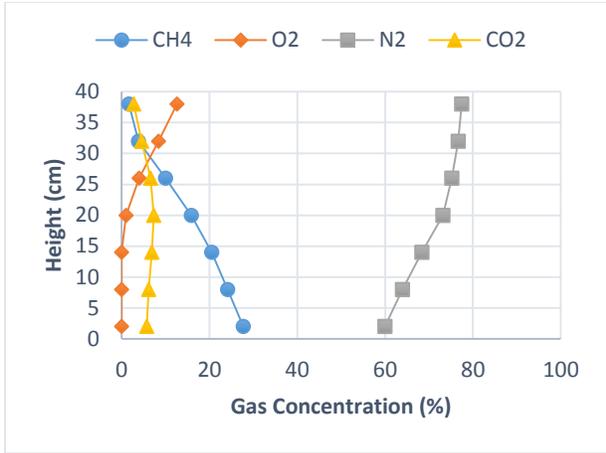
Figure 4-11: CH₄ removal efficiency (%) over time for 6 columns operated at different pHs

4.3.2 Gas Concentration Profiles

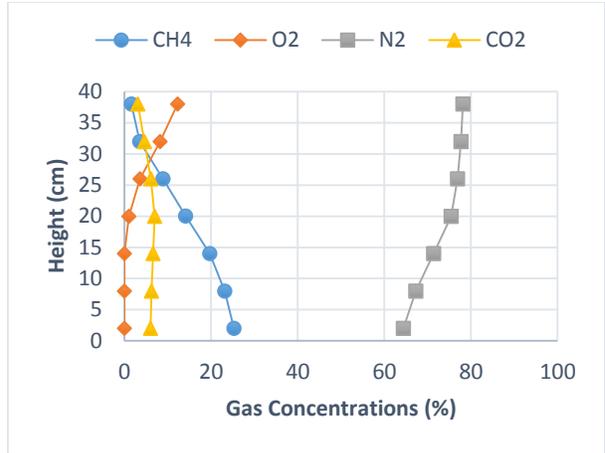
Vertical gas concentration profiles in each of the biofilter columns are presented in sections 4.3.2.1 and 4.3.2.2 for a single day at steady state condition before and after sulfuric acid addition, respectively.

4.3.2.1 Concentration Profiles for the first stage

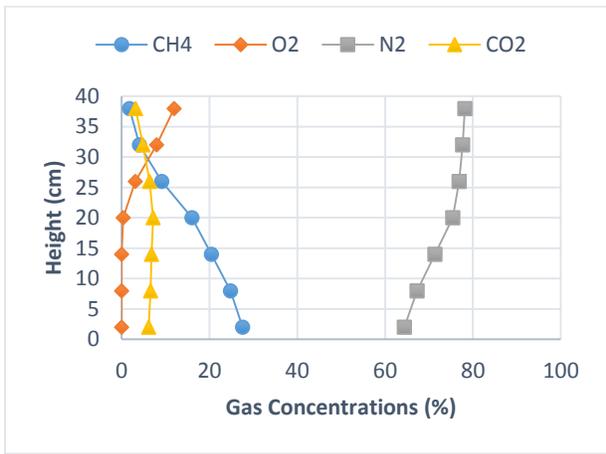
Figure 4-12a through Figure 4-12f present vertical gas concentration profiles of 6 columns measured on day 25. All biofilter columns were run without any sulfuric acid in them at compost initial pH (8.5) for 25 days. Afterwards, all 6 columns achieved their maximum removal efficiency at steady state condition.



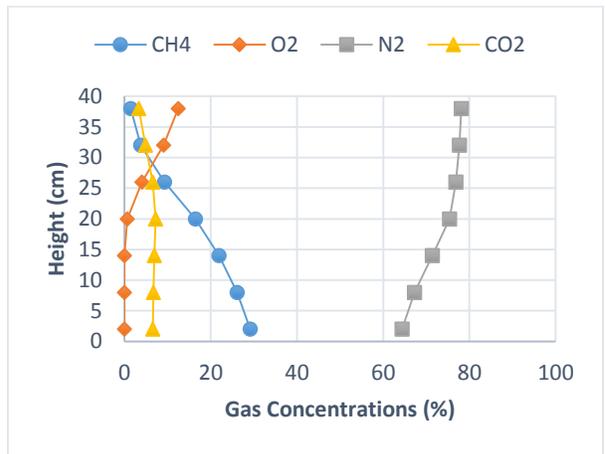
a) Concentration Profiles - Column 1



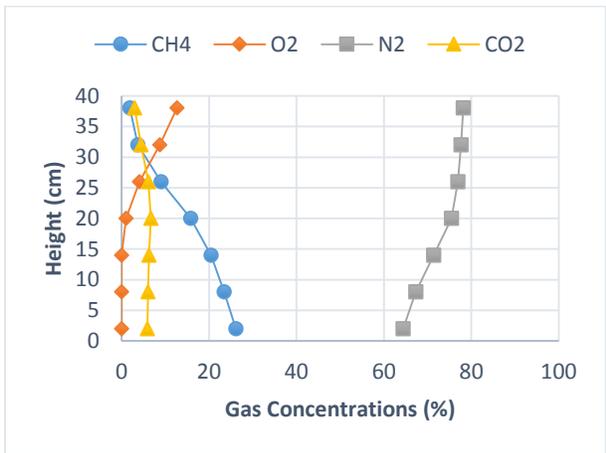
b) Concentration Profiles - Column 2



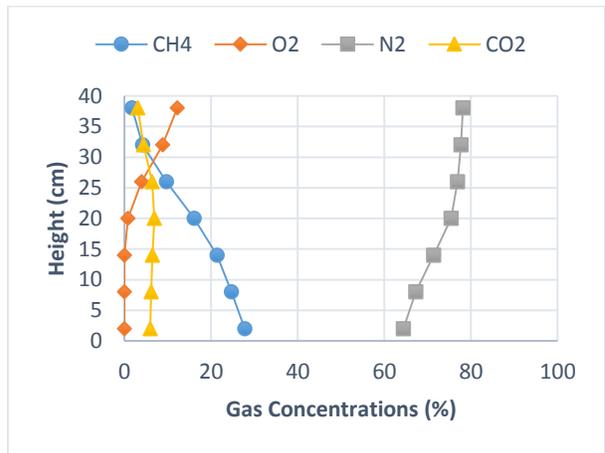
c) Concentration Profiles - Column 3



d) Concentration Profiles - Column 4



e) Concentration Profiles - Column 5



f) Concentration Profiles - Column 6

Figure 4-12: Gas Concentration Profile for day 25 (first stage) for 6 columns at steady state condition

Since all the 6 columns were operated at the same condition (same pH) until day 25, a very similar gas concentration profile was observed for all of them. As Figure 4-12 for all 6 columns suggests, the majority of the oxidation activities occur at a height of 14-38 cm, where CH₄ concentration drops sharply. Although an decrease in O₂ concentration along the columns length is expected due to CH₄ oxidation from bottom to the top, air injection from the top of columns makes the oxygen concentrations higher at top layers. Lower oxygen levels are seen close to the bottom because penetration is difficult due to the aeration regime being passive and because of bacterial activity. An increase in CO₂ concentration was observed until the depth of 20 cm from the top. Afterwards, CO₂ concentration was decreased due to air injection from the top of columns (passive aeration).

4.3.2.2 Concentration Profiles for the second stage

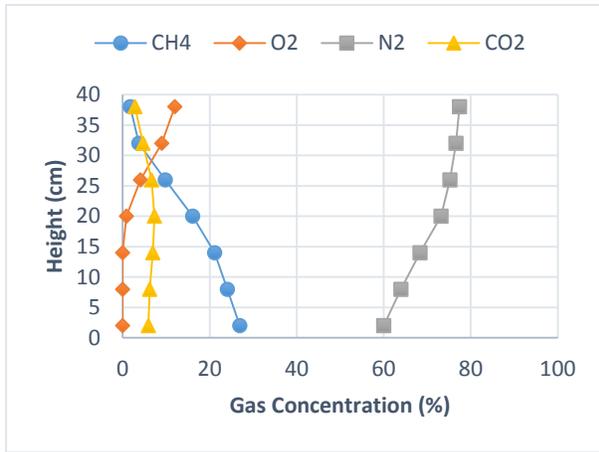
Figure 4-13a through 4-13f present gas concentration profiles for 6 columns on day 56 of the second stage (after sulfuric acid addition and pH adjustment). All 6 columns were achieved a steady oxidation rate on day 56. No change was observed in concentration profiles of columns 1 and 2 on day 56, since they were continued operating at their initial condition (no sulfuric acid addition on day 25). However, for other 4 columns which were operated at different pHs in the second stage, a significant change was observed in the gas concentration profiles.

Figure 4-13c and Figure 4-13d present the gas concentration profile in columns 3 and 4 operated at pH 4.5. As seen in these figures, the oxygen penetration depth was more than 30 cm. Oxygen penetration depth was increased from 18 cm (Figures 4-12c and 4-12d) to 30 cm as a result of pH changes (see Figures 4-13c and 4-13d). Since a very low pH inhibits methane oxidation, less oxygen is needed for the oxidation process. Therefore, more oxygen is present in columns operated at lower pHs. Furthermore, a reduction in pH from 7.5 (columns 1 and 2) to 4.5 (columns 3 and 4)

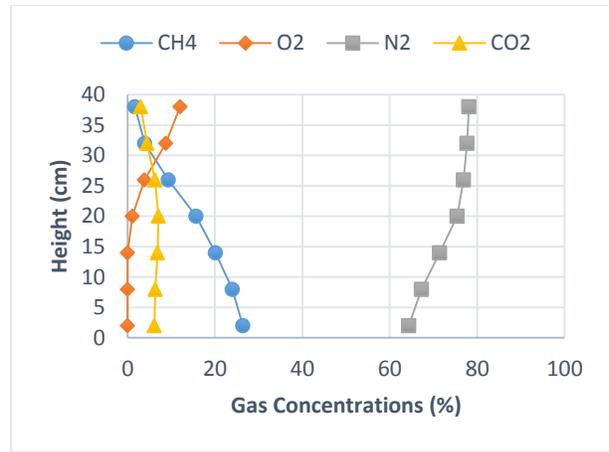
increased the methane concentration in different depth of columns (compare Figure 4-13a with Figure 4-13c). Methane oxidation rate decreases as a result of pH reduction, so less methane is consumed in the oxidation process. The methane concentration at the bottom of columns 3 and 4 (at pH 4.5) was close to 45% at the end of experiment, while it was around 28% for columns 1 and 2 (at pH 7.5).

The gas concentration profiles for columns operated at pH 2.5 are presented in Figure 4-13e and Figure 4-13f. The oxygen penetration depth was 36 cm, when the pH was adjusted to 2.5 (Figures 4-13e and 4-13f). The oxygen penetration depth was found to increase with pH reduction. Moreover, as seen in Figures 4-13e and 4-13f, the methane concentration at the bottom was close to 70%. As a result of inhibitory effect of low pH on methane removal efficiency, methane is not consumed in the oxidation process and much more methane is present at different depth of these two columns. Further increase in methane concentration was observed at the bottom of the columns, when the pH was decreased from 4.5 (Figures 4-13c and 4-13d) to 2.5 (Figures 4-13e and 4-13f).

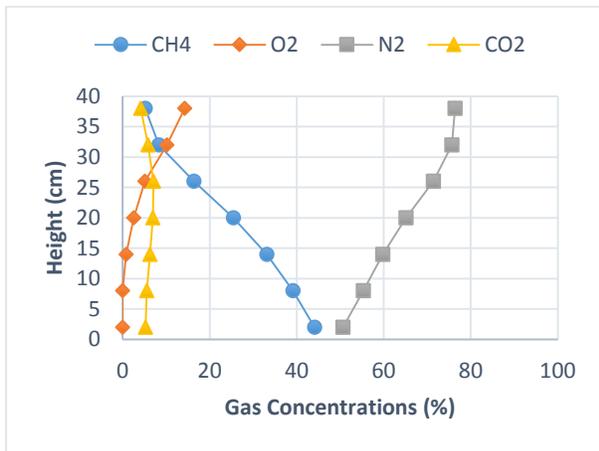
At the end of the experiment, the oxygen concentrations at top surface were different at columns operated at different pHs. The oxygen concentration at top surface was 11.8% for columns operated at pH 7.5, but was found to increase to 14% for columns operated at pH 4.5, and 15% for columns operated at pH 2.5. The increase in O₂ concentrations were observed at a height of 8-38 cm, and 2-38 cm for columns at pH 4.5 and 2.5, respectively. Furthermore, the CH₄ concentration at top surface was 1.5% for columns operated at pH 7.5 (80% removal efficiency), 5.3% for columns operated at pH 4.5 (30% removal efficiency), and 6.7% for columns operated at pH 2.5 (zero removal efficiency).



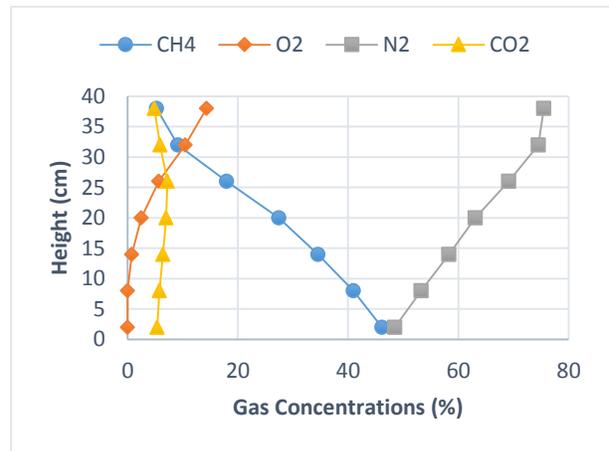
a) Concentration Profiles - Column 1



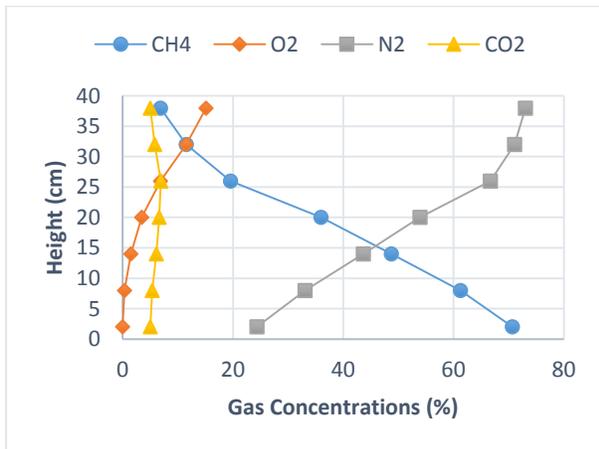
b) Concentration Profiles - Column 2



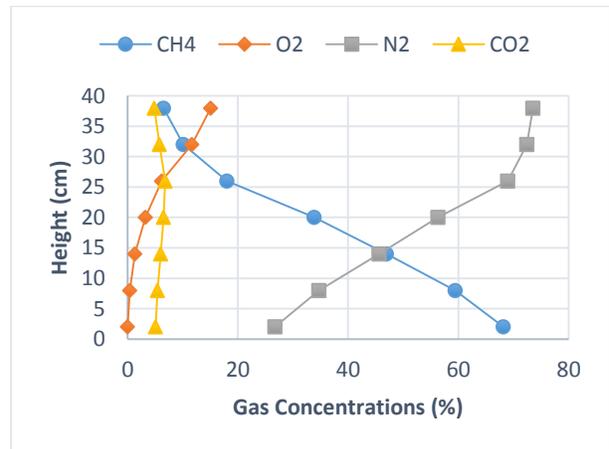
c) Concentration Profiles - Column 3



d) Concentration Profiles - Column 4



e) Concentration Profiles - Column 5



f) Concentration Profiles - Column 6

Figure 4-13: Gas Concentration Profile for day 56 (second stage) for 6 columns at steady state condition

4.4 Microbiological Analysis

After the biofilter columns were dismantled at the end of experiments, samples were extracted from the top, middle and bottom portions of each column. Then, 1g of the extracted samples was mixed and sent to the biological science group at the University of Calgary. Using the DNA sequencing technique, the samples were analyzed in order to identify the diversity of the microbial community. The results are presented in Table 4-6 in which C2T, C2M, C2B stand for top, middle, and bottom section samples of the column operated at pH 7.5, respectively. C4T, C4M, C4B are top, middle, and bottom section samples of the column operated at pH 4.5, respectively. C6T, C6M, C6B represent the analysis for top, middle, and bottom sections of the column operated at pH 2.5.

Table 4-6 summarises the microbial population in biofilter columns with special attention to methanotrophic community count. Columns are labelled as previously defined. Values in the table are percentages of the total microbial population. For instance, in column C4T (top section of the column operated at pH 4.5), 33.4% of the microbial community is comprised of methanotrophs Type I, 0.88% are methanotrophs Type II and the rest (65.72%) are other bacteria.

Table 4-6 suggests that with respect to the total methanotrophic population the biofilter operated at pH 7.5 has the highest population. This is justified especially as explained in section 4.3.1, this biofilter also has the highest methane oxidation rate. Also, the columns were operated at pH 4.5 contain around 30% of methanotrophs population in top, middle and bottom portions. This is expected since these biofilters were able to oxidize methane at 30% removal efficiency (section 4.3.1). However, biofilters operated at pH 2.5 have only 2% methanotrophs population indicating that very low pH killed the methanotrophic bacteria and inhibited methane oxidation.

As explained in section 4.3.1, these two biofilters were unable to oxidize methane, and their removal efficiencies were zero at the end of experiments.

Table 4-7: Methanotrophs community in the biofilter columns

Population (%)	C6T	C6M	C6B	C4T	C4M	C4B	C2T	C2M	C2B
Type I	2.65	2.18	2.24	33.4	31.83	28.91	56.91	55.15	51.79
Methylobacter	2.47	2.04	1.74	32.63	31.24	28.11	56.24	54.44	51.24
Methylomonas	0.01	0	0	0	0	0	0.04	0.03	0.01
Methylosarcina	0	0	0	0.07	0.05	0.01	0.05	0.07	0.02
Methylomicrobium	0.01	0.04	0.06	0.46	0.29	0.48	.41	.45	.36
Methylosphaera	0	0	0	0	0	0	0	0.01	0.01
Methylocaldum	0.16	0.1	0.44	0.22	0.23	0.31	0.15	0.11	0.14
Unclassified	0	0	0	0.02	0.02	0	0.02	0.04	0.01
Methylococcales									
Type II	0	0	0.03	0.88	1.32	0.54	1.54	1.71	1.58
Methylocystis	0	0	0.02	0.81	1.29	0.54	1.54	1.68	1.57
Methylocella	0	0	0	0	0.01	0	0	0	0
unclassified	0	0	0.01	0.07	0.02	0	0	0.03	0.01
Methylocystaceae									
Others	97.35	97.82	97.73	65.72	66.85	70.55	41.55	43.14	46.63

Figure 4-14 shows the bar chart plot of microbial population in biofilter columns. In all of the biofilter columns, O₂ concentrations were higher in the top portion due to sweep air injection from the top (passive aeration). Type I methanotrophs are more prevalent in zones where O₂ is present sufficiently (Hanson and Hanson, 1996; Mancinelli, 1995), therefore, are expected to be more prevalent in the top section as confirmed by Figure 4-14. Also Figure 4-14 indicates that the population of methanotrophs type II is quite similar in different portions of all three biofilters.

Therefore, it is concluded that low pH value could not affect the growth of methanotrophs type II. In contrast, the population of methanotrophs type I is reasonably different in three biofilter columns. Column 6 which was operated at pH 2.5 had only 2% methanotrophs type I, compared to column 2 (pH 7.5) in which methanotrophs type I allocated more than 55% of the whole microbial population.

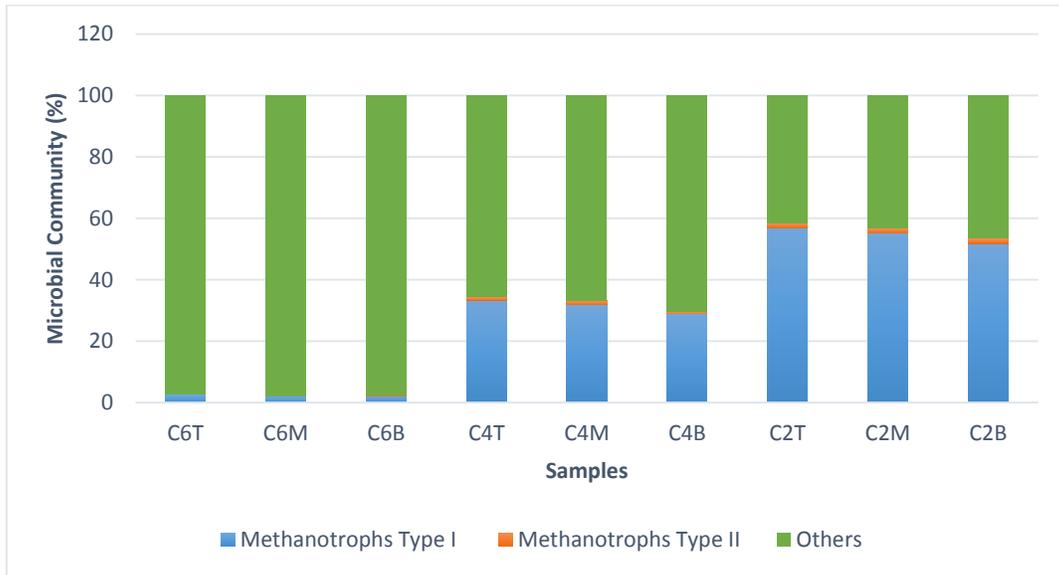


Figure 4-14: Bar chart plot of microbial population in the biofilter columns

Chapter 5: CONCLUSIONS

In this study, the effect of pH on methane oxidation efficiency was investigated. Two sets of batch experiments as well as laboratory column experiments were performed to highlight the inhibitory effect of acidic pHs on methane oxidation. The objectives of this study have been met as detailed below.

5.1 Conclusions

From the batch experiments we were able to determine:

- Compost at pH values below 4.5 is not able to provide the characteristics that methanotrophs need to live and convert CH₄ to CO₂. The corresponding V_{max} value at pH 4.5 was 3.885 μmol/g.hr. The V_{max} values for experiments at pH 3.5 and 2.5 were close to zero.
- Neutral conditions were found to give the highest methane oxidation rate. Maximum methane oxidation rate (V_{max}) for the batch experiments performed around neutral conditions were 19.153 μmol/g.hr and 21.129 μmol/g.hr at MC of 30% and 38%, respectively.
- Changing the moisture content from 30% to 44% at pH 4.5 increased the V_{max} value from 3.885 μmol/g.hr to 10.414 μmol/g.hr which indicates that diverse native population of MOB (types I and II) have the ability to adapt to changing acidic conditions at high moisture values.
- From the ANOVA analysis we know that changing the MC provides the most drastic change in results, followed by the square effect of moisture content (MC²), and pH. However, the interaction effect between MC and pH as well as the square effect of pH (pH²) were not statistically significant.

From the column experiments, we concluded:

- Compost columns operated at pH 7.5 achieved the highest oxidation rate since they were run under optimum conditions (neutral) which allow a proper growth of methanotrophic bacteria.
- Average removal efficiency for columns operated at different pHs were 80%, 30%, and 1% (negligible) for pHs 7.5, 4.5, and 2.5, respectively.
- A decrease in oxidation rates of columns operated at pH 4.5 was observed until day 47, in which they reached a steady oxidation rate of 54.95 g/m²/day (137.375 g/m³/day) after 22 days of sulfuric acid addition.
- Regarding the columns operated at pH 2.5, too acidic conditions caused a sharp decrease in oxidation rates. After 14 days of operation, they exhibited no oxidation activity leading to the fact that methanotrophs were not able survive at too acidic environment.
- Considering the results from this study, we conclude that the most suitable condition to provide bacteria with a favorable environment to oxidize CH₄ happens at neutral pH and optimum moisture content of 38%.
- Microbiological analysis confirmed that too acidic conditions tend to hinder the growth and activity of methanotrophs type I while being ineffective to the growth of methanotrophs type II.
- Considering the DNA sequencing results, it is concluded that too acidic environment compromised the methanotrophs capacity to oxidize methane by changing their

population. Columns operated at pH 2.5 had only 2% methanotrophs, compared to 30% in columns operated at pH 4.5 and 55% in columns operated at pH 7.5.

5.2 Recommendations for future research

- It is recommended that experiments be performed to evaluate the effect of pH on methane oxidation efficiency at variable air flow rates.
- Additional experiments should be performed at variable O₂ concentrations to investigate the inhibitory effect that low O₂ concentrations seem to have on the sulfuric acid oxidation as well as the development of methanotrophs population.
- Further experiments should be performed in field scale to evaluate the behavior of methane biofilters at acidic conditions in a very long period of time.
- A mathematical model should be developed to enhance the understanding of passively-aerated systems under different unfavorable conditions such as low pHs.

References:

- Amaral, J. A., T. Ren, and R. Knowles (1998). Atmospheric Methane Consumption by Forest Soils and Extracted Bacteria at Different pH Values. *Applied and Environmental Microbiology*, 64(7): 2397-2402.
- ASTM International. 2014. *ASTM D2974-14, Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils*. West Conshohocken, PA. www.astm.org.
- Auman, A. J., and M. E. Lidstrom (2002). Analysis of sMMO-Containing Type I Methanotrophs in Lake Washington Sediment. *Environmental Microbiology*, 4(9): 517-524. doi:10.1046/j.1462-2920.2002.00323.x.
- Barratt, P. A. (1995). Microbial Methane Oxidation and the Effective Biological Treatment of Landfill Generated Methane. *Waste Disposal by Landfill: GREEN93*, Rotterdam.
- Bender, M., and R. Conrad (1995). Effect of CH₄ Concentrations and Soil Conditions on the Induction of CH₄ Oxidation Activity. *Soil Biology and Biochemistry*, 27(12): 1517-1527. doi:10.1016/0038-0717(95)00104-M.
- Boeckx, P., and O. Van Cleemput (1996). Methane Oxidation in a Neutral Landfill Cover Soil: Influence of Moisture Content, Temperature, and Nitrogen-Turnover. *Journal of Environmental Quality*, 25(1): 178-183.
- Bogner, J., K. Spokas, and E. Burton (1997). Kinetics of Methane Oxidation in a Landfill Cover Soil: Temporal Variations, a Whole-Landfill Oxidation Experiment, and Modeling of Net CH₄ Emissions. *Environmental Science and Technology*, 31(9): 2504-2514.
- Borjesson, G. (2001). Inhibition of Methane Oxidation by Volatile Sulfur Compounds (CH₃SH and CS₂) in Landfill Cover Soils. *Waste Management & Research*, 19(4): 314-319.
- Borjesson, G., I. Sundh, A. Tunlid, Å. Frostegård, and B. H. Svensson (1998). Microbial Oxidation of CH₄ at High Partial Pressures in an Organic Landfill Cover Soil under Different Moisture Regimes. *FEMS Microbiology Ecology*, 26(3): 207-218.
- Borjesson, G., I. Sundh, and B. H. Svensson (2004). Microbial Oxidation of CH₄ at Different Temperatures in Landfill Cover Soils. *FEMS Microbiology Ecology*, 48(3): 305-312. doi:10.1016/j.femsec.2004.02.006.
- Bowman, J.P., L. Jimenez, I. Rosario, T. C. Hazen, and G. S. Sayler (1993). Characterization of the Methanotrophic Bacterial Community Present in a Trichloroethylene-Contaminated Subsurface Groundwater Site. *Applied and Environmental Microbiology*, 59(8): 2380-2387.

- Cabral, A. R., P. Tremblay, and G. Lefebvre (2004). Determination of the Diffusion Coefficient of Oxygen for a Cover System Including a Pulp and Paper by-Product. *Geotechnical Testing Journal*, 27(2): 184-197. doi:10.1520/GTJ11233.
- Cáceres, M., J. C. Gentina, and G. Aroca (2014). Oxidation of Methane by Methylococcoides Burmannii and Methylocystis sp. in the Presence of H₂S and NH₃. *Biotechnology Letters*, 36(1): 69-74.
- Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, et al. (2010). QIIME Allows Analysis of High-throughput Community Sequencing Data. *Nature Methods*, 7(5): 335-336.
- Caulton, D. R., P. B. Shepson, R. L. Santoro, J. P. Sparks, R. W. Howarth, A. R. Ingraffea, M. O. L. Cambaliza, et al. (2014). Toward a Better Understanding and Quantification of Methane Emissions from Shale Gas Development. *Proceedings of the National Academy of Sciences*, 111(17): 6237-42. doi:10.1073/pnas.1316546111.
- Chanton, J., and K. Liptay (2000). Seasonal Variation in Methane Oxidation in Landfill Cover Soils as Determined by an in Situ Stable Isotope Technique. *Global Biogeochemical Cycles*, 14(1): 51-60.
- Christophersen, M., L. Linderud, P. E. Jensen, and P. Kjeldsen (2000). Methane Oxidation at Low Temperatures in Soil Exposed to Landfill Gas. *Journal of Environmental Quality*, 29(6): 1989-1997.
- Ciais, P., C. Sabine, G. Bala, L. Bopp, V. Brovkin, J. Canadell, et al. (2014). Carbon and Other Biogeochemical Cycles. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 465-570), Cambridge University Press.
- Conrad, R. (1996). Soil Microorganisms as Controllers of Atmospheric Trace Gases (H₂, CO, CH₄, OCS, N₂O, and NO). *Microbiological Reviews*, 60(4): 609-640.
- Cossu, R., R. Raga, and M. Zane (2003). Methane Oxidation and Attenuation of Sulphurated Compounds in Landfill Top Cover Systems: Lab-Scale Tests. *Proceedings of Ninth International Waste Management and Landfill Symposium*, Cagliari, Italy.
- Cubasch, U., D. Wuebbles, D. Chen, M. C. Facchini, D. Frame, N. Mahowald, and J. G. Winther (2013). Climate Change 2013: The Physical Science Basis. *Contribution of Working Group I to the 5th Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge and New York, 119-158.
- Czepiel, P. M., B Mosher, P M Crill, and R C Harriss (1996). Quantifying the Effect of Oxidation on Landfill Methane Emissions. *Journal of Geophysical Research*, 101(D11): 721-29.

- Dammann B., J. Streese, R. Stegmann (1999). Microbial Oxidation of Methane from Landfills in Biofilters. *Proceedings of Sardinia 99, 7th International Waste Management and Landfill Symposium*, Cagliari, Italy.
- Delhomenie, M. C., and M. Heitz (2005). Biofiltration of Air: A Review. *Critical Reviews in Biotechnology*, 25(1-2): 53-72.
- Delhomenie, M. C., L. Bibeau, N. Bredin, S. Roy, S. Broussau, R. Brzezinski, et al. (2002). Biofiltration of Air Contaminated with Toluene on a Compost-Based Bed. *Advances in Environmental Research*, 6(3): 239-254.
- Dever, S. A., G. E. Swarbrick, and R. M. Stuetz (2005). Passive Drainage and Biofiltration of Landfill Gas Using Recycled Waste Materials, under Australian Conditions. *Tenth International Waste Management and Landfill Symposium*, Cagliari, Italy.
- Deviny, J., M. Deshusses, and T. Webster (1999). Biofiltration for Air Pollution Control. *Lewis: Boca Raton, FL*.
- De Visscher, A., D. Thomas, P. Boeckx, and O. Van Cleemput (1999). Methane Oxidation in Simulated Landfill Cover Soil Environments. *Environmental Science and Technology*, 33: 1854-1859.
- Dlugokencky, E. J., K. A. Masarie, P. M. Lang, and P. P. Tans (1998). Continuing Decline in the Growth Rate of the Atmospheric Methane Burden. *Nature*, 393(6684): 447-450.
- Eadie, G. S. (1942). The Inhibition of Cholinesterase by Physostigmine and Prostigmine. *Journal of Biological Chemistry*, 146(1): 85-93.
- Environment Canada. 2014. *Canada's Sixth National Report on Climate Change 2014*.
- EPA (2006). Global Anthropogenic Emissions of Non-CO₂ Greenhouse Gases : 1990-2020. *U. S. EPA*.
- EPA (2010). Overview of Climate Change Science. *U. S. EPA*.
- Etheridge, D. M., L. P. Steele, R. J. Francey, and R. L. Langenfelds (1998). Atmospheric Methane between 1000 A.D. and Present: Evidence of Anthropogenic Emissions and Climatic Variability. *Journal of Geophysical Research-D*, 103(13): 15979-15995.
- Figuroa, R. (1993). Methane Oxidation in Landfill Top Soils. *Proceedings of Fourth International Waste Management and Landfill Symposium*, Sardinia, 701-715.
- Fornés, I., C. Ott, and J. Jager (2003). Development of a Landfill Cover with Capillary Barrier for Methane Oxidation-Methane Oxidation in a Compost Layer. *Proceedings of Ninth International Waste Management and Landfill Symposium*, Cagliari, Italy.

- Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D. W. Fahey, et al. (2007). Changes in Atmospheric Constituents and in Radiative Forcing. Chapter 2. In *Climate Change 2007. The Physical Science Basis*.
- Gebert, J., A. Groenigroeft, and G. Meehlich (2003). Kinetics of Microbial Landfill Methane Oxidation in Biofilters. *Waste Management*, 23: 609-619.
- Gebert, J., and A. Groenigroeft (2006). Passive Landfill Gas Emission-influence of Atmospheric Pressure and Implications for the Operation of Methane-oxidising Biofilters. *Waste Management*, 23(3): 245-251.
- Gerrity, S. (2015). The Microbial Ecology of Novel Horizontal Flow Biofilm Reactors (HFBRs) Used to Treat Methane, Hydrogen Sulphide and Ammonia Contaminated Airstreams at 10° C. *Doctoral Dissertation*, National University of Ireland-Galway.
- Giani, L., J. Bredekamp, and I. Edem (2002). Temporal and Spatial Variability of the CH₄ Dynamics of Landfill Cover Soils. *Journal of Plant Nutrition and Soil Science*, 165: 205-210.
- Hanes, C. S. (1932). Studies on Plant Amylases : The Effect of Starch Concentration upon the Velocity of Hydrolysis by the Amylase of Germinated Barley. *Biochemical Journal*, 26: 1406-1421.
- Hanson, R. S. and T. E. Hanson (1996). Methanotrophic Bacteria. *Microbial Review*, 60: 439-471.
- Haththotuwa, C. (2005). Controlling Factors Affecting Microbial Methane Oxidation in Different Soil Environments. In *7th World Congress on Recovery, Recycling and Re-integration, Poster Presentation, Abstract* (No. 164).
- Haubrichs, R., and R. Widmann (2006). Evaluation of Aerated Biofilter Systems for Microbial Methane Oxidation of Poor Landfill Gas. *Waste Management*, 26(4): 408-416. doi:10.1016/j.wasman.2005.11.008.
- Henckel, T., U. Jäckel, S. Schnell, and R. Conrad (2000). Molecular Analyses of Novel Methanotrophic Communities in Forest Soil that Oxidize Atmospheric Methane. *Applied and Environmental Microbiology*, 66(5): 1801-1808.
- Hettiarachchi, V. C., P. J. Hettiaratchi, A. K. Mehrotra, and S. Kumar (2011). Field-scale Operation of Methane Biofiltration Systems to Mitigate Point Source Methane Emissions. *Environmental pollution*, 159(6): 1715-1720.
- Hilger, H. A., A.G. Wollum, and M. A. Barlaz (2000). Landfill Methane Oxidation Response to Vegetation, Fertilization and Liming. *Journal of Environmental Quality*, 29(1): 324-334.
- Hilger, H. A., S. K. Liehr, and M. A. Barlaz (1999). Exopolysaccharide Control of Methane Oxidation in Landfill Cover Soil. *Journal of Environmental Engineering*, 125(12): 1113-1123.

- Hofstee, B. H. J. (1952). Specificity of Esterases I. Identification of Two Pancreatic Aliesterases. *Journal of Biological Chemistry*, 199(1): 357-364.
- Hogan, K. B., J. F. Hoffman, and A. M. Thompson (1991). Methane on the Greenhouse Agenda. *Nature*, 354(21): 181-182.
- Huber-Humer, M., J. Gebert, and H. Hilger (2008). Biotic Systems to Mitigate Landfill Methane Emissions. *Waste Management & Research*, 26(1): 33-46. doi:10.1177/0734242X07087977.
- Humer, M. and P. Lechner (1999). Alternative Approach to the Elimination of Greenhouse Gases from Old Landfills. *Waste Management & Research*, 17: 443-452.
- Hutsch, B. W., C. P. Webster, and D. S. Powlson (1994). Methane Oxidation in Soil as Affected by Land Use, Soil pH and N Fertilization. *Soil Biology and Biochemistry*, 26(12): 1613-1622.
- Imandi, S. B., V. R. Bandaru, S. R. Somalanka, and H. R. Garapati (2007): Optimization of Medium Constituents for the Production of Citric Acid from Byproduct Glycerol Using Doehlert Experimental Design. *Enzyme and Microbial technology*, 40(5): 1367-1372.
- IPCC, (2013): Climate Change 2013: The Physical Science Basis. *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P. M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.
- IPCC, (2014): Climate Change 2014: Synthesis Report. *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, R. K. Pachauri and L. A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.
- Jaffrin, A., N. Bentounes, A. M. Joan, and S. Makhoulf (2003). Landfill Biogas for Heating Greenhouses and Providing Carbon Dioxide Supplement for Plant Growth. *Biosystems Engineering*, 86(1): 113-123.
- Johnson, M. R., and A. R. Coderre (2012). Compositions and Greenhouse Gas Emission Factors of Flared and Vented Gas in the Western Canadian Sedimentary Basin. *Journal of the Air & Waste Management Association*, 62(9): 992–1002. doi:10.1080/10962247.2012.676954.
- Kightley, D., D. B. Nedwell, and M. Cooper (1995). Capacity for Methane Oxidation in Landfill Cover Soils Measured in Laboratory-Scale Soil Microcosms. *Applied and Environmental Microbiology*, 61(2): 592-601.
- Kim, K. H., Y. J. Choi, E. C. Jeon, and Y. Sunwoo (2005). Characterization of Malodorous Sulfur Compounds in Landfill Gas. *Atmospheric Environment*, 39(6): 1103-1112.

- Kim, J., W. Kim, and K. Yong (2012). CuO/ZnO Heterostructured Nanorods: Photochemical Synthesis and the Mechanism of H₂S Gas Sensing. *The Journal of Physical Chemistry C*, 116(29): 15682-15691.
- Kirschke, S., P. Bousquet, P. Ciais, M. Saunois, J. G. Canadell, E. J. Dlugokencky, et al. (2013). Three Decades of Global Methane Sources and Sinks. *Nature Geoscience*, 6(10): 813-823.
- Klute, A. (1986). Water Retention: Laboratory Methods. In: A. Klute (Ed.) *Methods of Soil Analysis*. 2.Ed. Madison: ASA, 1986. p.635-662.
- Knief, C., A. Lipski, and P. F. Dunfield (2003). Diversity and Activity of Methanotrophic Bacteria in Different Upland Soils. *Applied and Environmental Microbiology*, 69(11): 6703-6714.
- Krumholz, L. R., J. L., Hollenback, S. J. Roskes, and D. B. Ringelberg (1995). Methanogenesis and Methanotrophy within a Sphagnum Peatland. *FEMS Microbiology Ecology*, 18(3): 215-224.
- Lebrero, R., J. C. López, I. Lehtinen, R. Pérez, G. Quijano, and R. Muñoz (2016). Exploring the Potential of Fungi for Methane Abatement: Performance Evaluation of a Fungal-bacterial Biofilter. *Chemosphere*, 144: 97-106.
- Lee, S. Y., H. U. Ryu, and K. S. Cho (2012). Effect of Ammonia on the Oxidation of Methane by Methanotrophs. *Journal of Korean Society of Odor Research and Engineering*, 1(11): 41-46.
- Leskovac, V. (2003). Comprehensive Enzyme Kinetics. *Kluwer Academic Publishers*, New York. pp. 438.
- Li, L., Y. Han, X. Yan, and J. Liu (2013). H₂S Removal and Bacterial Structure along a Full Scale Biofilter Bed Packed with Polyurethane Foam in a Landfill Site. *Bioresource Technology*, 147: 52-58.
- Limbri, H., C. Gunawan, B. Rosche, and J. Scot (2013). Challenges to Developing Methane Biofiltration for Coal Mine Ventilation Air: A Review. *Water, Air, & Soil Pollution*, 224(6), 1566. doi:10.1007/s11270-013-1566-5.
- Lineweaver, H., and D. Burk (1935). The Determination of Enzyme Dissociation Constants. *Journal of American Chemical Society*, 56: 658-666.
- Lopez, M. E., E. R. Rene, M. C. Veiga, and C. Kennes (2013). Biogas Upgrading. Air Pollution Prevention and Control: *Bioreactors and Bioenergy*, 293-318.
- Malashenko, Y.R., T. P. Pirog, V. A. Romanovskaia, I. G. Sokolov, and T. A. Gringerg (2001). Search for Methanotrophic Producers of Exopolysaccharides. *Applied Biochemistry and Microbiology*, 37(6): 599-602.

- Mancebo, U. (2011). Compost Physiochemical Characteristics Influencing Methane Biofiltration. *Doctoral Dissertation*, University of Calgary.
- Mancebo, U., and J. P. A. Hettiaratchi (2015). Rapid Assessment of Methanotrophic Capacity of Compost-Based Materials Considering the Effects of Air-Filled Porosity, Water Content and Dissolved Organic Carbon. *Bioresource Technology*, 177: 125-133.
- Mancebo, U., P. Hettiaratchi, P. Jayasinghe, and R. Surampalli (2016). Determination of Water Retention Capacity of Granular Media of Methane Biofilters: A Simplified Approach. *Environmental Earth Sciences*, 75(1): 74. doi:10.1007/s12665-015-4907-4.
- Mancinelli, R. L. (1995). The Regulation of Methane Oxidation in Soil. *Annual Review of Microbiology*, 49: 581-605.
- Melse, R. W., and A. W. van der Werf (2005). Biofiltration for Mitigation of Methane Emission from Animal Husbandry. *Environmental Science and Technology*, 39(14): 5460-5468. doi:10.1021/es048048q.
- Menard, C., A. A. Ramirez, J. Nikiema, and M. Heitz (2012). Biofiltration of Methane and Trace Gases from Landfills: A Review. *Environmental Reviews*, 20(1): 40-53.
- Mingxing, W., and L. Jing (2002). CH₄ Emission and Oxidation in Chinese Rice Paddies. *Nutrient Cycling in Agroecosystems*, 64(1): 43-55.
- Mor, S., A. De Visscher, K. Ravindra, R. P. Dahiya, A. Chandra, and O. Van Cleemput (2006). Induction of Enhanced Methane Oxidation in Compost: Temperature and Moisture Response. *Waste Management*, 26(4): 381-388.
- MSDS. 1996. Material Safety Data Sheet for Hydrogen Sulfide. Murray Hill, NJ: BOC Gases. Available from <http://www.vngas.com/pdf/g94.pdf> (accessed on June 20, 2006).
- Mudliar, S., B. Giri, K. Padoley, D. Satpute, R. Dixit, P. Bhatt, et al. (2010). Bioreactors for Treatment of VOCs and Odours: A Review. *Journal of Environmental Management*, 91(5): 1039-1054.
- Myhre, G., D. Shindell, F. M., Bréon, W. Collins, J. Fuglestedt, J. Huang, et al. 2013. Anthropogenic and Natural Radiative Forcing. *Climate Change 2013, The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, 423.
- Nikiema, J., L. Bibeau, J. Lavoie, R. Brzezinski, J. Vigneux, and M. Heitz (2005). Biofiltration of Methane: An Experimental Study. *Chemical Engineering Journal*, 113(2): 111-117.
- Nikiema, J., R. Brzezinski, and M. Heitz (2007). Elimination of Methane Generated from Landfills by Biofiltration: A Review. *Reviews in Environmental Science and Biotechnology*, 6(4): 261-284. doi:10.1007/s11157-006-9114-z.

- Omel'chenko, M. V., N. D. Savel'eva, L. V. Vasil'eva, and G. A. Zavarzin (1993). Pathways of Primary and Intermediary Metabolism in a Psychrophilic Methanotroph. *Mikrobiologiya*, 62(5): 849-854.
- Ottengraf, S. P. (1986). Exhaust Gas Purification. *Biotechnology*, 8(425): 425-52.
- Park, S., K. W. Brown, and J. C. Thomas (2002). The Effect of Various Environmental and Design Parameters on Methane Oxidation in a Model Biofilter. *Waste Management & Research*, 20(5): 434-444. doi:10.1177/0734242X0202000507.
- Pedersen, G. B., C. Scheutz, and P. Kjeldsen (2011). Availability and Properties of Materials for the Fakse Landfill Biocover. *Waste Management*, 31(5): 884-894.
- Pokhrel, D., P. Hettiaratchi, and S. Kumar (2011). Methane Diffusion Coefficient in Compost and Soil-Compost Mixtures in Gas Phase Biofilter. *Chemical Engineering Journal*, 169(1): 200-206.
- Pokhrel, D. (2006). Compost Based Biocap Performance. *Doctoral Dissertation*, University of Calgary.
- Pratt, C., A. S. Walcroft, K. R. Tate, D. J. Ross, R. Roy, M. H. Reid, and P. W. Veiga (2012). Biofiltration of Methane Emissions from a Dairy Farm Effluent Pond. *Agriculture, Ecosystems & Environment*, 152: 33-39.
- Prieme, A. and S. Christensen (1997). Seasonal and Spatial Variation of Methane Oxidation in a Danish Sorce Forest. *Soil Biology and Biochemistry*, 29(8): 1165-1172.
- Quigley, C., C. Easter, P. Burrowes, and J. Witherspoon (2004). Biotechnology-based Odour Control: Design Criteria and Performance Data. *Water Science and Technology*, 50(4): 319-326.
- Roth, S. H. (1993). Hydrogen Sulfide. *Handbook of Hazardous Materials*, 367-376.
- Rudolph, F. B., and H. J. Fromm (1979). Plotting Methods for Analyzing Enzyme Rate Data. *Methods in Enzymology*, 63, 138.
- Scheutz, C., and P. Kjeldsen (2004). Environmental Factors Influencing Attenuation of Methane and Hydrochlorofluorocarbons in Landfill Cover Soils. *Journal of Environmental Quality*, 33(1): 72-79.
- Scheutz, C., P. Kjeldsen, J. E. Bogner, A. De Visscher, J. Gebert, H. A. Hilger, M. Huber-Humer, and K. Spokas (2009). Microbial Methane Oxidation Processes and Technologies for Mitigation of Landfill Gas Emissions. *Waste Management & Research*, 27(5): 409-455. doi:10.1177/0734242X09339325.

- Schumacher, B. A. (2002). Methods for the Determination of Total Organic Carbon in Soils and Sediments. *Ecological Risk Assessment Support Center*, 1-23.
- Semrau, J. D. A. A. DiSpirito, and S. Vuilleumier (2011). Facultative Methanotrophy: False Leads, True Results, and Suggestions for Future Research. *FEMS Microbiology Letters*, 323(1): 1-12.
- Sly, L.I., L.J. Bryant, J.M. Cox, and J.M. Anderson (1993). Development of a Biofilter for the Removal of Methane from Coal Mine Ventilation Atmospheres. *Applied Microbiology and Biotechnology*, 39(3), 400-404. doi:10.1007/BF00192101.
- Stein, V. B. and J. P. A. Hettiaratchi (2001). Methane Oxidation in Three Alberta Soils: Influence of Soil Parameters and Methane Flux Rates. *Environmental Technology*, 22:101-111.
- Straka, F., J. Crha, M. Musilova, M. Kuncarova (1999). LFG-biofilters on Old Landfills. In: *Proceedings of the Sardinia 99, 7th International Waste Management and Landfill Symposium*, Cagliari, Italy.
- Stralis-Pavese, N., L. Bodrossy, T. G. Reichenauer, A. Weilharter, and A. Sessitsch (2006). 16S rRNA Based T-RFLP Analysis of Methane Oxidising Bacteria - Assessment, Critical Evaluation of Methodology Performance and Application for Landfill Site Cover Soils. *Applied Soil Ecology*, 31(3): 251-266.
- Streese, J. and R. Stegmann (2003). Microbial Oxidation of Methane from Old Landfills in Biofilters. *Waste Management*, 23: 573-580.
- Sundh, I., C. Mikkela, M. Nilsson, and B. H. Svensson (1995). Potential Aerobic Methane Oxidation in a Sphagnum-Dominated Peatland: Controlling Factors and Relation to Methane Emission. *Soil Biology and Biochemistry*, 27(6): 829-837.
- Syed, M., G. Soreanu, P. Falletta, and M. Béland (2006). Removal of Hydrogen Sulfide from Gas Streams Using Biological Processes: A Review. *Canadian Biosystems Engineering*, 48, 2.
- Syed, R., S. Saggar, K. Tate, B.H.A. Rehm (2014). Characterising a Field Methane Oxidation Biofilter - Treating Farm Methane Emissions from Massey University No.4 Dairy Pond. In *Conference: Nutrient Management for the Farm, Catchment and Community*, Massey University, Palmerston North, New Zealand.
- Syed, R., S. Saggar, K. Tate, and B. H. Rehm (2016). Does Acidification of a Soil Biofilter Compromise its Methane-Oxidising Capacity?. *Biology and Fertility of Soils*, 52(4): 573-583.
- Trotsenko, Y. A., and J. C. Murrell (2008). Metabolic Aspects of Aerobic Obligate Methanotrophy. *Advances in Applied Microbiology*, 63: 183-229.

- Trotsenko, Y. A., and V. N. Khmelenina (2002). Biology of Extremophilic and Extremotolerant Methanotrophs. *Archives of Microbiology*, 177(2): 123-131.
- Veillette, M., M. Girard, P. Viens, R. Brzezinski, and M. Heitz (2012). Function and Limits of Biofilters for the Removal of Methane in Exhaust Gases from the Pig Industry. *Applied Microbiology and Biotechnology*, 94(3): 601-611.
- Visvanathan, C., D. Pokhrel, W. Chiemchaisri, J. P. A. Hettiaratchi, and J. Wu (1999), Methanotrophic Activities in Tropical Landfill Cover Soils: Effects of Temperature, Moisture Content, and Methane Concentration. *Waste Management & Research*, 17: 313-323.
- Whalen, S. C., W. S. Reeburgh, and K. A. Sandbeck (1990). Rapid Methane Oxidation in Landfill Cover Soil. *Applied and Environmental Microbiology*, 56: 3405-3411.
- Whalen, S. C., and W. S. Reeburgh (1996). Moisture and Temperature Sensitivity of CH₄ Oxidation in Boreal Soils. *Soil Biology and Biochemistry*, 28(10): 1271-1281.
- Wilshusen, J. H., J. P. A. Hettiaratchi, A. De Visscher, and R. Saint-Fort (2004). Methane Oxidation and Formation of EPS in Compost: Effect of Oxygen Concentration. *Environmental Pollution*, 129(2): 305-314.
- Wu, W., G. Cui, and B. Lu (2000). Optimization of Multiple Evariables: Application of Central Composite Design and Overall Desirability. *Chinese Pharmaceutical Journal*, 35(8): 530-532.
- Yang, T. and E. R. Allen. (1994). Biofiltration Control of Hydrogen Sulfide. I- Design and Operational Parameters. *Air and Waste*, 44: 863-868.
- Ying, D., C. Chuanyu, H. Bin, X. Yueen, Z. Xuejuan, C. Yingxu, and W. Weixiang (2012). Characterization and Control of Odorous Gases at a Landfill Site: A Case Study in Hangzhou, China. *Waste Management*, 32(2): 317-326.
- Yu, H. X., H. Min, Z. M. Lv, and J. Liu (2009). Influence of Poisonous Gases on the Bio-Oxidation of Coalmine Gas. *International Journal of Mining, Reclamation and Environment*, 23(2): 121-131.

Appendix A: V_{max} plots for OFAT batch experiments at different pHs.

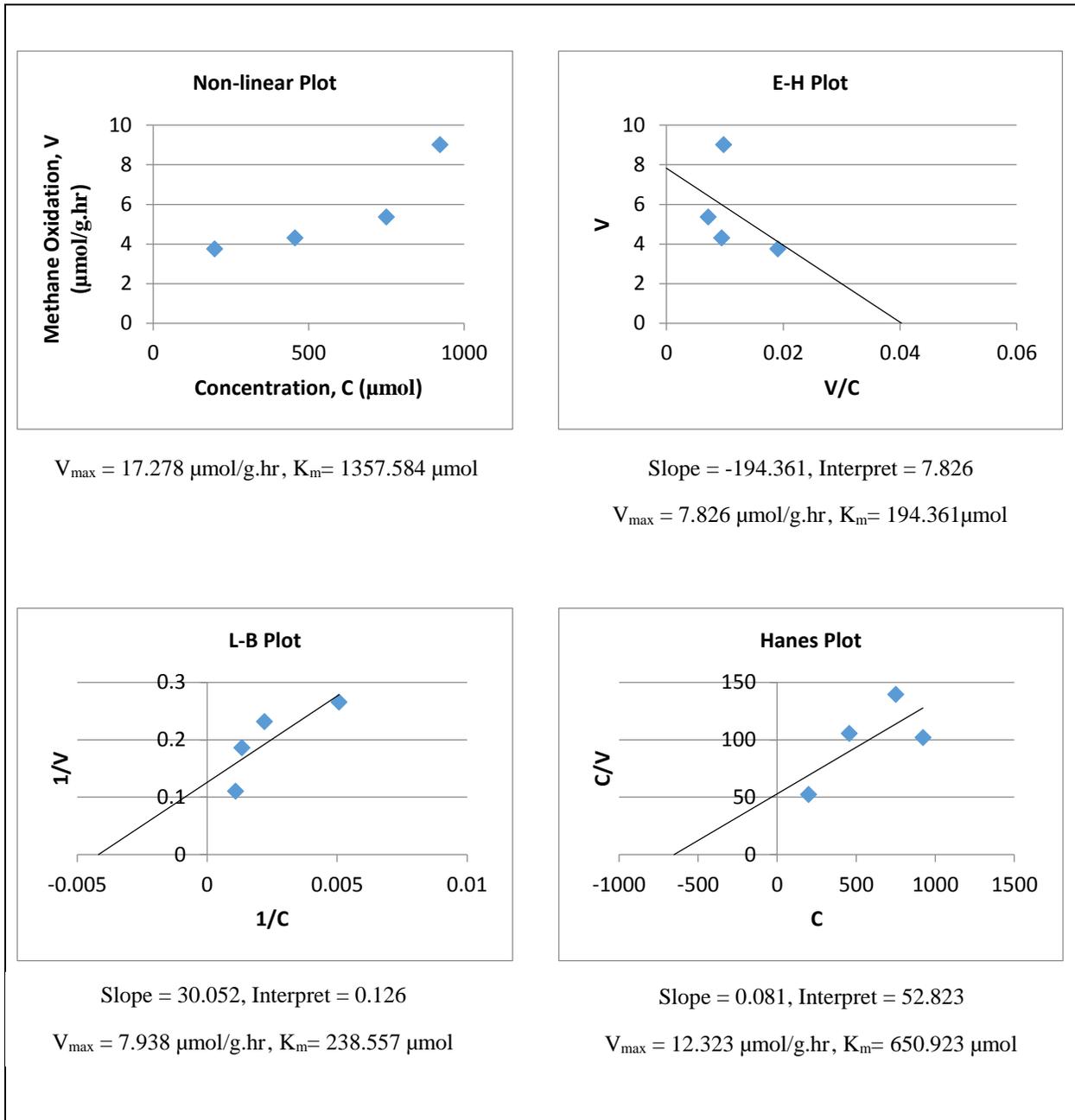


Figure A-1: Michaelis-Menten standard and linearized graphs and V_{max} values for the OFAT experiment at pH=7.7

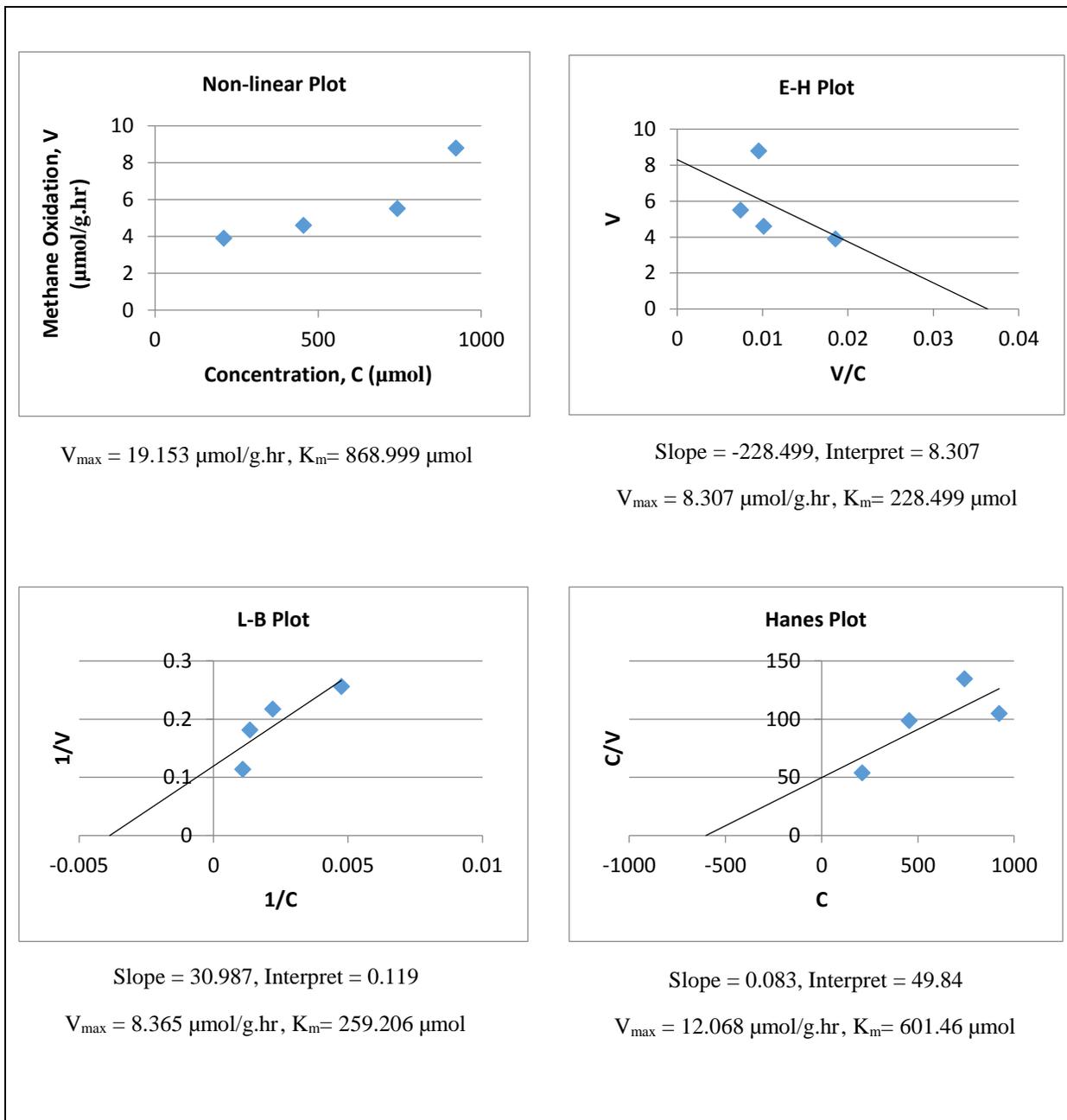


Figure A-2: Michaelis-Menten standard and linearized graphs and V_{max} values for the OFAT experiment at pH=7.2

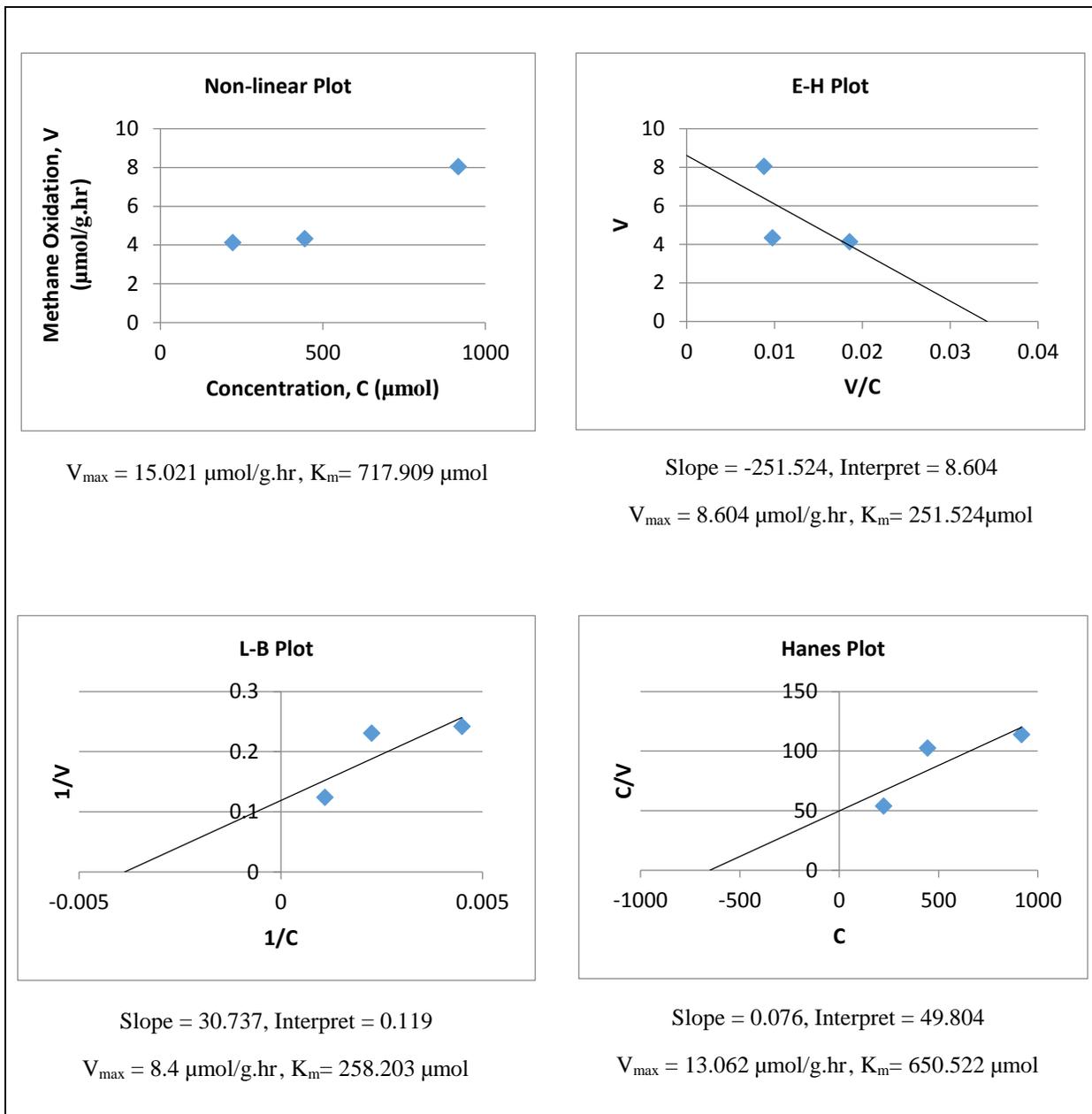


Figure A-3: Michaelis-Menten standard and linearized graphs and V_{\max} values for the OFAT experiment at pH=6.7

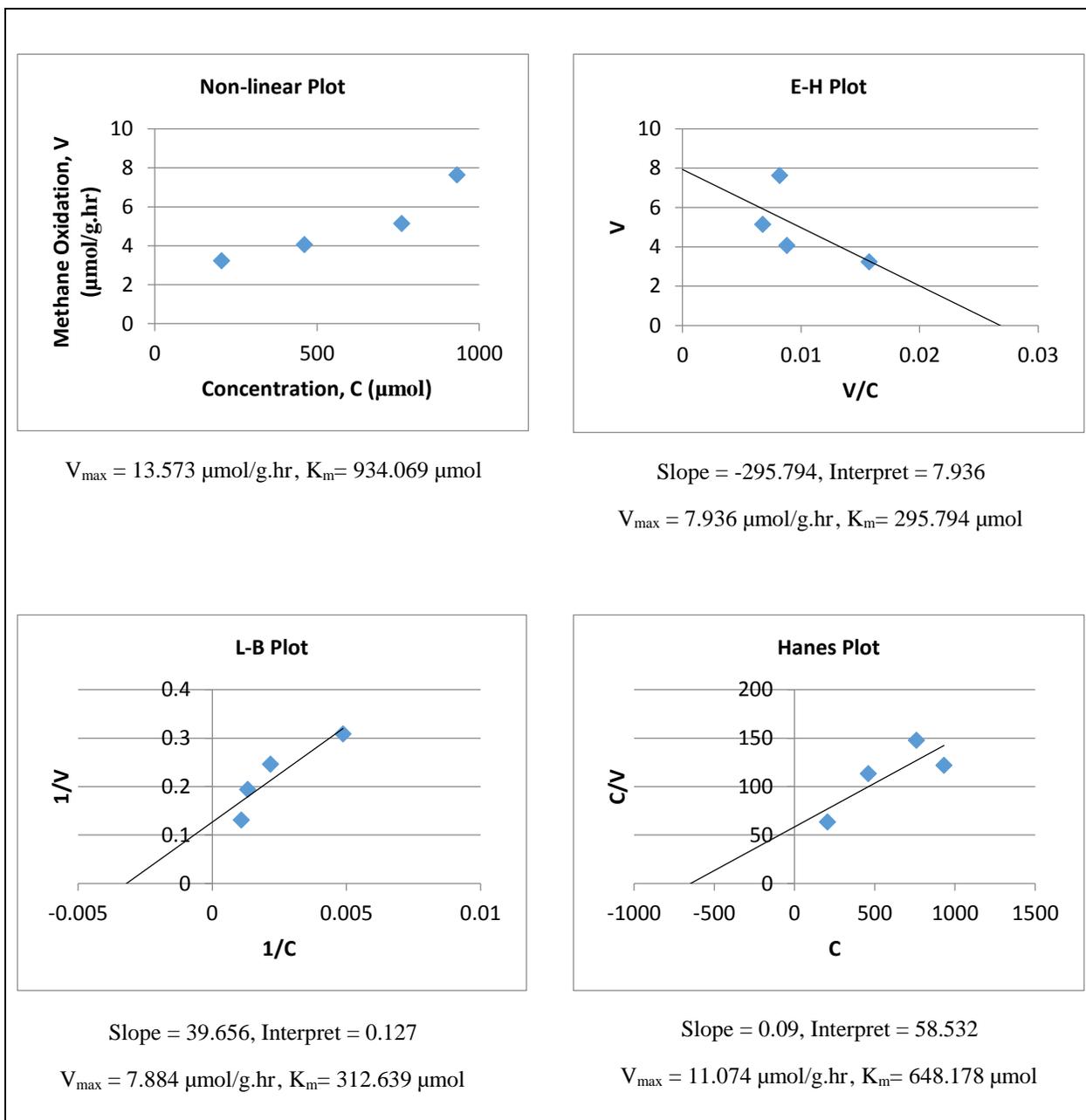
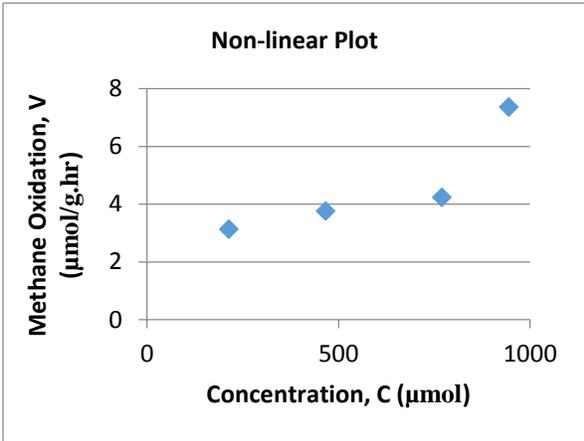
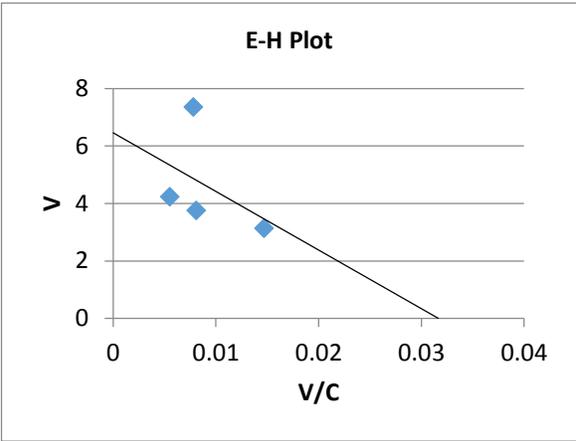


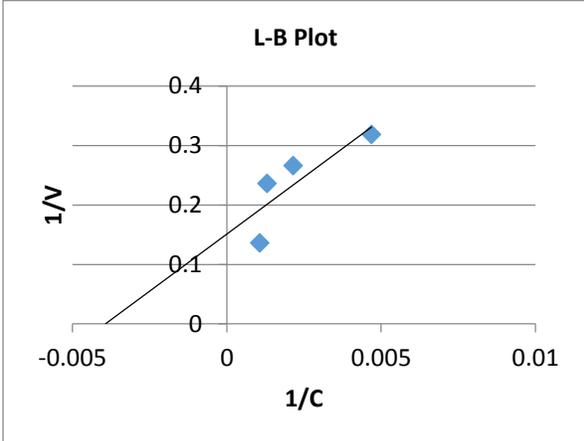
Figure A-4: Michaelis-Menten standard and linearized graphs and V_{max} values for the OFAT experiment at pH=6.2



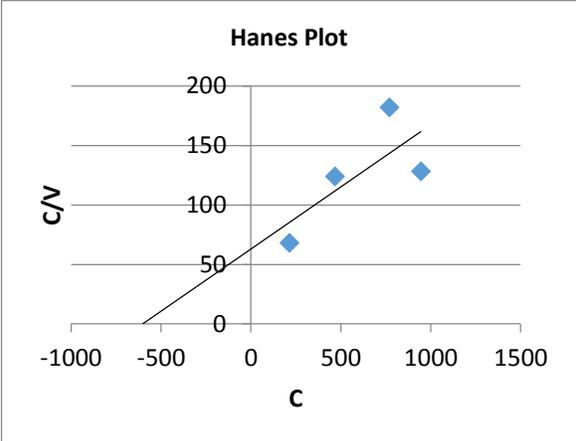
$V_{max} = 12.112 \mu\text{mol/g.hr}$, $K_m = 995.01 \mu\text{mol}$



Slope = -203.927, Interpret = 6.456
 $V_{max} = 6.456 \mu\text{mol/g.hr}$, $K_m = 203.927 \mu\text{mol}$

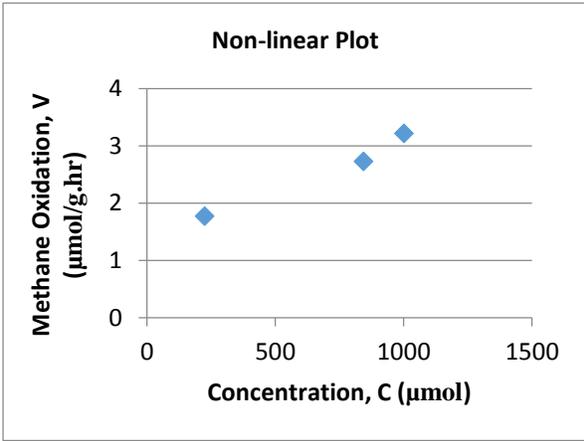


Slope = 38.426, Interpret = 0.151
 $V_{max} = 6.617 \mu\text{mol/g.hr}$, $K_m = 254.272 \mu\text{mol}$

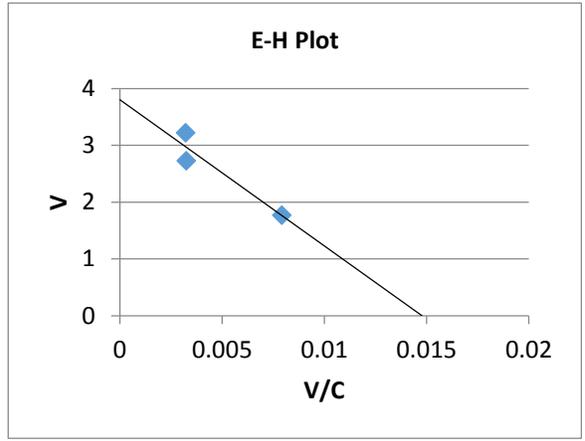


Slope = 0.105, Interpret = 62.972
 $V_{max} = 9.545 \mu\text{mol/g.hr}$, $K_m = 601.376 \mu\text{mol}$

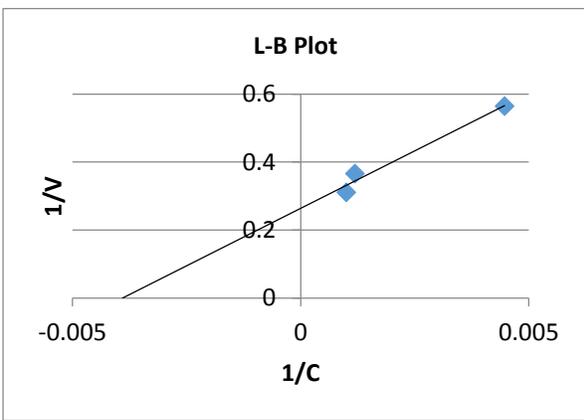
Figure A-5: Michaelis-Menten standard and linearized graphs and V_{max} values for the OFAT experiment at pH=5.5



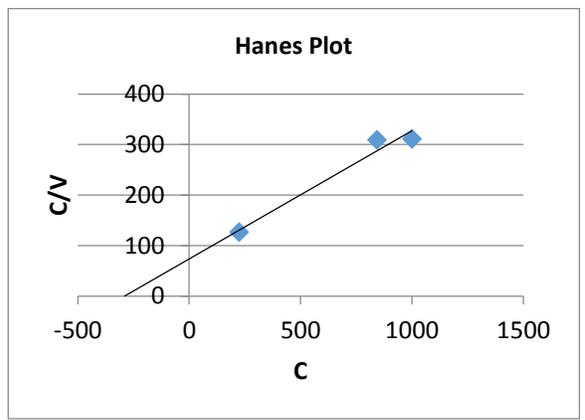
$V_{\max} = 3.885 \mu\text{mol/g.hr}$, $K_m = 275.711 \mu\text{mol}$



Slope = -257.244, Interpret = 3.805
 $V_{\max} = 3.805 \mu\text{mol/g.hr}$, $K_m = 257.244 \mu\text{mol}$

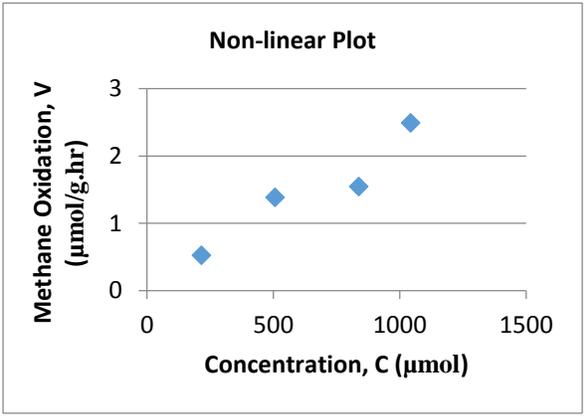


Slope = 67.545, Interpret = 0.264
 $V_{\max} = 3.787 \mu\text{mol/g.hr}$, $K_m = 255.783 \mu\text{mol}$

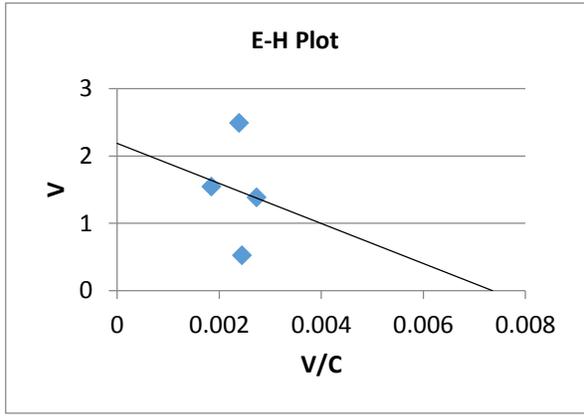


Slope = 0.254, Interpret = 73.894
 $V_{\max} = 3.943 \mu\text{mol/g.hr}$, $K_m = 291.354 \mu\text{mol}$

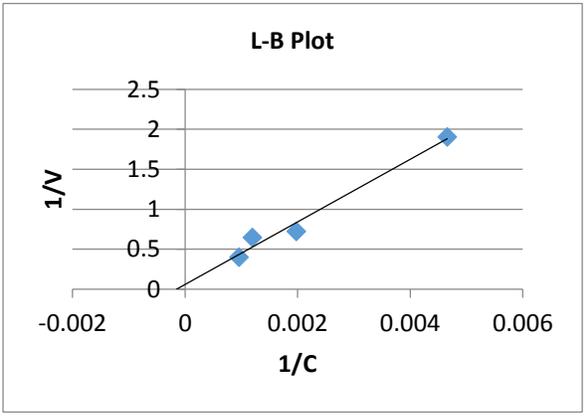
Figure A-6: Michaelis-Menten standard and linearized graphs and V_{\max} values for the OFAT experiment at pH=4.5



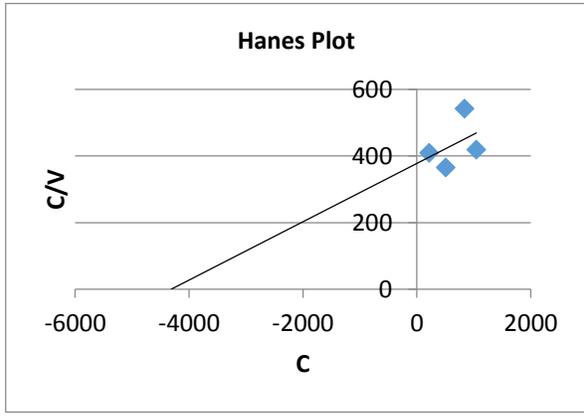
$V_{\text{max}} = 1.281 \mu\text{mol/g.hr}$, $K_m = 859.959 \mu\text{mol}$



Slope = -297.042, Interpret = 2.185
 $V_{\text{max}} = 2.185 \mu\text{mol/g.hr}$, $K_m = 297.042 \mu\text{mol}$



Slope = 391.596, Interpret = 0.059
 $V_{\text{max}} = 10.905 \mu\text{mol/g.hr}$, $K_m = 1987.042 \mu\text{mol}$



Slope = 0.087, Interpret = 377.079
 $V_{\text{max}} = 4.531 \mu\text{mol/g.hr}$, $K_m = 1204.032 \mu\text{mol}$

Figure A-7: Michaelis-Menten standard and linearized graphs and V_{max} values for the OFAT experiment at pH=3.5

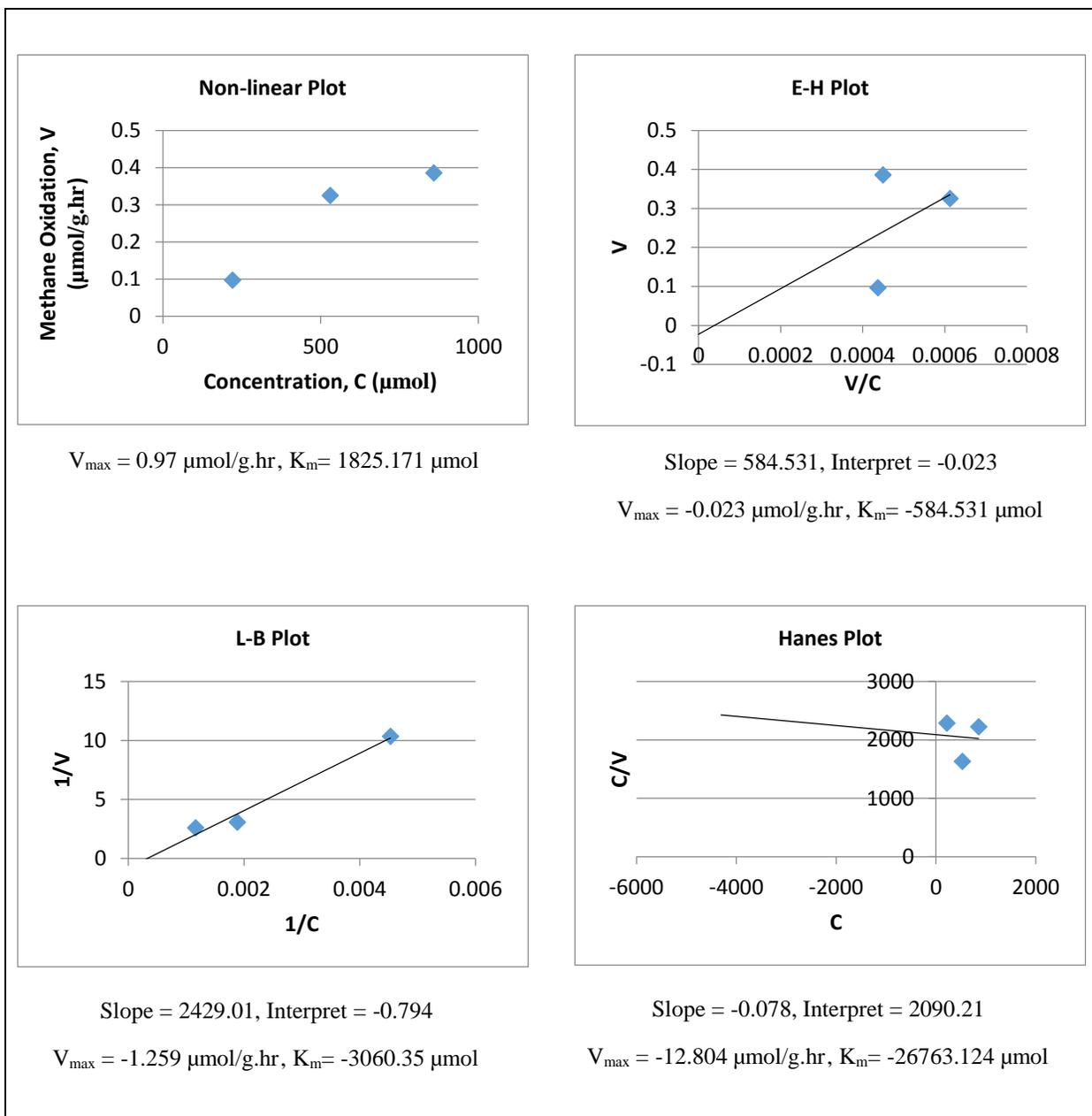


Figure A-8: Michaelis-Menten standard and linearized graphs and V_{max} values for the OFAT experiment at pH=2.5

Appendix B: V_{\max} plots for CCD batch experiments at different pH and MC.

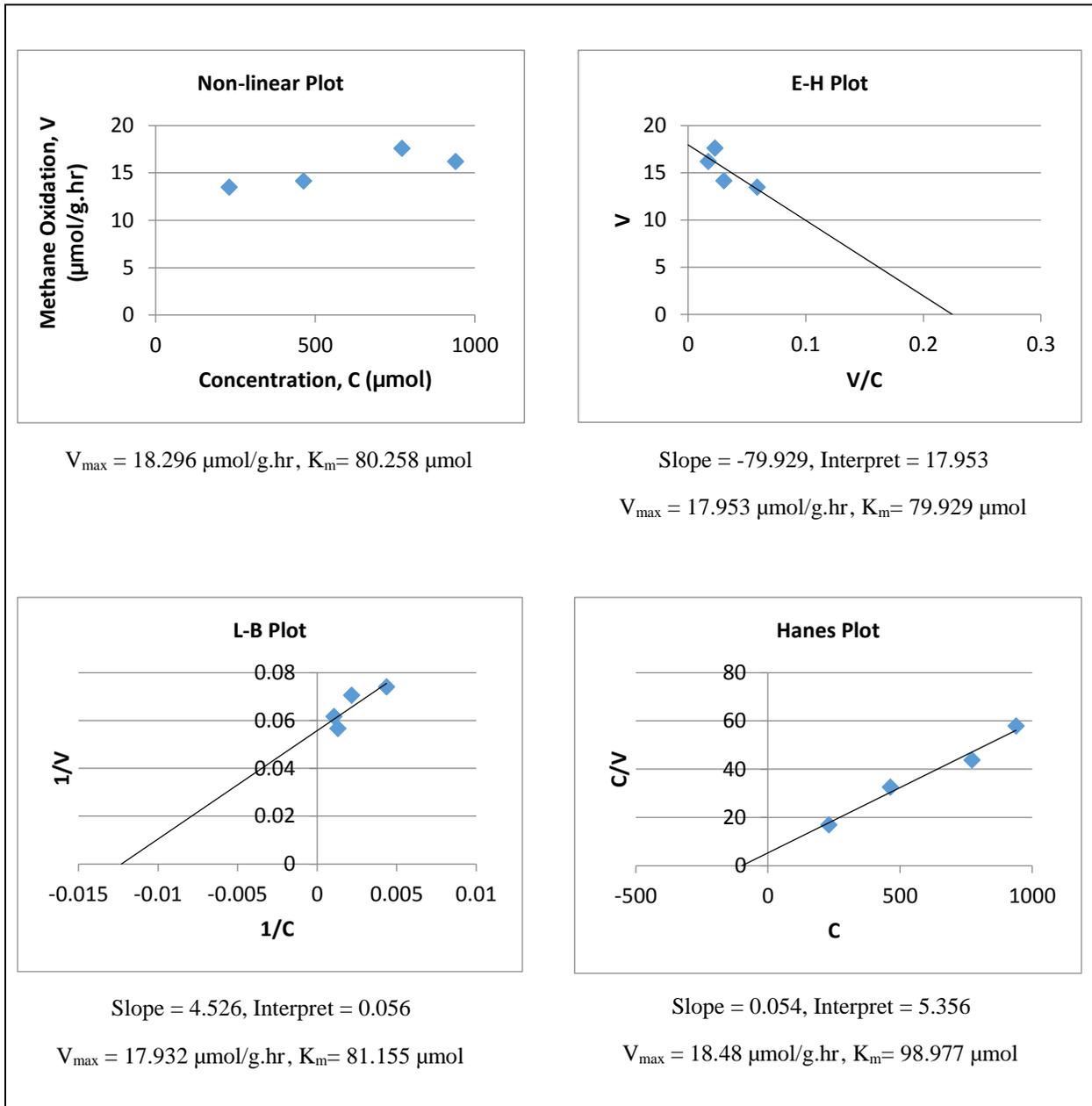
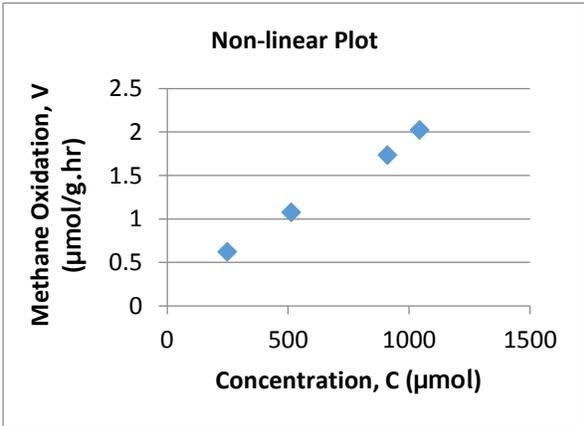
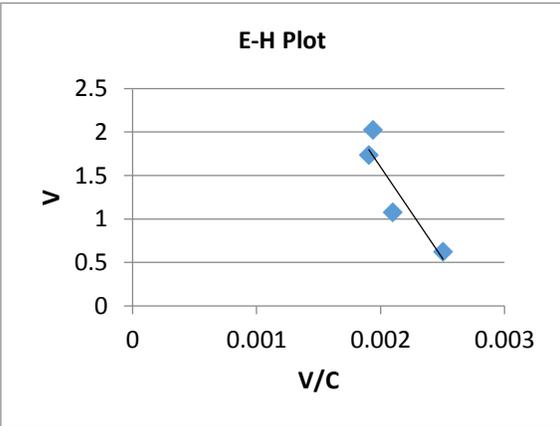


Figure B-1: Michaelis-Menten standard and linearized graphs and V_{\max} values for the CCD experiment at pH=7.9 and MC=34%

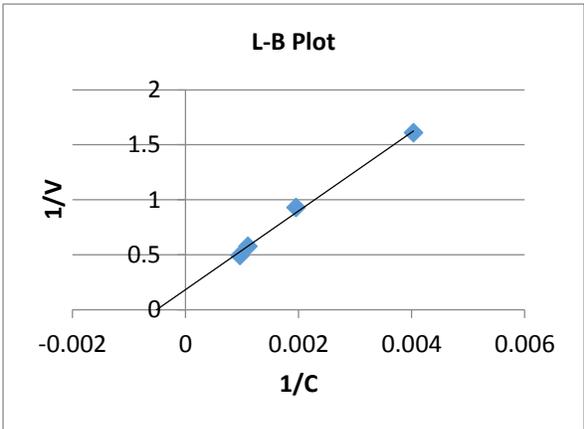


$V_{max} = 8.783 \mu\text{mol/g.hr}$, $K_m = 1825.263 \mu\text{mol}$



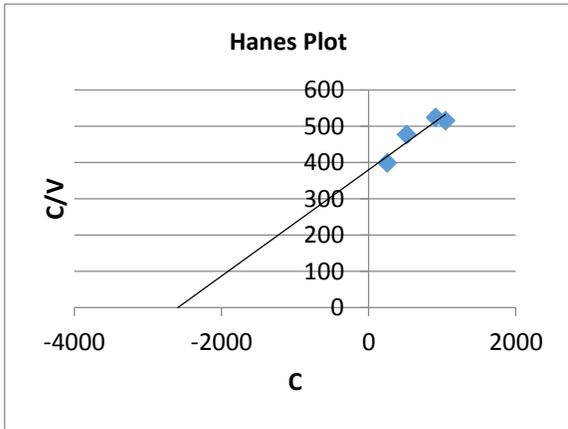
Slope = -2113.352, Interpret = 5.827

$V_{max} = 5.827 \mu\text{mol/g.hr}$, $K_m = 2113.352 \mu\text{mol}$



Slope = 357.737, Interpret = 0.184

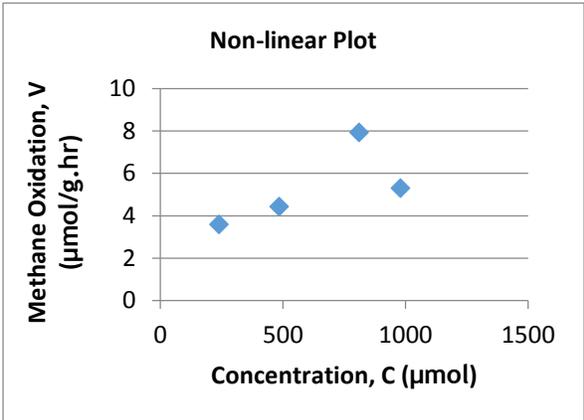
$V_{max} = 5.443 \mu\text{mol/g.hr}$, $K_m = 1947.151 \mu\text{mol}$



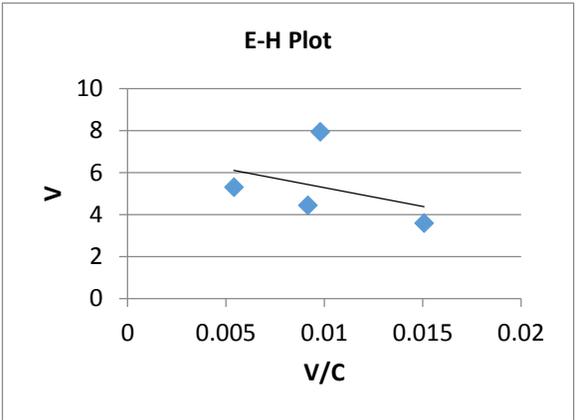
Slope = 0.146, Interpret = 379.751

$V_{max} = 6.832 \mu\text{mol/g.hr}$, $K_m = 2592.423 \mu\text{mol}$

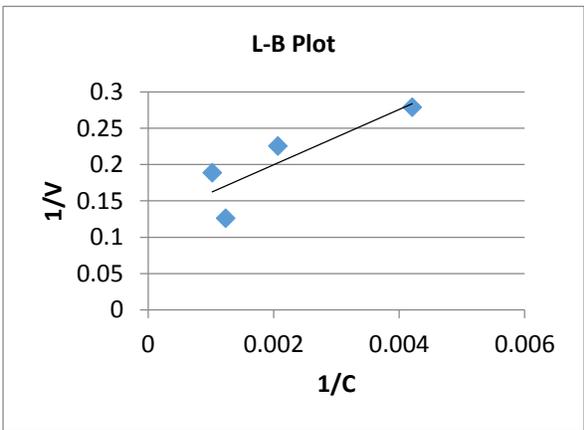
Figure B-2: Michaelis-Menten standard and linearized graphs and V_{max} values for the CCD experiment at pH=5.1 and MC=54%



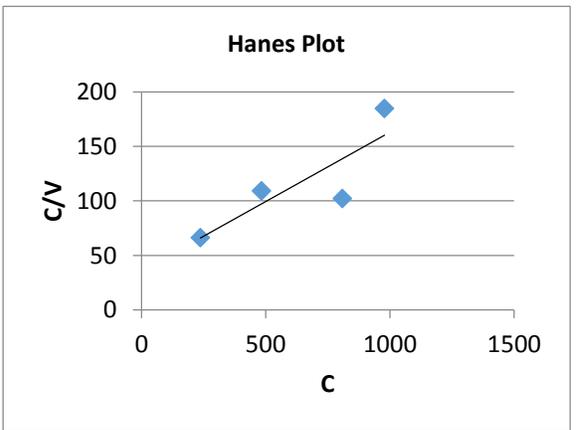
$V_{max} = 8.994 \mu\text{mol/g.hr}$, $K_m = 311.254 \mu\text{mol}$



Slope = -179.72, Interpret = 7.083
 $V_{max} = 7.083 \mu\text{mol/g.hr}$, $K_m = 179.72 \mu\text{mol}$



Slope = 38.034, Interpret = 0.124
 $V_{max} = 8.079 \mu\text{mol/g.hr}$, $K_m = 307.289 \mu\text{mol}$



Slope = 0.127, Interpret = 35.647
 $V_{max} = 7.851 \mu\text{mol/g.hr}$, $K_m = 279.863 \mu\text{mol}$

Figure B-3: Michaelis-Menten standard and linearized graphs and V_{max} values for the CCD experiment at pH=7.9 and MC=54%

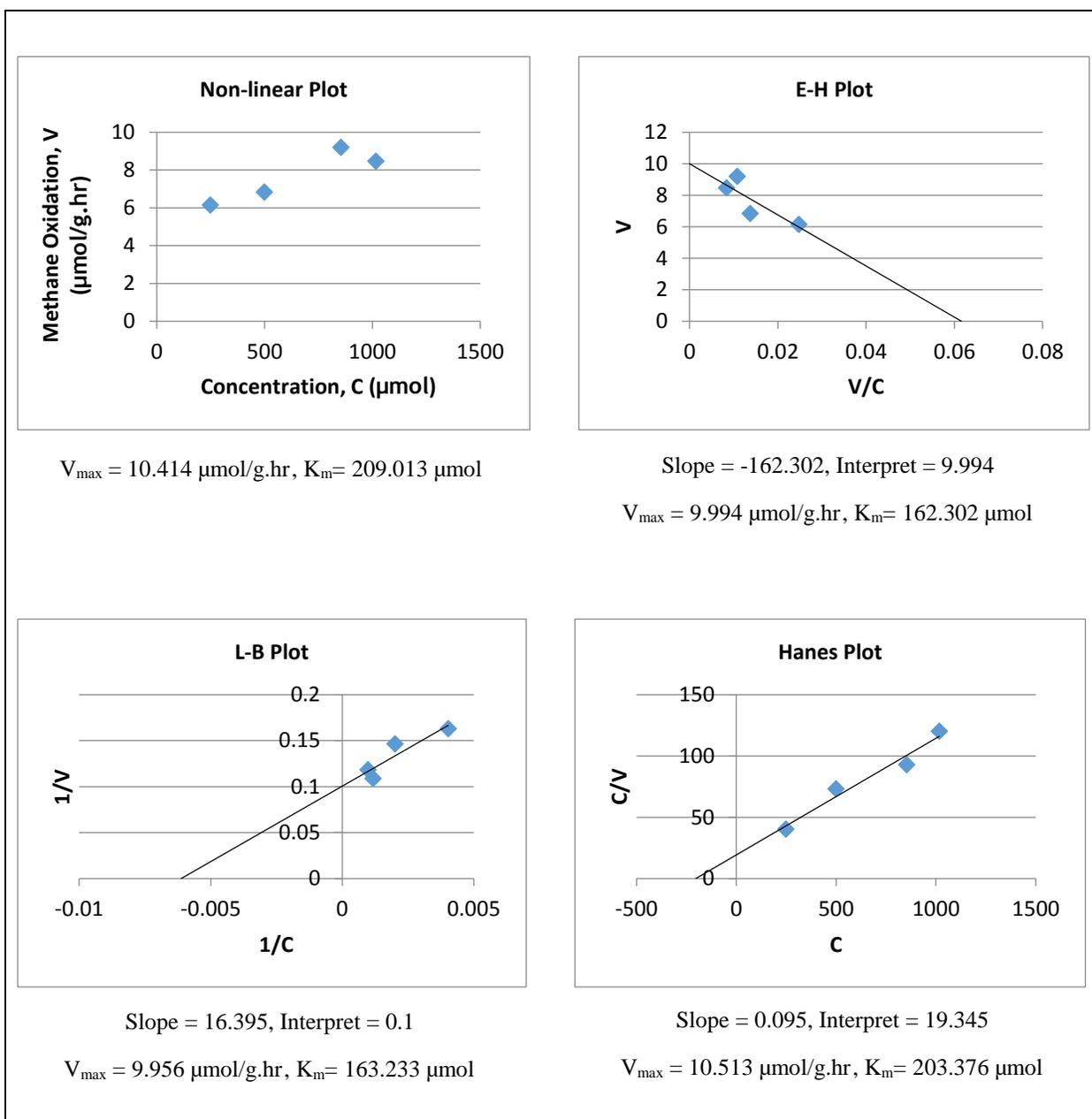


Figure B-4: Michaelis-Menten standard and linearized graphs and V_{max} values for the CCD experiment at pH=4.5 and MC=44%

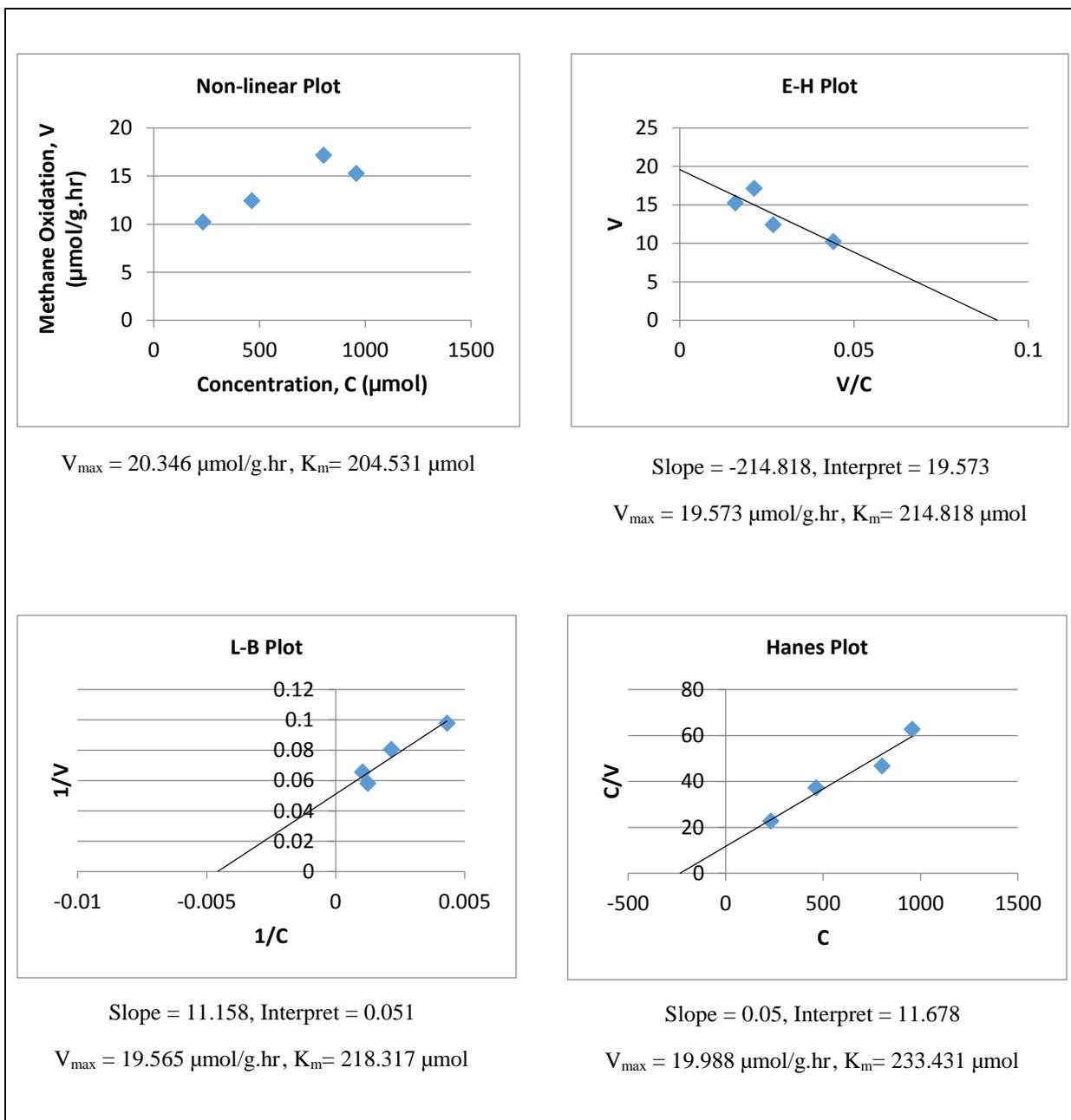
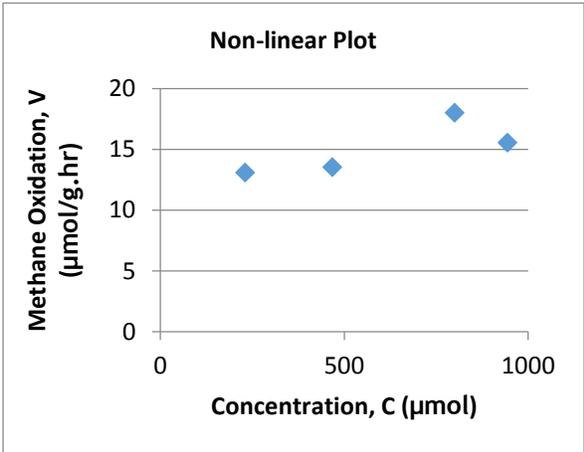
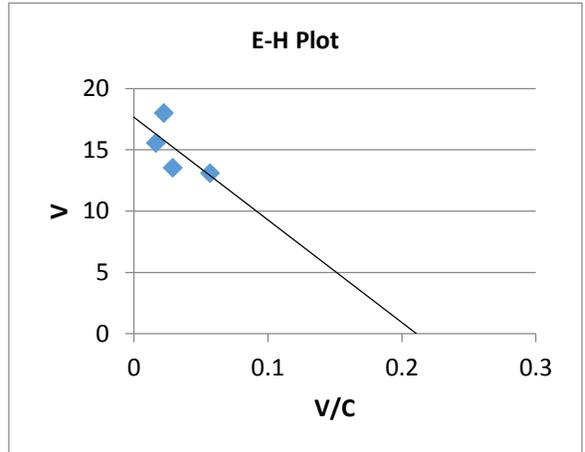


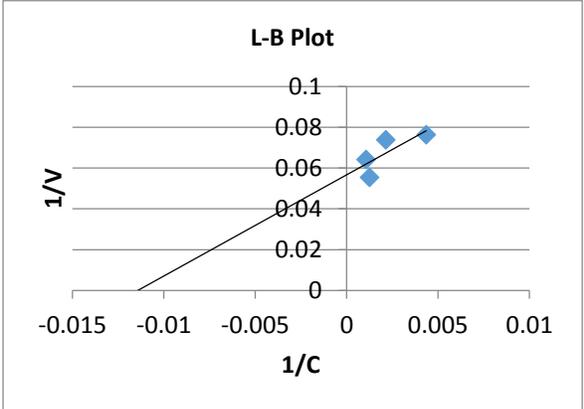
Figure B-5: Michaelis-Menten standard and linearized graphs and V_{max} values for the CCD experiment at pH=8.5 and MC=44%



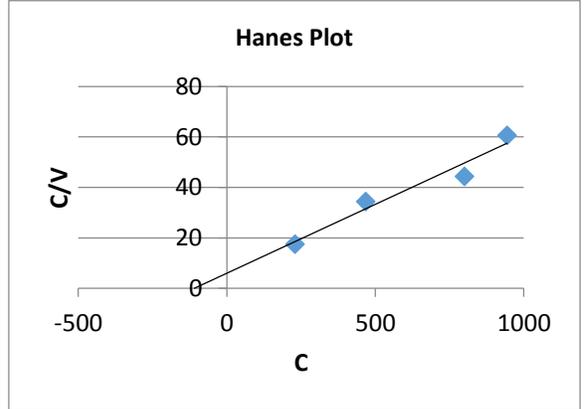
$V_{max} = 18.234 \mu\text{mol/g.hr}$, $K_m = 194.878 \mu\text{mol}$



Slope = -83.799, Interpret = 17.661
 $V_{max} = 17.661 \mu\text{mol/g.hr}$, $K_m = 83.799 \mu\text{mol}$



Slope = 4.954, Interpret = 0.057
 $V_{max} = 17.658 \mu\text{mol/g.hr}$, $K_m = 87.488 \mu\text{mol}$



Slope = 0.055, Interpret = 5.977
 $V_{max} = 18.313 \mu\text{mol/g.hr}$, $K_m = 109.457 \mu\text{mol}$

Figure B-6: Michaelis-Menten standard and linearized graphs and V_{max} values for the CCD experiment at pH=6.5 and MC=30%

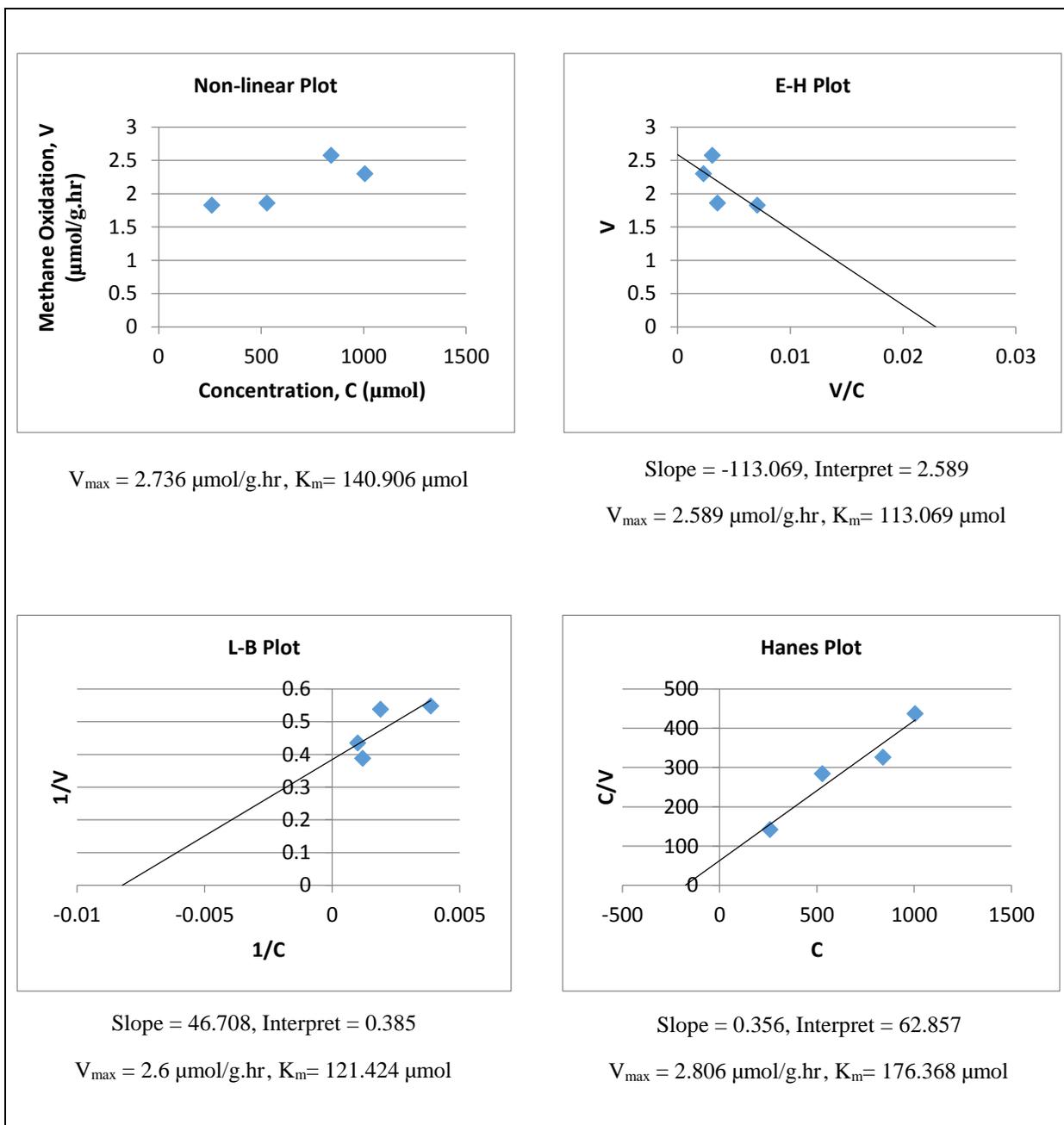
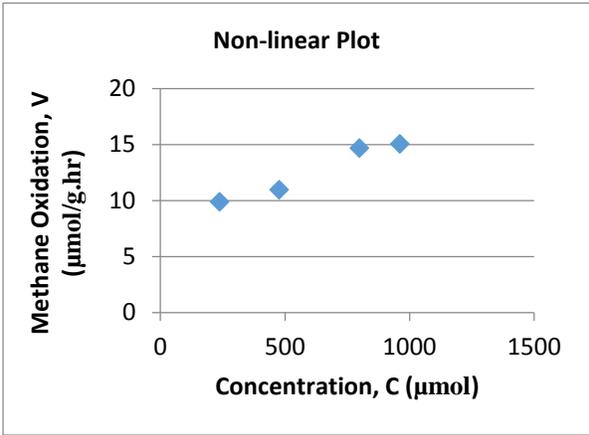
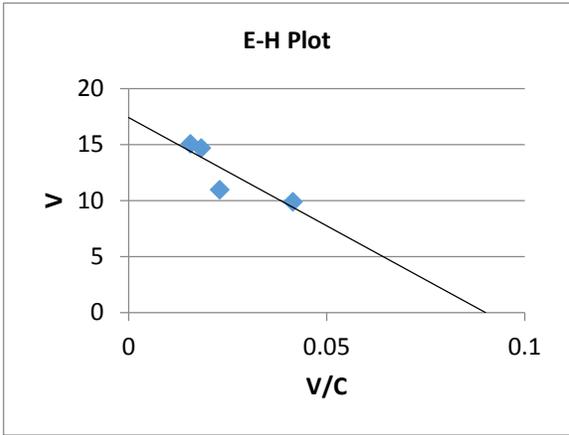


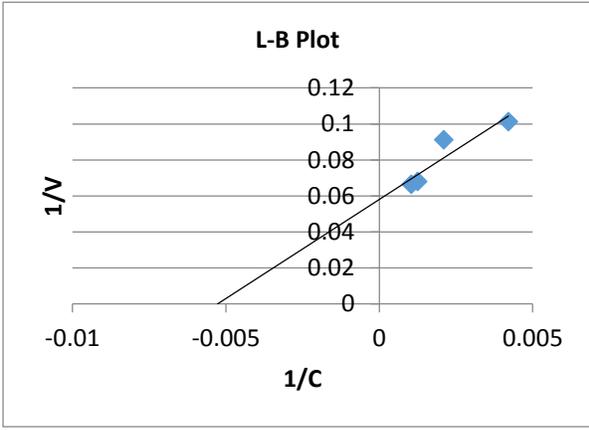
Figure B-7: Michaelis-Menten standard and linearized graphs and V_{max} values for the CCD experiment at pH=6.5 and MC=58%



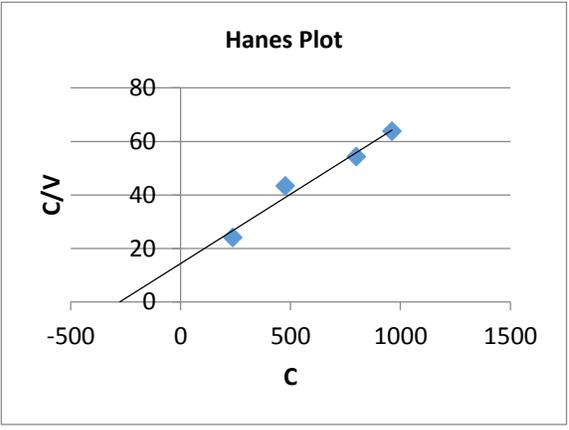
$V_{\max} = 18.648 \mu\text{mol/g.hr}$, $K_m = 211.098 \mu\text{mol}$



Slope = -193.052, Interpret = 17.4
 $V_{\max} = 17.4 \mu\text{mol/g.hr}$, $K_m = 193.052 \mu\text{mol}$

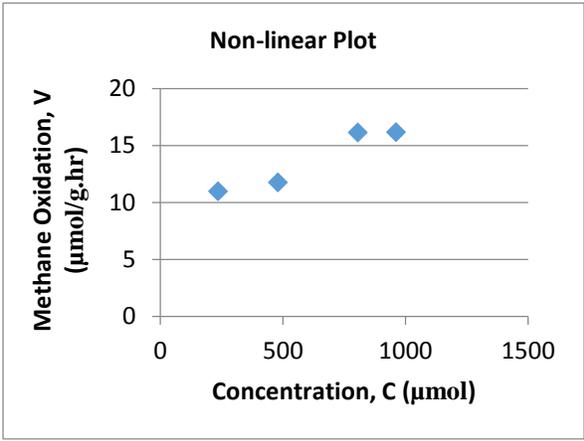


Slope = 11.022, Interpret = 0.058
 $V_{\max} = 17.219 \mu\text{mol/g.hr}$, $K_m = 189.781 \mu\text{mol}$

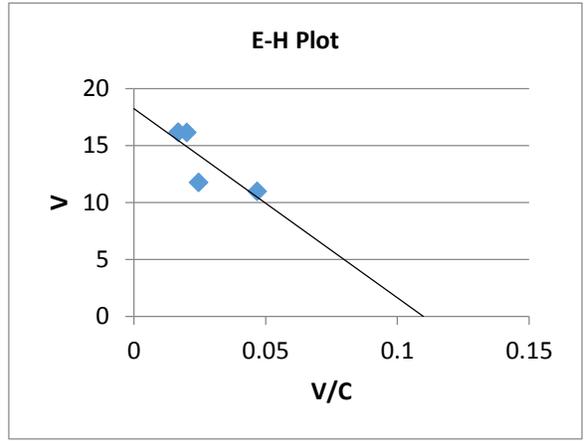


Slope = 0.052, Interpret = 14.388
 $V_{\max} = 19.294 \mu\text{mol/g.hr}$, $K_m = 277.61 \mu\text{mol}$

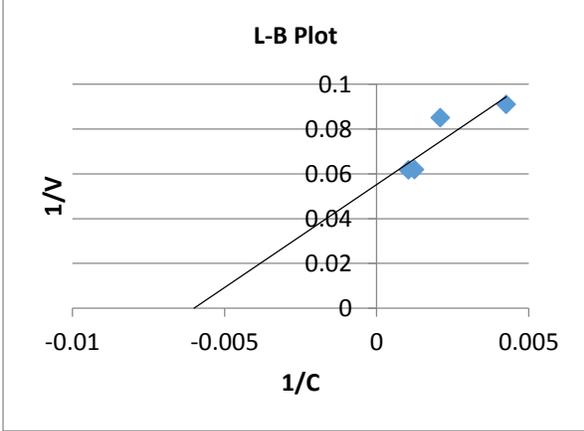
Figure B-8: Michaelis-Menten standard and linearized graphs and V_{\max} values for the CCD experiment at pH=6.5 and MC=44%



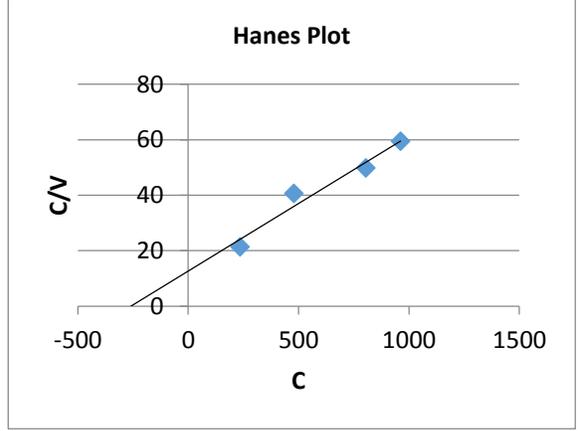
$V_{max} = 19.683 \mu\text{mol/g.hr}$, $K_m = 234.432 \mu\text{mol}$



Slope = -166.201, Interpret = 18.251
 $V_{max} = 18.251 \mu\text{mol/g.hr}$, $K_m = 166.201 \mu\text{mol}$

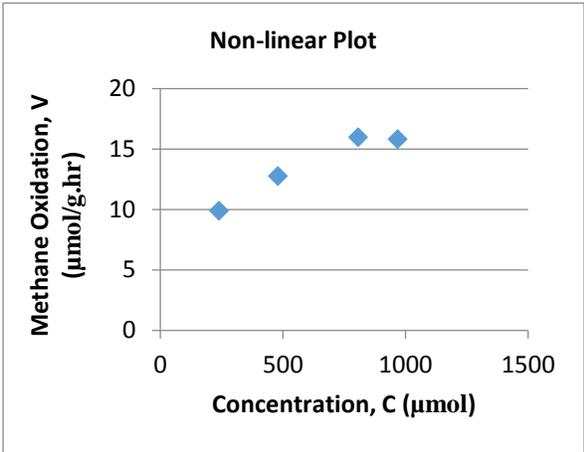


Slope = 9.185, Interpret = 0.055
 $V_{max} = 18.119 \mu\text{mol/g.hr}$, $K_m = 166.422 \mu\text{mol}$

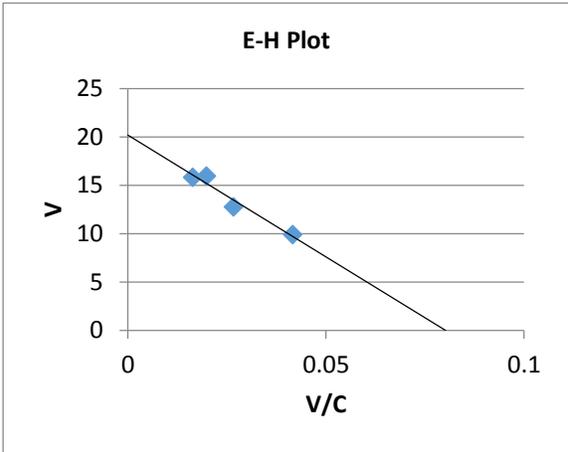


Slope = 0.049, Interpret = 12.681
 $V_{max} = 20.535 \mu\text{mol/g.hr}$, $K_m = 260.411 \mu\text{mol}$

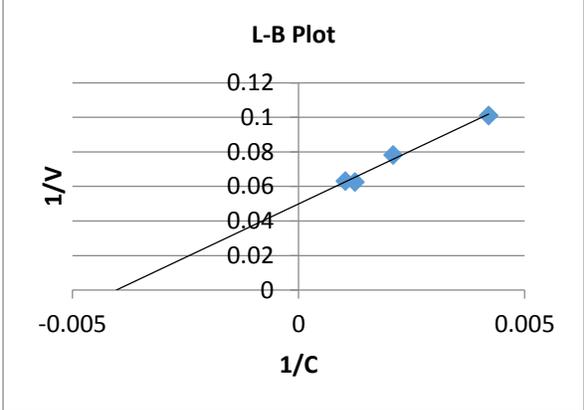
Figure B-9: Michaelis-Menten standard and linearized graphs and V_{max} values for the CCD experiment at pH=6.5 and MC=44% (replicate 1)



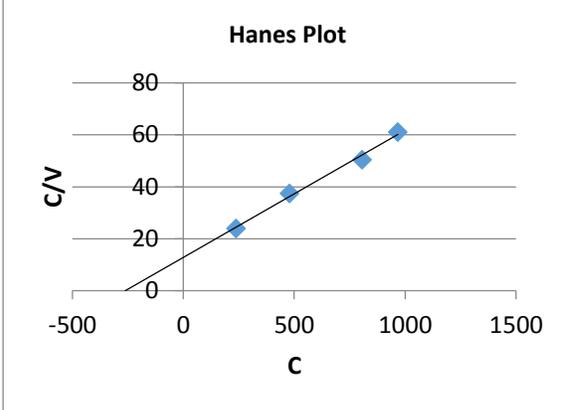
$V_{max} = 20.46 \mu\text{mol/g.hr}$, $K_m = 250.411 \mu\text{mol}$



Slope = -251.753, Interpret = 20.182
 $V_{max} = 20.182 \mu\text{mol/g.hr}$, $K_m = 251.753 \mu\text{mol}$



Slope = 12.358, Interpret = 0.05
 $V_{max} = 20.06 \mu\text{mol/g.hr}$, $K_m = 247.912 \mu\text{mol}$



Slope = 0.05, Interpret = 12.834
 $V_{max} = 20.433 \mu\text{mol/g.hr}$, $K_m = 262.238 \mu\text{mol}$

Figure B-10: Michaelis-Menten standard and linearized graphs and V_{max} values for the CCD experiment at pH=6.5 and MC=44% (replicate 2)

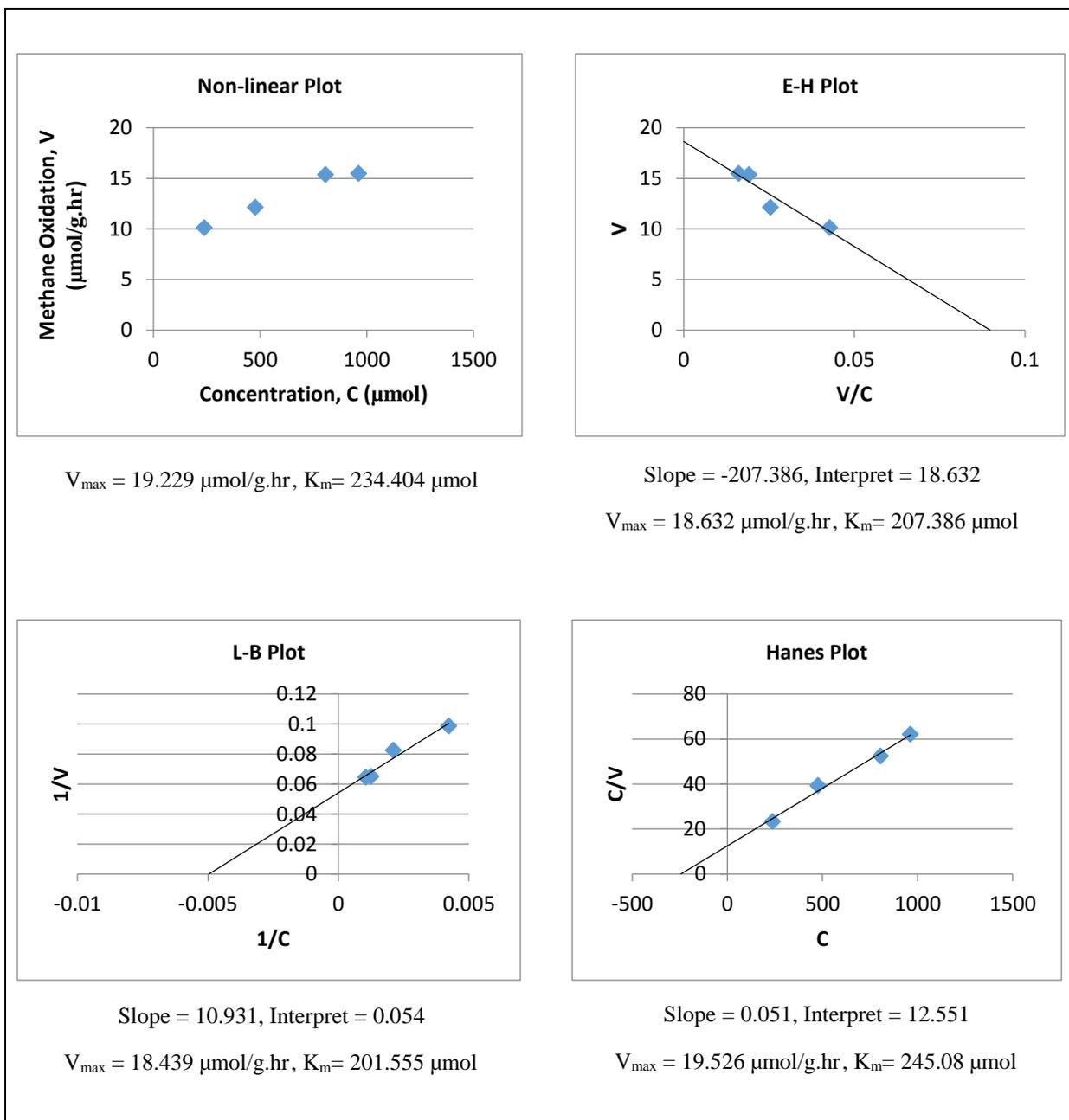
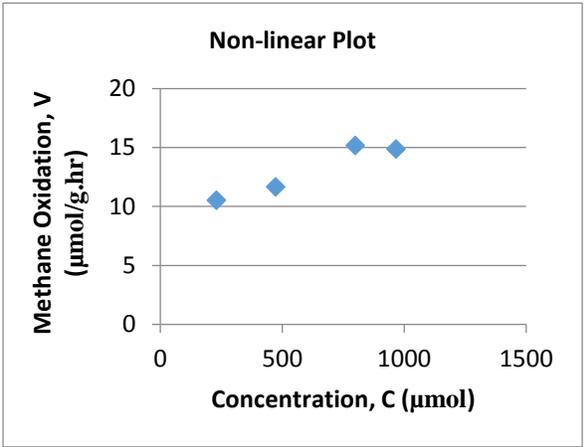
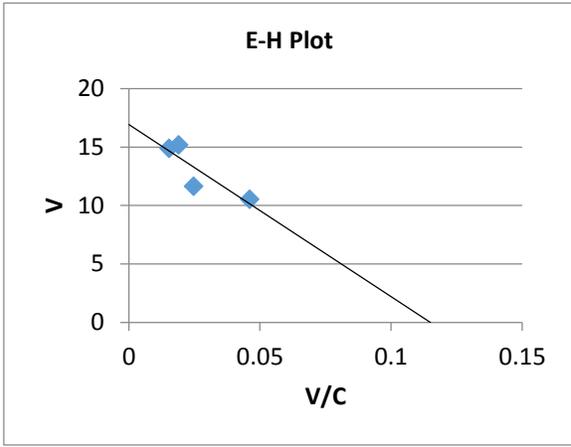


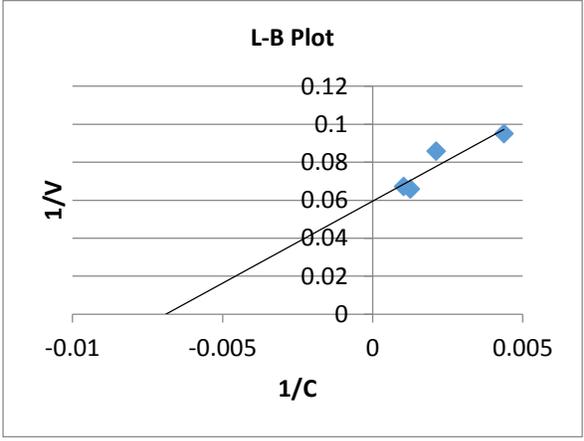
Figure B-11: Michaelis-Menten standard and linearized graphs and V_{max} values for the CCD experiment at pH=6.5 and MC=44% (replicate 3)



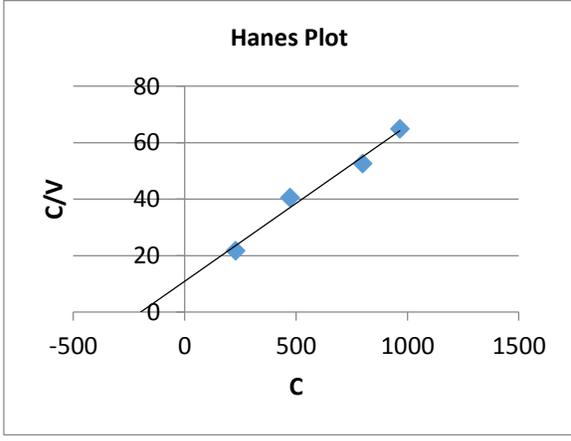
$V_{\max} = 17.598 \mu\text{mol/g.hr}$, $K_m = 176.812 \mu\text{mol}$



Slope = -147.005, Interpret = 16.913
 $V_{\max} = 16.913 \mu\text{mol/g.hr}$, $K_m = 147.005 \mu\text{mol}$



Slope = 8.64, Interpret = 0.059
 $V_{\max} = 16.791 \mu\text{mol/g.hr}$, $K_m = 145.073 \mu\text{mol}$



Slope = 0.055, Interpret = 10.914
 $V_{\max} = 18.096 \mu\text{mol/g.hr}$, $K_m = 197.498 \mu\text{mol}$

Figure B-12: Michaelis-Menten standard and linearized graphs and V_{\max} values for the CCD experiment at pH=6.5 and MC=44% (replicate 4)