Diversity and Activity of Methanotrophs in Biogeochemical Landfill Cover

BY

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THESIS

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CONTRIBUTIONS OF AUTHORS

Chapter 1 is an introduction to the background, problem statement, and goal of this research. Chapter 2 represents a published conference paper [Rai, R.K., Chetri, J.K., Green, S.J., and Reddy, K.R. (2019). "Identifying active methanotrophs and mitigation of CH_4 emissions in landfill cover soil." In: Zhan L., Chen Y., Bouazza A. (eds) Proc. 8th International Congress on Environmental Geotechnics Volume 2. ICEG 2018. Environmental Science and Engineering. Springer, Singapore] of which I was the primary author and performed all the experiments, microbial sequencing was performed at the University of Illinois at Chicago Sequencing Core (UICSQC), and basic bioinformatics processing of the data were performed at the UIC Research Informatics Core (RIC). My colleague Ms. Jyoti K. C contributed in reviewing and editing of the paper. Dr. Stefan J. Green provided valuable feedback and revisions of the paper, along with my mentor Dr. Krishna Reddy. Chapter 3 represents a conference paper [Rai, R. K., and Reddy, K. R. " Role of Landfill Cover Materials in Mitigating GHG Emissions in Biogeochemical Landfill Cover System." Proc. World Environmental and Water Resources Congress, Pittsburgh, PN, USA, May 19th – May 23rd, 2019]. I was the principal author for writing the conference paper, as well as conducting the experiments. My colleague Ms. Jyoti K. C and my mentor Dr. Krishna Reddy contributed in reviewing and editing of the paper. Chapter 4 represents a conference paper [Rai, R. K., and Reddy, K. R. "Methanotrophic methane oxidation in new biogeochemical landfill cover system." Proc. 34th International Conference on Solid Waste Technology and Management, Annapolis, MD, USA, March 31st – April 3rd, 2019]. I was the principal author for writing the conference paper, as well as conducting the experiments. My colleague Ms. Jyoti K. C and my mentor Dr. Krishna Reddy contributed in reviewing and editing of the paper. The chapters

(Chapter 5, 6, and 7) represent a series of my own unpublished experiments with the assistance of my colleague Ms. Jyoti K. C. These chapters were reviewed and edited by Dr. Stefan J. Green and Dr. Krishna Reddy. All the microbial sequencing was performed at the University of Illinois at Chicago Sequencing Core (UICSQC), and basic bioinformatics processing of the data were performed at the UIC Research Informatics Core (RIC). Chapter 8 represents a conference paper [Rai. R. K, Chetri, J. K, and Reddy, K. R. (2019). "Effect of methanotrophic-activated biochar amended soil in mitigating CH_4 emissions from landfills." Proc. 4th International Conference on Civil and Environmental Geology and Mining Engineering, Trabzon, Turkey, 20-22 April 2019] of which I was the primary author. The experiment was performed by me and Ms. Jyoti K. C. This paper was reviewed and edited by Ms. Jyoti K.C and my mentor Dr. Krishna Reddy. Chapter 9, represents overall conclusions and recommendations for future work.

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CHAPTER 1 - INTRODUCTION

1.1 BACKGROUND

Municipal solid waste (MSW) landfills are considered to be one of the third largest anthropogenic source of methane emissions in the United States (USEPA 2019). MSW in landfills undergo anaerobic decomposition typically generating ~50% methane (CH_4) and ~50% carbon dioxide (CO_2) with traces of hydrogen sulfide (H_2S) into the atmosphere. Landfill cover soils are usually dominated by methane oxidizing bacteria (MOB) that uses CH_4 as a sole source of carbon and energy. Most of the CH_4 produced during decomposition of the waste in the landfills are converted to CO_2 by MOBs before reaching into the atmosphere. In recent years, biocovers consisting of organic-rich amendments such as compost, sewage sludge, and biosolids to landfill cover soils have enhanced the CH_4 oxidation activity, thereby reducing CH_4 emissions from landfills.

Previous study at UIC on biochar-amended soil has demonstrated potential in enhancing CH_4 oxidation in both column and field scale studies. Biochar, a solid by-product obtained by organic mass pyrolysis, has shown promising results in proliferation of microbial community due to its physico-chemical characteristics (internal porosity, large surface area and stable organic C content) and enhancement of CH_4 oxidation in MSW landfills (Reddy et al. 2014; Yargicoglu and Reddy, 2017 a, b). Although the CH_4 emissions are mitigated by microbial oxidation, the resulting CO_2 emissions by microbial oxidation of CH_4 as well as CO_2 that prevails from MSW decomposition is continuously emitted into the atmosphere in undesirable amounts.

In order to mitigate CO_2 emissions from the landfills, a recent study has shown potential use of Basic Oxygen Furnace (BOF) slag, in sequestering CO_2 from landfill gas emissions (Reddy et al. 2018; Reddy et al. 2019). A biogeochemical landfill cover system is therefore proposed that uses biochar amended soil along with BOF slag to achieve simultaneous mitigation of CH_4 by

microbial oxidation and CO_2 sequestration by carbonation in BOF slag with an aim to achieve zero emissions from the MSW landfills.

1.2 PROBLEM STATEMENT

A biogeochemical landfill cover, consisting of biochar-amended soil and BOF slag is being developed to mitigate both CH_4 and CO_2 emissions from landfills (Reddy et al. 2019; Chetri et al. 2019). Previous research on the use of biochar-amended soil has shown potential in enhancing methane oxidation in the landfills. Recently, BOF slag has been used for sequestration of CO_2 from landfill gas emissions. However, the effect of high alkalinity of BOF slag (pH >12) on the survival and activity of methane oxidizing bacteria is not well understood. It is crucial to investigate the synergistic effects from the coexistence of BOF slag and microbially rich biochar-amended soil for effective performance of the biogeochemical cover. This study focuses on studying the role of CH_4 oxidizing bacteria in CH_4 oxidation in the proposed cover materials individually as well as in combinations. Further, the effect of critical factors that influence CH_4 oxidation process in the biogeochemical landfill cover system is investigated. The results from this study will help in designing cover profiles for scaling long term and field scale studies so as to achieve simultaneous CH_4 oxidation and CO_2 sequestration in the biogeochemical landfill cover system with an overall goal to achieve zero emissions from the landfills.

1.3 RESEARCH GOALS AND OBJECTIVES

The overall goal of this research is to systematically investigate the effect of various system variables that affects CH_4 oxidation and CO_2 sequestration in the newly proposed biogeochemical landfill cover system. The specific objectives of this research were to (i) characterize each cover

materials individually and in combinations suitable for CH_4 oxidation and CO_2 sequestration in the biogeochemical landfill cover, and (ii) to study the critical factors such as pH, BOF slag leachate and temperature that may affect CH_4 oxidation and microbial diversity in the biogeochemical landfill cover.

1.4 THESIS ORGANIZATION

With this chapter serving as the introduction, this thesis is organized into following chapters:

Chapter 2: Identify active methanotrophs using culture dependent and culture independent techniques in mitigation of methane emissions in Zion landfill cover soil. This chapter was previously published in the conference proceedings by the author as a part of this thesis work. [Rai, R.K., Chetri, J.K., Green, S.J., and Reddy, K.R. (2019). "Identifying active methanotrophs and mitigation of CH_4 emissions in landfill cover soil." In: Zhan L., Chen Y., Bouazza A. (eds) *Proc. 8th International Congress on Environmental Geotechnics Volume 2. ICEG 2018. Environmental Science and Engineering*. Springer, Singapore]

Chapter 3: Batch scale experiments to study the role of landfill cover materials individually in mitigating GHG emissions in biogeochemical landfill cover system was investigated. This chapter was previously published in the conference proceedings by the author as a part of this thesis work. [Rai, R. K., and Reddy, K. R. " Role of Landfill Cover Materials in Mitigating GHG Emissions in Biogeochemical Landfill Cover System." Proc. World Environmental and Water Resources Congress, Pittsburgh, PN, USA, May19th – May 23rd, 2019]

Chapter 4: Batch scale experiments to study the effect of biogeochemical landfill cover materials (soil, biochar and slag) in combinations on methane oxidation and carbon dioxide

sequestration is studied. This chapter was previously published in the conference proceedings by the author as a part of this thesis work.

[Rai, R. K., and Reddy, K. R. "Methanotrophic methane oxidation in new biogeochemical landfill cover system." *Proc. 34th International Conference on Solid Waste Technology and Management*, Annapolis, MD, USA, March 31st – April 3rd, 2019]

Chapter 5: Batch scale experiments were conducted to study the effect of pH on methane oxidation and microbial community composition in the landfill cover soil.

Chapter 6: Batch scale experiments were conducted to study the effect of temperature on methane oxidation and microbial community composition in the landfill cover soil.

Chapter 7: Batch scale experiments were conducted to study the effect of BOF slag leachate on methane oxidation and microbial community composition in the landfill cover soil.

Chapter 8: Long term batch scale experiments were conducted to study the effect of methanotrophic-activated biochar amended soil and non-methanotrophic activated biochar amended soil in mitigating methane emissions from landfills. This chapter was previously published in the conference proceedings by the author as a part of this thesis work

[Rai. R. K, Chetri, J. K, and Reddy, K. R. (2019). "Effect of methanotrophic-activated biochar amended soil in mitigating CH_4 emissions from landfills." *Proc. 4th International Conference on Civil and Environmental Geology and Mining Engineering*, Trabzon, Turkey, 20-22 April 2019]

Chapter 9: Overall summary, conclusions and recommendation of this study.

1.5 CITED REFERENCES

Chetri, J. K., Reddy, K. R., & Grubb, D. G. (2019, March). “Effect of Moisture Content on CO_2 Sequestration by BOF Slag in Landfill Cover”. *Proc. Geo-Congress 2019:*

Geoenvironmental Engineering and Sustainability (pp. 158-166). Reston, VA: American Society of Civil Engineers.

Reddy, K.R., Yargicoglu, E.N., Yue, D., and Yaghoubi, P. (2014). "Enhanced microbial methane oxidation in landfill cover soil amended with biochar." *J. Geotech. Geoenviron. Eng.*, ASCE, 140(9).

Reddy, K.R., Grubb, D.G. and Kumar, G. (2018). "Innovative biogeochemical soil cover to mitigate landfill gas emissions," Proceedings of the *International Conference on Protection and Restoration of the Environment XIV*, Thessaloniki, Greece.

Reddy, K. R., Gopakumar, A., Rai, R. K., Kumar, G., Chetri, J. K., and Grubb, D. G. (2019). "Effect of basic oxygen furnace slag particle size on sequestration of carbon dioxide from landfill gas". *Waste Management & Research,* 0734242X18823948.

Yargicoglu, E. N., and Reddy, K. R. (2017 a). "Effects of biochar and wood pellets amendments added to landfill cover soil on microbial methane oxidation: A laboratory column study." *J. Environ. Manag.*, 193, 19-31.

Yargicoglu, E. N., & Reddy, K. R. (2017 b). Biochar-amended soil cover for microbial methane oxidation: effect of biochar amendment ratio and cover profile. Journal of Geotechnical and Geoenvironmental Engineering, 144(3), 04017123.

United States Environmental Protection Agency (USEPA). (2019). "Landfill methane outreach program: Basic information about landfill gas". Available at: https://www.epa.gov/lmop/basic-information-about-landfill-gas (accessed on 25th February, 2019)

CHAPTER 2 - IDENTIFYING ACTIVE METHANOTROPHS AND MITIGATION OF CH_4 EMISSIONS IN LANDFILL COVER SOIL

2.1 INTRODUCTION

The content of this chapter has been previously published by Rai et al. (2018) during the author's master's thesis work [Previously published as Rai, R.K., Chetri, J.K., Green, S.J., and Reddy, K.R. (2018) Identifying active methanotrophs and mitigation of CH_4 emissions in landfill cover soil, In the International Congress on Environmental Geotechnics (pp. 308-316). Springer, Singapore.]

In the USA, landfills are estimated to be the third largest anthropogenic source of CH_4 emissions making up 16.4% of the total CH_4 emissions in 2016 (USEPA 2018). Despite significant amount of CH_4 emitted from landfills, it is estimated that between 10 and 90% is actively been consumed by the methane oxidizing bacteria (MOB) in landfill cover soils as reviewed by Semrau et al. (2010). MOB, also known as methanotrophs, are a subset of a larger microbial community called Methylotrophs. The methanotrophs utilize CH_4 as a sole source of carbon and energy, whereas the methylotrophs use C1-compounds as their source of carbon and energy (Hanson and Hanson 1996). Methanotrophs within the phylum Proteobacteria are classified into three phylogenetically distinct groups: Type I, Type II and Type X methanotrophs, where Type I and Type X are grouped in Gamma proteobacteria and Type II within Alpha proteobacteria. Type I, Type II and Type X methanotrophs are ubiquitous in nature and are usually found in abundance where low to high concentration of CH_4 prevails.

Landfill cover soils are typically dominated with either by Type-I or Type-II methanotrophs as reported in many studies (Yargicoglu and Reddy 2017, Cébron et al. 2007). Molecular ecology studies have utilized two distinct approaches for identification of methanotrophs from the environmental samples: cultivation-based enrichment and isolation

approaches, and cultivation-independent molecular tools (Murrell et al.1998) employing targeted amplification and sequencing of functional or structural genes or shotgun sequencing approaches. In some cases, both approaches are used concurrently, with molecular tools used to monitor and characterize enrichments and isolates. Targeted amplification protocols typically target structural genes such as the microbial small subunit ribosomal RNA genes (*i.e.*, 16S rRNA gene), as well as methane monooxygenase (MMO) genes and genes involved in C1 compound oxidation.

This study focuses on adopting PCR-based high-throughput amplicon sequencing technique to analyze microbial structure in landfill cover soil (LFCS) and in microcosms studies inoculated with LFCS. The specific objectives of this research were to: (1) characterize methanotrophic communities in landfill cover soil using 16S rRNA gene analysis, (2) Conduct microcosm batch tests using soil suspension and methanotrophic enrichment culture from LFCS using 16S rRNA gene analysis, and (3) assess the relationship between CH_4 oxidation rates and the relative abundance of methanotrophic community in LFCS. This study is a part of broader on-going study funded by the U.S. National Science Foundation with ultimate goal to evaluate system parameters that control microbial diversity and activity and design optimal and efficient biocover systems to mitigate CH_4 emissions at landfills.

2.2 MATERIALS AND METHODS

2.2.1 Soil Enrichment

Soil was collected from Zion landfill site, located in Greater Chicago area, Illinois, USA. Soil samples were collected from an interim cover layer at a depth of ~1 to 2 feet and were shipped to the Geotechnical and Geoenvironmental Engineering Laboratory at the University of Illinois at Chicago (UIC) where it was stored at room temperature (23°C). Soil samples were air dried

(moisture content <0.5%), pulverized and screened through 2 mm sieve prior to the inoculation of batch reactors. To obtain methanotroph-enriched consortia, approximately 5g of sieved soil was mixed with 100 mL of modified NMS medium (Whittenbury et al.1970) in a 500 mL serum vial and stoppered using long sleeved rubber septa. Approximately 80 mL of air from the headspace was replaced with equal volume of mix gas CH_4 /CO_2 to achieve a headspace concentration of 7% CH_4 (v/v) and 7% CO_2 (v/v) balanced in air (86%) and were incubated for 20 days at 23°C. To determine the activity of Methanotrophs and CH_4 oxidation rates, gas samples were analyzed at regular intervals using Gas Chromatography (GC) and were monitored until the CH_4 concentration dropped to less than 1%. To enrich the methanotrophic enrichment culture the mix gas (CH_4/CO_2) was replenished twice throughout the enrichment. The soil after enrichment was stored in micro-centrifuge tubes and frozen at -20°C for DNA extraction and molecular analysis. Similarly, the supernatant enriched with methanotrophic cells were also pelletized in 2 mL micro-centrifuge tubes by centrifuging at 12500 RPM for 15 min, decanting the supernatant and freezing at -20°C for DNA extraction and molecular analysis.

2.2.2 Enrichment Culture Batch Tests

Prior to culture experiments, serum vials, rubber septa and pipettes were sterilized using a Napco Model 8000-DSE autoclave operated at >120°C for a minimum of 60 minutes to ensure complete sterilization. The supernatant obtained from soil enrichment as mentioned above was used in the experimental sets. 1 mL of enrichment culture was inoculated in 9 mL modified NMS medium (total of 10 ml), placed in 125 mL serum vials and sealed air tight using butyl rubber septa followed by crimp cap. Approximately 20 mL of air from the headspace was replaced with equal volume of synthetic landfill gas comprising of 50% (v/v) CH_4 and 50% (v/v) CO_2 to achieve a headspace

concentration of 5% CH_4 (v/v) and 5% CO_2 (v/v) balanced in air (90%). To determine changes in the headspace concentration, gas samples were analyzed every alternate day using Gas Chromatography (GC) until the headspace concentration dropped to less than 1%. All the experiments were conducted in triplicates along with the controls (media-NMS). The rates of CH_4 oxidation were determined from linear regression analysis of CH_4 concentration with respect to time based on zero-order kinetics observed during testing. pH of the culture along with controls (NMS only) were also measured at the beginning and end of the experiment to examine any changes in the pH due to microbial activity.

2.2.3 DNA extraction, PCR Amplification and Next Generation Sequencing

To measure microbial diversity in enriched soils and in enrichment culture, genomic DNA was extracted from the samples using DNeasy Power Soil Kit (Qiagen). Extractions were performed according to the manufacturer's instructions, with slight modifications. Briefly, samples were heated at 65 °C for 10 min before homogenization with FastPrep-24 5G bead-beating device (MP Biomedicals) at 6 m/s for 40 sec. Genomic DNA was used as template for PCR amplification with primers 515F-modified and 926R (Walters et al. 2016), targeting the V4-V5 variable region of the microbial small subunit ribosomal RNA gene using a two-stage "targeted amplicon sequencing (TAS)" protocol (Green et al. 2015, Bybee et al. 2011). The primers contained 5' common sequence tags (known as common sequence 1 and 2, CS1 and CS2) as described previously (Moonsamy et al. 2013). The CS1_515F and CS2_926R primer sequences were ACACTGACGACATGGTTCTACAGTGYCAGCMGCCGCGGTAA and TACGGTAGCAGAGACTTGGTCTCCGYCAATTYMTTTRAGTTT, respectively, with the underlined regions indicating the common sequence tags.

First stage PCR amplifications were performed in 10 microliter reactions in 96-well plates, using the MyTaq HS 2X mastermix (Bioline, Taunton, MA). PCR conditions were 95°C for 5 minutes, followed by 28 cycles of 95°C for 30", 50°C for 60" and 72°C for 90". Subsequently, a second PCR amplification was performed in 10 microliter reactions in 96-well plates. Each well received a separate primer pair with a unique 10-base barcode, obtained from the Access Array Barcode Library for Illumina (Fluidigm, South San Francisco, CA; Item# 100-4876), as well as 1 microliter of 1st stage PCR product. Cycling conditions were as follows: 95°C for 5 minutes, followed by 8 cycles of 95°C for 30", 60°C for 30" and 72°C for 30". Libraries were loaded onto a MiSeq v3 flow cell and sequenced using an Illumina MiSeq sequencer. Raw sequence data were processed and merged using software package PEAR (Zhang et al. 2013), followed by quality checking (Q20), length trimming (>300 bp) and chimera checking using the UCHIME algorithm as compared with the Silva 119 16S.97database (Edgar 2010). After chimera removal, the software package QIIME (Caporaso et al. 2010) was used to annotate sequences and generate annotation tables using a sub-OTU protocol. Briefly, all sequences were pooled, and unique sequences were de-replicated from the combined sequenced. Those sequences with counts greater than 10 were used as seed or master sequences for clustering. Low abundance sequences (fewer than 10) were queried against the master sequences using USEARCH to find the master sequence with a minimum percent identity of 98%; for matching sequences, the counts of the low abundance sequences were incorporated into the counts for the cluster. Taxonomic annotations were assigned to each seed and independent low abundance sequence using USEARCH and the Silva 119 reference database. Taxonomic and abundance data were merged into a single sequence table (seq table. biome) and summaries of absolute abundances of taxa were generated for all phyla, classes, orders, families, genera, and species present in the dataset. Library preparation, pooling and

Illumina sequencing were performed at the University of Illinois at Chicago Sequencing Core (UICSQC), and basic bioinformatics processing of the data were performed at the UIC Research Informatics Core (RIC).

2.2.4 Gas Analysis

Gas samples were collected at regular time intervals and analyzed for CH_4, CO_2 and O_2 concentrations using an SRI 9300 Gas Chromatography (GC) equipped with thermal conductivity detector (TCD) and CTR-1 column that separates N_2 and O_2 for simultaneous analysis of CO_2, CH_4, O_2 and N_2 as previously described (Yargicoglu and Reddy, 2017). Gas samples were withdrawn using 1-mL syringe where 0.5mL of gas sample was injected into the GC equipped with TCD. A calibration curve for a minimum of three points was established using high purity standard gas mixtures ranging from 1% to 50% CH_4 and 5% to 50% CO_2.

2.3 RESULTS AND DISCUSSION

2.3.1 Methane Oxidation

Figs.2-1(a) and 2-1(b) show a decrease in the headspace CH_4 concentration with time in both soil-suspension and enrichment culture tests. An initial lag phase of four days was observed in soil-suspension batch tests, after which there was a rapid decrease in the CH_4 headspace concentration, consistent with microbial CH_4 oxidation. This is in agreement with prior studies showing similar lag phases (10-12 days) in microcosms inoculated with field samples without pre-incubation (Spokas et al. 2011). Enrichment culture tests also showed a lag phase of 24 hours. Lag phases are generally due to the inoculation of microorganisms into the fresh media (NMS) and the time taken for responding or adjusting to the new environment. The CH_4 oxidation rates were calculated using

linear regression that followed zero-order kinetics. Maximum oxidation rates in the soil suspension and enrichment culture tests resulted to be 123.3 µg CH_4 g^{-1} dry-soil d^{-1} and 73.1µg CH_4 $mL^{-1}d^{-1}$, respectively. The pH of the enrichment culture sample was also measured at the beginning and end of the experiment to examine if changes in the pH had any impacts on CH_4 oxidation rates. Results showed that the pH remained stable and was in the range of 6.7-7.1 throughout the experiment.

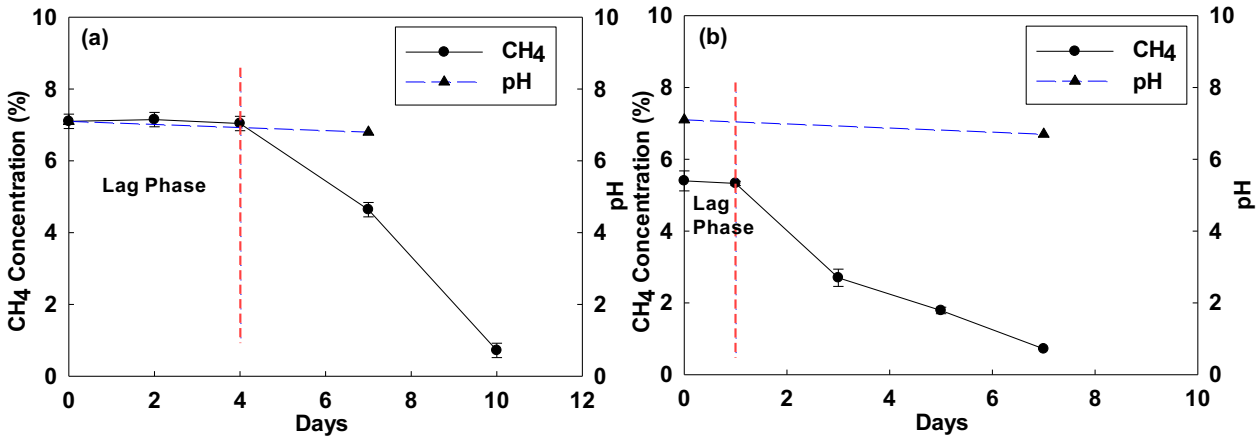

Fig. 2-1. Methane oxidation in: (a) Soil Suspension, and (b) Enrichment Culture

2.3.2 Microbial Community Composition

The microbial community present in the enriched soil and enrichment culture consortium were analyzed using PCR amplification and high-throughput next generation sequencing of 16S rRNA genes. The results of the taxonomical classification for both samples are summarized below, with methanotrophic and methylotrophic taxa indicated.

Fig.2-2. shows the phylum-level composition of enriched soil and culture consortium microbial communities. The enriched soil sample was dominated by the phyla Proteobacteria (73%) and Bacteriodetes (9.1%), with lower levels of Acidobacteria (4.1%), Actinobacteria (2.8%), Verricomicrobia (2.31%), Firmicutes (1.1%), Planctomycetes (0.8%) and others. In the

culture consortium, 79% of all sequences were annotated to the phylum Proteobacteria followed by Verrucomicrobia (5.7%), Bacteriodetes (2.9%) and others (< 1%).

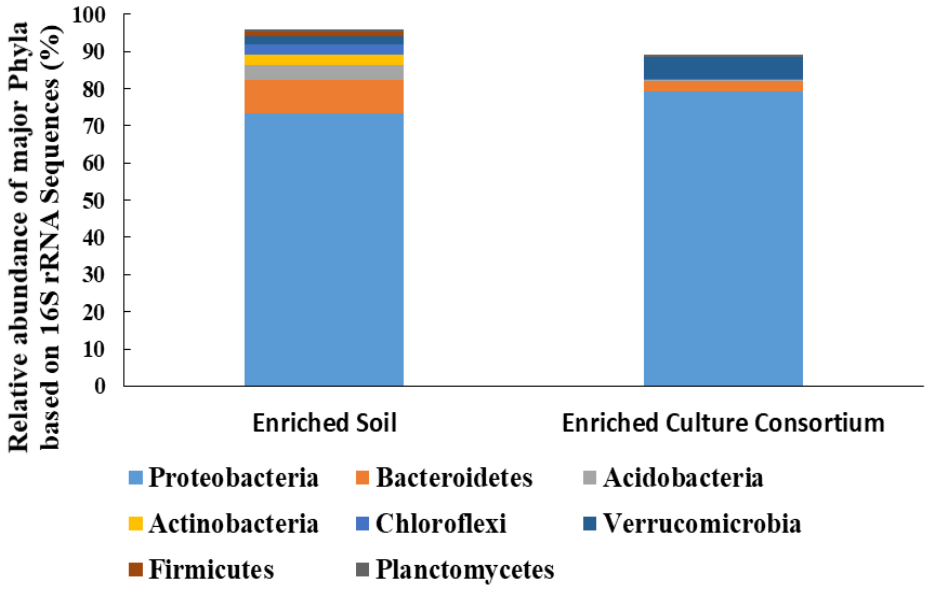

Fig. 2-2. Relative abundance (%) of sequences related to dominant phyla

Fig.2-3. shows the genus-level composition of enriched soil and culture consortium microbial communities. Sequences from different clades of CH_4 oxidizing bacteria, along with non- CH_4 oxidizing methylotrophs, were identified in these samples. The most abundant methanotrophic taxa detected in both soil and cultures were *Methylobacter* accounting for 31% and 39% of the total 16S rRNA sequences identified, followed by *Methylovorus* (6.6% in soil and 4.7% in culture), and *Methylocystis* (2.6% in soil and 1.6 % in culture). Some sequences recovered from these samples were not annotated at the taxonomic level of genus but could still be identified as being derived from putative methylotrophs. These sequences were derived from members of the families Methylophilaceae, Methylocystaceae, Methylococcacea, Crenotrichaceae, and Methylobacteriaceae. The majority (7.4%) of these sequences were derived from bacteria belonging to the family Methylophilaceae.

Kallistova et al. (2012) and Han et al. (2016) detected *Methylovorus* in landfill cover soil and were successful in cultivating them in laboratory, suggesting that their occurrence in landfill cover soils is not unusual and is consistent with the community composition found in the current study. *Methylobacter* has been identified as one of the major genera of methanotrophic community present in abundance in most of the landfill cover soil (Yargicoglu and Reddy 2017, Cébron et al. 2007). *Methylobacter* are Type I methanotrophs that were found in abundance in the cover soil in the present study and could be responsible for oxidizing CH_4 at faster rates, but this cannot be affirmed with our results alone as the 16S rRNA gene analysis does not determine the active members or function of the microbes/ microbial community (Cébron et al. 2007). However, DNA profiling of microbial community structure can identify active microorganisms when sampling is performed longitudinally, and strong shifts are observed, or if analysis of RNA is performed.

Overall, a number of methanotrophic genera (Type-I and Type-II) were detected in both soil and culture samples including *Methylobacter, Methylocystis, Methylomicrobium, Pleamorphomonas, Methylomonas, Methylobacterium, Methylosarcina, Methylocaldum, and Crenothrix*. More broadly, methylotrophic bacteria from the genera *Methylovorus, Methyloversatilis, Methylobacillus, Microvirga*, and from *OM43 clade* were detected. Sequences derived from methanotrophs were most frequently annotated as belonging to the genus *Methylobacter*, and second most frequently annotated as *Methylovorus glucosetrophus SIP3-4*. Due to the limited taxonomic resolution of the V4-V5 region of the microbial 16S rRNA gene, species-level annotation could not be obtained. Future studies in which shotgun metagenome sequencing (*i.e.*, non-targeted, PCR-independent deep sequencing of microbial genomic DNA) will be performed, that will be used to identify taxa to the level of species. Shotgun metagenomic

sequence data will also be used to identify taxon-specific DNA sequences of functional genes that will be used to develop quantitative assays for MMO messenger RNA molecules.

Of the total microbial community analyzed, the landfill cover soil that was enriched in laboratory with modified NMS showed a relative abundance of 39.5% of Type I methanotrophs, 1.5% of Type II methanotrophs, and 18.9% of methylotrophs in the culture consortium. Similarly, 33% of Type I methanotrophs, 2.8% of Type II methanotrophs, and 7.6% of methylotrophs were identified in enriched soil. From **Fig.2-4**, it can be concluded that enrichment favored for cultivating majority of Type I methanotrophic bacteria and methylotrophic bacteria. The reason for not cultivating Type II methanotrophs in this study could possibly be due to substrate (methane) limitation, as Type II methanotrophs usually dominate at high CH_4 concentration and low O_2 concentration or the nutrient rich NMS that usually promotes the growth of Type I methanotrophs inhibiting the growth of Type II methanotrophs (Wise et al. 1999). In addition, high CH_4 oxidation potential from both enrichment and culture batch tests could possibly confirm the presence of abundant methanotrophic/methylotrophic community.

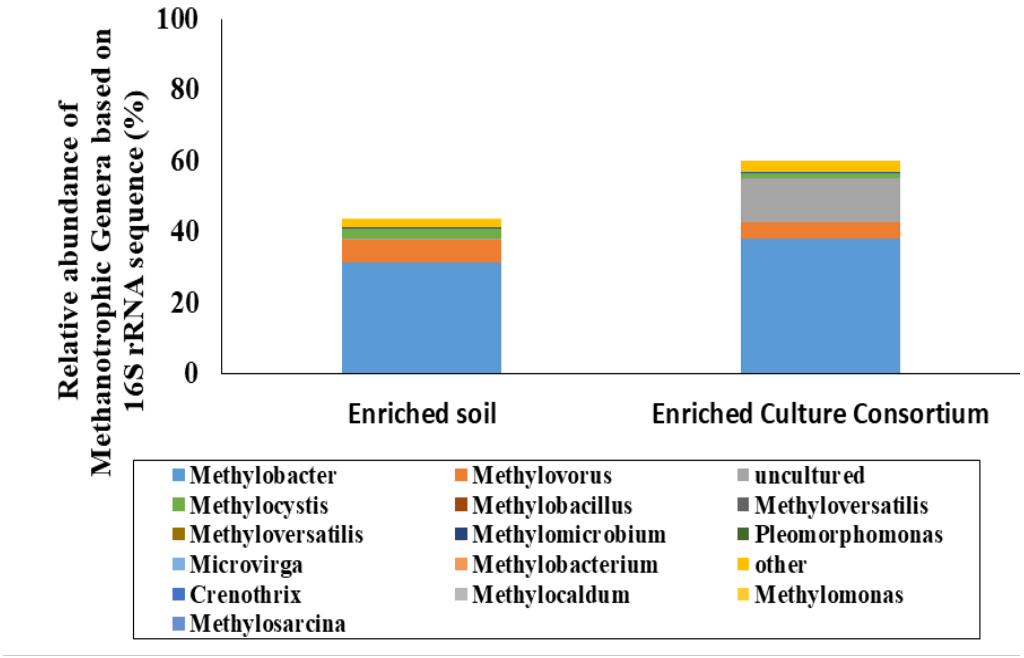

Fig. 2-3. Relative abundance (%) of sequences related to Methanotrophic genera

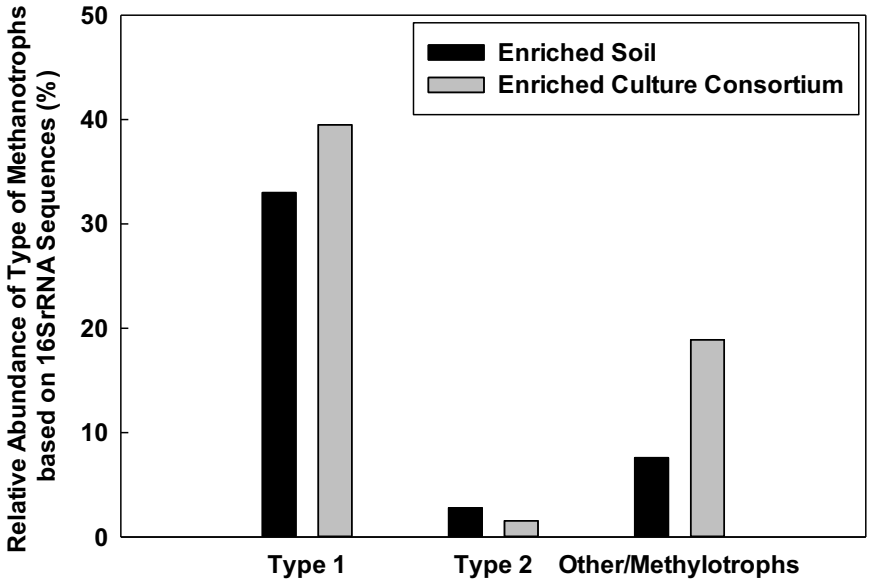

Fig. 2-4. Relative abundance (%) of sequences based on the types of methanotrophs

2.4 CONCLUSIONS

The following conclusions can be drawn from this study:

- Enrichment technique favored the maximum growth of Type I methanotrophs of the genera *Methylobacter* that were present in abundance constituting about 31% (enriched soil) and 39% (culture) of the total 16S rRNA gene amplicon sequences identified. The growth of Type II methanotrophs were possibly inhibited due to lack of substrates needed for growth (1.5 -2.6%). In future, shotgun metagenome sequencing shall be performed to identify taxa at the species level.
- High CH_4 oxidation rates of 123.3 μg CH_4 g^{-1} dry-soil d^{-1} (soil suspension) and 73.1μg CH_4 $mL^{-1}d^{-1}$ (enrichment culture) were observed in this study that correlate to the relative abundance of methanotrophic and methylotrophic community in both the enriched LFCS and the culture consortium.
- Overall, the enriched landfill cover soil was dominated by Type I methanotrophs (33%) followed by Methylotrophs (7.6%) and Type II methanotrophs (2.6%).

The present study provided initial insight into microbial diversity in the Zion landfill cover soil under typical field condition. Additional DNA profiling and/or RNA analyses are being performed to identify active microorganisms. Series of microcosms are also being tested under variable moisture, pH, and temperature conditions to assess the resiliency of methanotrophs for CH_4 oxidation. In addition, enhanced CH_4 oxidation with an organic amendment such as biochar in the cover soil is also being investigated.

2.5 CITED REFERENCES

Bybee, S. M., Bracken-Grissom, H., Haynes, B. D., Hermansen, R. A., Byers, R. L., Clement, M. J., and Crandall, K. A. (2011). "Targeted amplicon sequencing (TAS): a scalable next-gen approach to multilocus, multitaxa phylogenetics". *Genome Biology and Evolution*, *3*, 1312-1323.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., and Huttley, G. A. (2010). "QIIME allows analysis of high-throughput community sequencing data". *Nature Methods*, 7(5), 335.

Cébron, A., Bodrossy, L., Chen, Y., Singer, A. C., Thompson, I. P., Prosser, J. I., and Murrell, J. C. (2007). "Identity of active methanotrophs in landfill cover soil as revealed by DNA-stable isotope probing". *FEMS Microbiology Ecology*, 62(1), 12-23.

Edgar, R. C. (2010). "Search and clustering orders of magnitude faster than BLAST". *Bioinformatics*, 26(19), 2460-2461.

Green, S. J., Venkatramanan, R., and Naqib, A. (2015). "Deconstructing the polymerase chain reaction: understanding and correcting bias associated with primer degeneracies and primer-template mismatches". *PloS one*, *10*(5), e0128122.

Han, J. S., Mahanty, B., Yoon, S. U., and Kim, C. G. (2016). "Activity of a Methanotrophic Consortium Isolated from Landfill Cover Soil: Response to Temperature, pH, CO2, and Porous Adsorbent". *Geomicrobiology Journal*, *33*(10), 878-885.

Kallistova, A. Y., Montonen, L., Jurgens, G., Münster, U., Kevbrina, M. V., and Nozhevnikova, A. N. (2013). "Culturable psychrotolerant methanotrophic bacteria in landfill cover soil". *Microbiology*, *82*(6), 847-855.

Moonsamy, P. V., Williams, T., Bonella, P., Holcomb, C. L., Höglund, B. N., Hillman, G., and Simen, B. B. (2013). "High throughput HLA genotyping using 454 sequencing and the Fluidigm Access Array™ system for simplified amplicon library preparation". *HLA*, *81*(3), 141-149.

Murrell, J. C., McDonald, I. R., and Bourne, D. G. (1998). "Molecular methods for the study of methanotroph ecology". *FEMS Microbiology Ecology*, *27*(2), 103-114.

Semrau, J. D., DiSpirito, A. A., and Yoon, S. (2010). "Methanotrophs and copper". *FEMS Microbiology Reviews*, *34*(4), 496-531.

Spokas, K. A., and Bogner, J. E. (2011). "Limits and dynamics of methane oxidation in landfill cover soils". *Waste Management*, *31*(5), 823-832.

United States Environmental protection Agency (USEPA 2018), Inventory of Greenhouse Gas Emissions and Sinks (1990-2016).

Walters, W., Hyde, E. R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., and Apprill, A. (2016). "Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys". *Msystems*, 1(1), e00009-15.

Wise, M. G., McArthur, J. V., and Shimkets, L. J. (1999). "Methanotroph diversity in landfill soil: isolation of novel type I and type II methanotrophs whose presence was suggested by culture-independent 16S ribosomal DNA analysis". *Applied and Environmental Microbiology*, 65(11), 4887-4897.

Whittenbury, R., Phillips, K. C., and Wilkinson, J. F. (1970). "Enrichment, isolation and some properties of methane-utilizing bacteria". *Microbiology*,*61*(2), 205-218.

Yargicoglu, E. N., and Reddy, K. R. (2017). "Microbial Abundance and Activity in Biochar-Amended Landfill Cover Soils: Evidence from Large-Scale Column and Field Experiments". *Journal of Environmental Engineering*, *143*(9), 04017058.

Yargicoglu, E. N., and Reddy, K. R. (2017). “Effects of biochar and wood pellets amendments added to landfill cover soil on microbial methane oxidation: A laboratory column study”. *Journal of Environmental Management*, 193, 19-31.

Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. (2013). “PEAR: a fast and accurate Illumina Paired-End reAd merger”. *Bioinformatics*, 30(5), 614-620.

CHAPTER 3 – ROLE OF LANDFILL COVER MATERIALS IN MITIGATING GHG EMISSISONS IN BIOGEOCHEMICAL LANDFILL COVER SYSTEM

3.1 INTRODUCTION

The content of this chapter has been previously published by Rai and Reddy (2019) during the author's master's thesis work [Previously published as Rai, R. K., and Reddy, K. R. (2019) Role of Landfill Cover Materials in Mitigating GHG Emissions in Biogeochemical Landfill Cover System, In World Environmental and Water Resources Congress 2019: Emerging and Innovative Technologies and International Perspectives (pp. 52-62). Reston, VA: American Society of Civil Engineers]

Municipal solid waste (MSW) landfills are regarded as the third largest anthropogenic source of methane (CH_4) emissions in the United States. The landfill gas (LFG), generated due to anaerobic biodegradation of organic fraction in MSW, typically comprises of 50% CH_4 and 50% CO_2, both of which are greenhouse gases impacting global climate change. The CH_4 emissions from the landfills are known to be partially converted to CO_2 by the naturally available CH_4 oxidizing bacteria (methanotrophs) present in the cover soil. For nearly two decades, many investigators investigated the CH_4 oxidation capacity of the landfill cover soils based on batch tests and small-scale to near full-scale column studies tests to field-scale test plots (Sadasivam and Reddy 2014). To further improve CH_4 oxidation and mitigate CH_4 emissions from landfills, organic amendments to the cover soil have also been proposed and investigated in recent years (Stern et al. 2007, Scheutz et al. 2011, Sadasivam and Reddy 2014). Due to the degradation potential of organic-rich materials such as compost in the landfill cover soils, the use of alternative stable materials such as biochar, which is a stable and recalcitrant material to microbial

degradation, is proposed for the long-term application (Yargicoglu and Reddy 2017a). Biochar is a solid product resulting from pyrolysis or gasification of organic wastes feed stocks such as waste wood, switchgrass, and corn stove, during bioenergy production. Recent studies have demonstrated that biochar derived from waste wood has great potential to oxidize CH_4 into CO_2 in the landfill covers (Reddy et al. 2014, Yargicoglu and Reddy 2017a). In spite of addressing CH_4 emissions, not much consideration has been given to control landfill CO_2 emissions, that typically range between 40 - 50% of the total landfill gas, and also the CH_4 oxidized CO_2 emissions.

The application of BOF slag as drainage material in landfill cover has been reported with respect to its geotechnical properties (Diener et al. 2010, Andreas et al. 2005). Furthermore, its application is widely being used in construction industry as an aggregate material and in environmental engineering applications as media for contaminant adsorption and CO_2 sequestration. It is investigated in treating heavy metals and TCE in soil and groundwater, phosphate removal from wastewater, and soil conditioner/fertilizer in agriculture as reviewed by Reddy et al. (2019). Recently, Reddy et al. (2018a) proposed the concept of an innovative biogeochemical cover to mitigate both CH_4 and CO_2 emissions from the landfills. Wherein, BOF slag, a byproduct from steel mills, is proposed for CO_2 sequestration due to the presence of various minerals such as CaO, portlandite ($Ca(OH)_2$) and larnite (Ca_2SiO_4) (Huijgen et al. 2005). The use of BOF slag as one of the landfill cover material in mitigating CO_2 emissions has not been considered to date. The BOF slag is proposed to be used along with other materials such as soil and biochar in the biogeochemical cover system in an optimal way to mitigate both CH_4 and CO_2 emissions.

This paper provides a brief overview of the concept of biogeochemical cover and then presents several series of batch experiments conducted to systematically evaluate the extent of CH_4

oxidation and CO_2 sequestration by the potential materials that could be used in it, specifically soil, BOF slag, biochar, and methanotrophic activated biochar.

3.1.1 Biogeochemical Cover Concept

Biogeochemical cover is an innovative, low-cost landfill cover system consisting of steel slag in combination with soil and biochar (Reddy et al. 2018a). Steel slag is a co-product of steel making process, and basic oxygen furnace (BOF) slag is a type of steel slag, which is rich in alkaline minerals such as CaO, MgO, etc. The alkaline metal oxides present in the slag react with CO_2, forming stable carbonates. Many studies have explored the carbonation potential of steel slag for the mineral CO_2 sequestration for different industrial applications. Moreover, several past studies have shown promising potential of biochar-amended soil to mitigate CH_4 emissions by the enhanced methanotrophic oxidation of CH_4 (Reddy et al. 2014; Yargicoglu and Reddy 2017b).

The biogeochemical cover aims to combine the carbonation potential of BOF slag along with the methanotrophic CH_4 oxidation potential of biochar-amended soil to mitigate both CH_4 and CO_2 emissions from the MSW landfills, ultimately leading to "Zero Emissions Landfill Cover". **Figure 3-1** shows the schematic of this steel slag and biochar-amended soil biogeochemical cover system. The proposed biogeochemical cover also has the potential to sequester hydrogen sulfide (H_2S) if present in the LFG as shown in **Figure 3-1**. The use of proposed biogeochemical cover in landfills will not only reduce the environmental concerns associated with the fugitive LFG emissions, but also provides new opportunity for the sustainable management of steel slags (especially finer slag) which are generally stockpiled in the steel industry or landfilled.

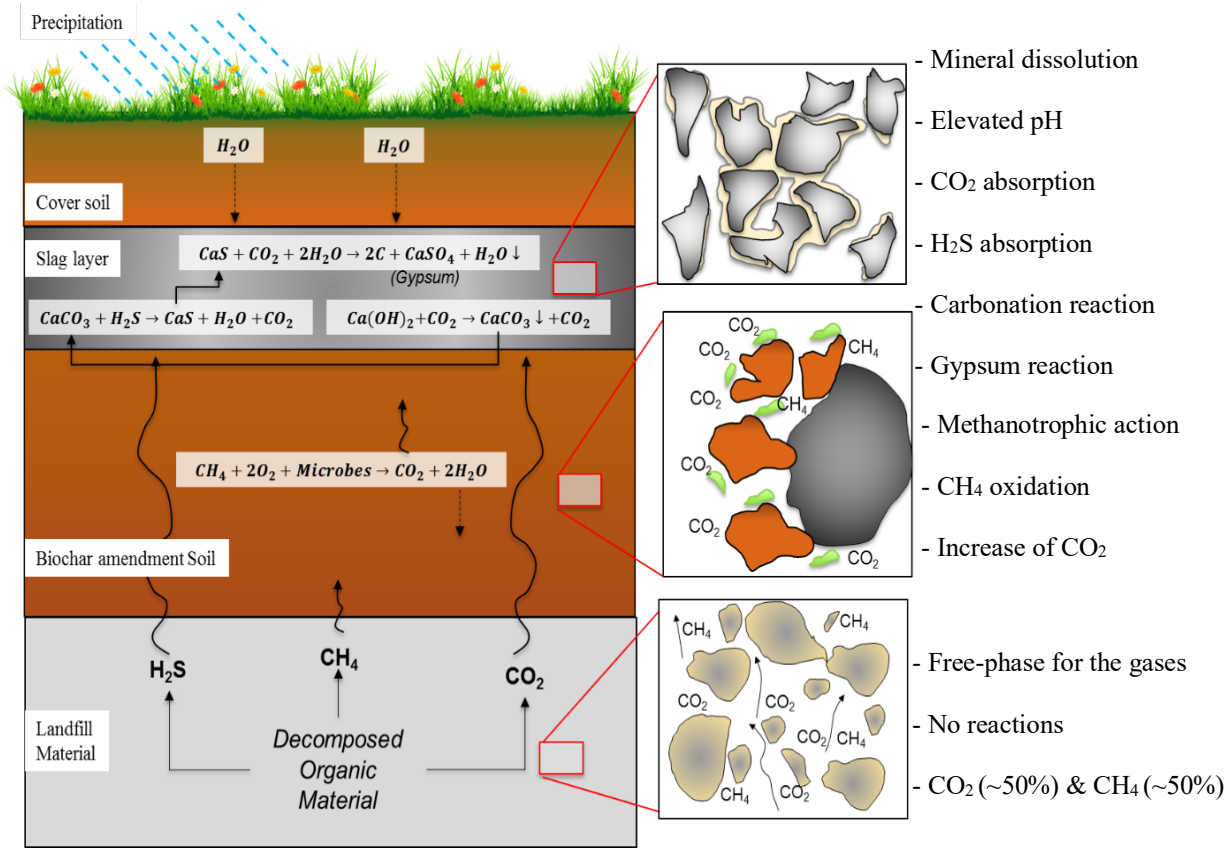

Figure 3-1. Schematic of biogeochemical cover system for zero emissions (Reddy et al. 2018a)

Although the proposed biogeochemical cover offers wide range of environmental as well as economic benefits, it is of utmost importance to analyze various system factors, which are crucial to the functioning of the coupled biogeochemical processes. A comprehensive laboratory testing program consisting of multiple tasks is undertaken for this purpose; this study presents the results from one of these tasks.

3.2 MATERIALS AND METHODS

3.2.1 Soil

Soil was collected from Zion landfill site, located in Zion, Illinois, USA. Soil samples were collected from an interim cover at a depth of ~1 to 2 feet and were shipped to the Geotechnical and Geoenvironmental Engineering Laboratory at the University of Illinois at Chicago (UIC) where it was stored at room temperature (23 ± 2°C). Soil samples were air dried (moisture content <0.5%), pulverized, and screened through a 2 mm sieve prior to conducting the experiments.

3.2.2 Biochar

Biochar was obtained from a commercial vendor in Illinois, USA. The biochar used in this study, designated as CE-WP2, was produced from waste pinewood subjected to gasification at a high temperature of ~520°C. In this study, biochar in pellet form was used with fines sieved and discarded. The biochar was oven-dried at 105°C to remove any moisture content before conducting the experiments.

3.2.3 BOF Slag

The BOF slag used in this experiment was obtained from Indiana Harbor East (IHE) of Arcelor Mittal steel plant, located in East Chicago, Indiana, USA. This slag, designated as IHE 9/17, is finer material being stockpiled at the plant; otherwise, requires landfill disposal. All the tests were performed using the bulk slag sample as obtained from the plant. The steel slag was also oven-dried at 105°C prior to conducting the experiments.

3.2.4 Materials Properties Testing

All the physical and chemical properties of the cover materials selected for this study were tested as per ASTM standards. ASTM D422 and ASTM D854 were the testing protocol followed for characterizing particle size distribution and specific gravity, respectively. Dry density was determined based on weight of the dry material compacted in the permeameter and volume of the permeameter. Hydraulic conductivity was tested per the ASTM D2434. The water holding capacity (WHC) of the material was conducted by placing a known mass of sample in a funnel lined with Whatman filter paper and adding known amount of deionized water. The sample was allowed to soak for 2-3 hours and drain under gravity. The WHC of the material was determined by calculating the moisture content retained by the sample (Yargicoglu et al. 2015). For chemical characterization, 10 g of each material under investigation was soaked in 0.01M $CaCl_2$ solution (L/S of 1:1) for 2 hours and pH, ORP and electrical conductivity were measured as per ASTM D4972. The pH meter was calibrated with standard buffers of pH 4, 7 and 10 prior to measurement. Organic matter content was determined based on loss-on-ignition (LOI) method as per ASTM D2974. All tests on each material were conducted in triplicate, and the results were averaged.

3.2.5 Mixed Methanotrophic Culture Consortium

The mixed methanotrophic culture was cultivated in the laboratory using enrichments from the landfill cover soil as described in Rai et al. (2018). The biochar was activated by inoculating 5-7 g of biochar in 10 mL of the mixed culture in the presence of ~5 - 6% CH_4 (v/v) and ~5 - 6% CO_2 (v/v) balanced in air and incubated at room temperature of 23ºC.

3.2.6 Batch Tests

For the batch testing, 10 g of the selected material (soil, biochar, methanotrophic activated biochar, or BOF slag) was placed in 125 mL-serum vials and the moisture was adjusted to 20% (w/w) using deionized water, except methanotrophic activated biochar that was soaked in the culture. The vials were sealed airtight using butyl rubber septa followed by crimp cap. 20 mL of air from the headspace was replaced with equal volume of synthetic landfill gas comprising of 50% (v/v) CH_4 and 50% (v/v) CO_2 to achieve a headspace concentration of ~5 - 6% CH_4 (v/v), ~5 - 6% CO_2 (v/v) and a balance (~88 - 90%) of air. The change in the headspace concentration was determined by collecting and analyzing the gas samples on a regular basis using gas chromatography (GC) until the headspace concentration dropped to less than 1%. All the experiments were conducted in triplicate along with the controls (with synthetic landfill gas without any material). The controls using soil (sterilized for 2 hours using Napco Model 8000-DSE autoclave) were also tested to discern the effects of any microbial activity in the soil. The CH_4 oxidation rates were calculated from the linear regression analysis of CH_4 concentration versus elapsed time, based on the zero-order kinetics.

3.2.7 Gas Analysis

The gas samples were analyzed at regular time intervals and analyzed for CH_4 and CO_2 concentrations using an SRI 9300 GC equipped with a thermal conductivity detector (TCD) and CTR-1 column that separates N_2 and O_2 for simultaneous analysis of CO_2, CH_4, O_2 and N_2 (Yargicoglu and Reddy, 2017). Gas samples were withdrawn using 1 mL syringe where 0.5 mL of the sample was discarded and remaining 0.5 mL was injected into the GC to reduce any pressure

effects due to sampling. A calibration curve for a minimum of three points was established using high purity standard gas mixtures ranging from 1% to 50% CH_4 and CO_2.

3.3 RESULTS AND DISCUSSION

Table 3-1 summarizes the physical and chemical properties of the cover materials tested. Based on sieve analysis, BOF slag and biochar consisted of 74% and 54% of sand-size fraction, respectively, and were classified as poorly graded sand (SP or SP-SM) equivalent as per the Unified Soil Classification System (USCS). Whereas, the cover soil consisted of more than 50% fines with plasticity index of 17% and classified as silty clay (CL). The materials showed slightly acidic to highly alkaline pH, measuring 6.7, 7.6 and 12.4 for biochar, soil and BOF slag, respectively. The organic content was found to be 96.7% in biochar, 1.6% in BOF slag, and 5.8% in cover soil. The negative oxidation reduction potential (ORP) values indicates higher reduction potential in the order of BOF slag (-313.3 mV), soil (-53.8 mV), and biochar (-6.3 mV). The water holding capacity was found to be 51.6% for biochar, 43% for soil, and 20% for BOF slag. The hydraulic conductivity of the soil was 5.4×10^{-8} cm/s which qualifies as a low permeable material, whereas BOF slag and biochar possessed high hydraulic conductivity of 1.1×10^{-3} and 2×10^{-4} cm/s, respectively. The specific gravity of BOF slag was 3.5, high due to high iron oxide content, and for biochar was 0.6, and for the soil was 2.57. The relatively lower specific gravity of the soil as compared to typical inorganic soils is due to its organic content of 5.8%. The high organic content implies that the soil is rich in biomass and can sustain microbial activity.

Table 3-1: Physical and chemical characteristics of BOF slag, cover soil and biochar

Properties	ASTM Method	BOF Slag	Soil	Biochar
Grain Size Distribution:	D422			
Gravel (%)		20.8	3.7	45
Sand (%)		74.2	14.7	54
Fines (%)		4.9	81.9	1
D_{50} (mm)			0.009	4.3
C_c		0.7	-	0.82
C_u		18	-	2.42
Atterberg Limits:	D4318			
Liquid Limit (%)		Non-	39	Non-Plastic
Plastic Limit (%)		Plastic	22	
Plasticity Index (%)			17	
USCS Classification	D2487	SP-SM	CL	SP
Specific Gravity	D854	3.5	2.57	0.6
Dry Density (g/cm^3)		1.72	1.8	1.15
Hydraulic Conductivity (cm/s)	D2434	1.1×10^{-3}	5.4×10^{-8}	2×10^{-4}
Loss-on-Ignition (%)	D2974	1.6	5.8	96.71
pH (1:1)	D4972	12.4	7.6	6.5
Electrical Conductivity (mS/cm)	D4972	13.3	0.55	0.8
Redox Potential (mV)	D4972	-313.3	-53.8	-6.3

C_c=Coefficient of curvature; C_u=Coefficient of uniformity

Figure 3-2 shows the plot of CH_4 and CO_2 gas uptake with time in batch tests with landfill cover soil. An increase in the CH_4 uptake with time confirms CH_4 oxidation by the CH_4 oxidizing bacteria in the cover soil. This observation was further bolstered with the observed no significant changes in gas concentrations in the controls (sterilized soil and LFG), thus confirming the CH_4 oxidation by the naturally existing CH_4 oxidizing bacteria in the cover soil. A minimal CO_2 adsorption by the cover soil with an uptake of 12% was noticed, after which an increase in the CO_2 levels, as a result of CH_4 oxidation, was observed. The CH_4 oxidation rate calculated based on zero-order kinetics is found to be 4.1 μg CH_4/g/h. Overall, the results suggest that the landfill cover soil used in this study was rich in CH_4 oxidizing bacteria that were able to perform CH_4 oxidation.

Many reported studies, involving laboratory batch experiments, have also shown significant CH_4 oxidation in the landfill cover soils (Scheutz et al. 2009; Sadasivam and Reddy 2014). At 5% (v/v) CH_4 concentration, studies have shown that the CH_4 oxidation rates can range from 0.0096 µg CH_4/g/h (Bender and Conrad 1994) to 173 µg CH_4/g/h (Borjesson et al.1998a, b). The results obtained in this study were in agreement with the results from these studies; however, many other studies have reported the CH_4 oxidation rates to be as low as 0.0024 µg CH_4/g/h (Boeckx et al. 1996) and as high as 118 µg CH_4/g/h (Scheutz and Kjeldsen 2004), showing differences in the CH_4 oxidation rates mainly due to variances in the experimental and site-specific conditions.

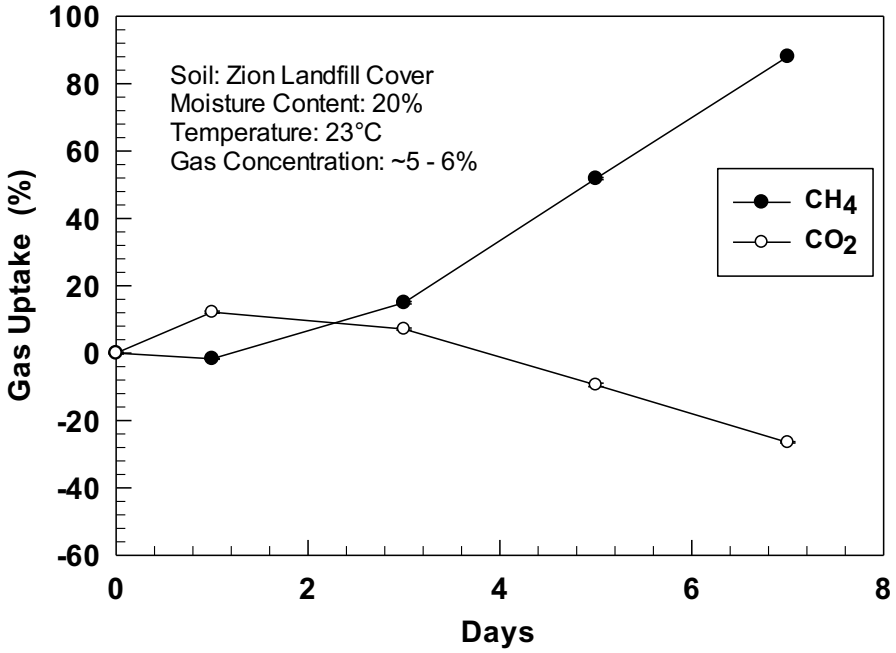

Figure 3-2. Methane and carbon dioxide uptake in landfill cover soil

Figure 3-3 shows the plot of CH_4 and CO_2 gas uptake with time in biochar. As biochar is free of any methanotrophs, the reduction in gas concentrations are presumed to be due to adsorption processes. The results show marginal adsorption of CH_4 on the biochar with a total CH_4 removal of 9.6%. The physical and chemical properties of the biochar are usually dictated by the

feedstock and production processes (Yargicoglu et al. 2015). The CH_4 adsorption capacity also varies depending upon the type of biochar used. Sadasivam and Reddy (2015) reported differences in the CH_4 adsorption capacity in seven wood-derived biochars and granulated activated carbon (GAC) concluding minimal CH_4 adsorbing capacity in biochars (0.04 - 0.18 mol/kg) when compared to GAC. In contrary, a study by Sethupathi et al. (2017) demonstrated no adsorption of CH_4 in four different types of biochar studied, suggesting the adsorption capacity of the biochar is highly dependent on its feedstock and its physicochemical properties.

Furthermore, the results in **Figure 3-3** showed significant CO_2 removal in the first 24 hours with an uptake of 21% but showed desorption in the consecutive days reaching an equilibrium after 5th day with an overall CO_2 removal of 5.3%. Biochar showed desorption followed by adsorption which could likely be due to the shaking of the vials before sampling or depressurization of the system due to sampling, resulting in the breakage of the weak intermolecular forces causing physisorption of CO_2 (Sethupathi et al. 2017).

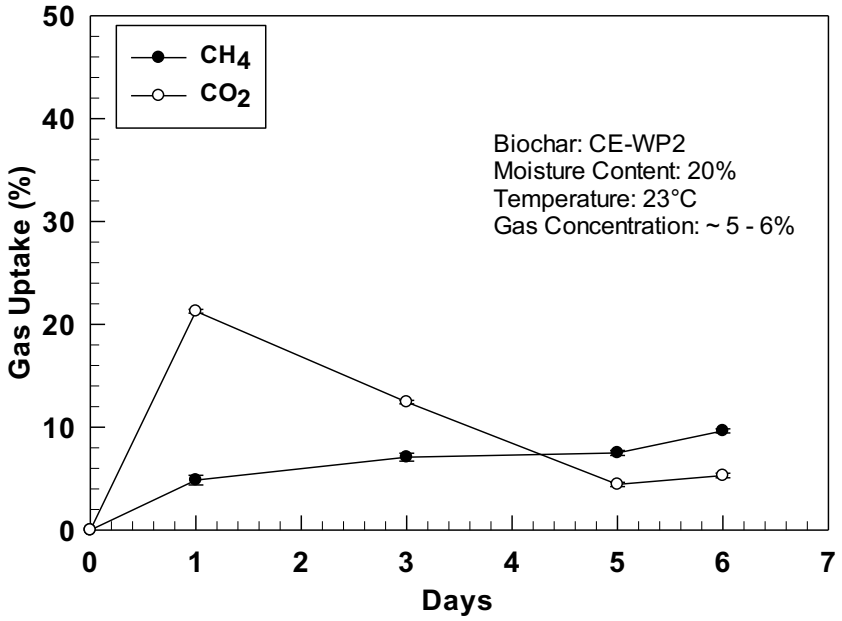

Figure 3-3. Methane and carbon dioxide uptake in biochar

Overall, these results demonstrate marginal adsorption of both CH_4 and CO_2 in the biochar. However, biochar is known to have potential in various agricultural and environmental applications due to its unique physicochemical properties such as water holding capacity, internal porosity, and surface area when amended with soil (Yargicoglu et al. 2015). It also has positive impacts on soil fertility including increasing soil pH, nutrient retention and cation exchange capacity (Chan et al. 2007). The effect of biochar amendment to the landfill cover soil is also under investigation by our research team, but it is beyond the scope of this paper.

Figure 3-4 shows the plot of CH_4 and CO_2 gas uptake with time in BOF slag. The trend in the CH_4 concentration shows minimal CH_4 adsorption capacity by the BOF slag with an uptake of 3.6%. However, significant removal of carbon dioxide with 75% of CO_2 uptake in 24 hours and 100% uptake in 5 days was observed. These results are consistent with the studies on CO_2 sequestration by BOF slag as discussed by Reddy et al. (2018b). The BOF slag is a highly reactive material due to the presence of high CaO (> 35%), making it conducive for CO_2 sequestration (Su et al. 2016). Due to the high alkaline nature of the BOF slag (pH 12.4), it is hypothesized that the BOF slag could induce negative impact on the CH_4 oxidation when amended with soil or biochar-amended soil. To confirm this hypothesis, the BOF slag in various combinations (mixed versus separated) with soil and biochar-amended soil are under investigation. The results from these investigations will be used for the design of a geochemical cover profile configuration for an effective CH_4 oxidation and simultaneous CO_2 sequestration.

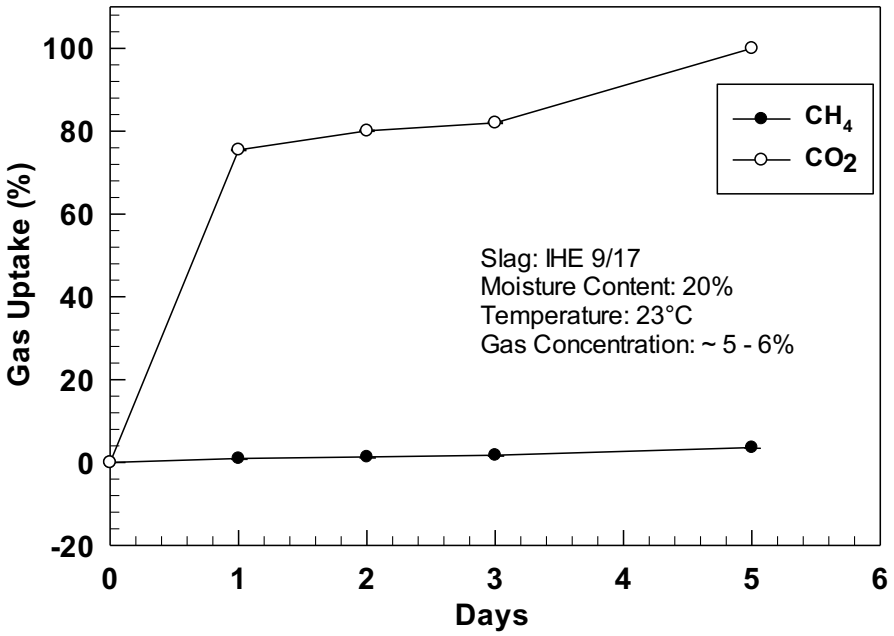

Figure 3-4. Methane and carbon dioxide uptake in BOF Slag

Biochar-amended soils have shown promising results in enhancing CH_4 oxidation in the landfills due to its favorable characteristics such as improved gas retention, water holding capacity, and habitable sites for proliferation of microbes as described by Reddy et al. (2014). However, the colonization of bacteria in the biochar takes longer time for acclimatizing and further improve oxidation rates. In this regard, this study evaluated the potential of methanotrophic activated biochar in the removal of CH_4. The plot of CH_4 and CO_2 gas uptake with time in methanotrophic activated biochar is shown in **Figure 3-5.**

An increase in the CH_4 uptake with time, as shown in **Figure 3-5**, is attributed to the performance of CH_4 oxidizing bacteria inoculated in the biochar. No significant CO_2 uptake by the methanotrophic activated biochar was detected but increase in the CO_2 levels due to CH_4 oxidation by the CH_4 oxidizing bacteria was observed. Overall, the results suggest that the methanotrophic activated biochar had colonized in the highly porous, large surface area of biochar and were able

to oxidize CH_4 without limitation to nutrients, showing CH_4 oxidation rate of 3.35 µg CH_4/g-biochar/h. We hypothesize that the methanotrophic activated biochar when amended with soil would mitigate CH_4 at faster rates when compared to non-activated biochar-amended soils. This study is being investigated by our research team and is beyond the scope of this paper.

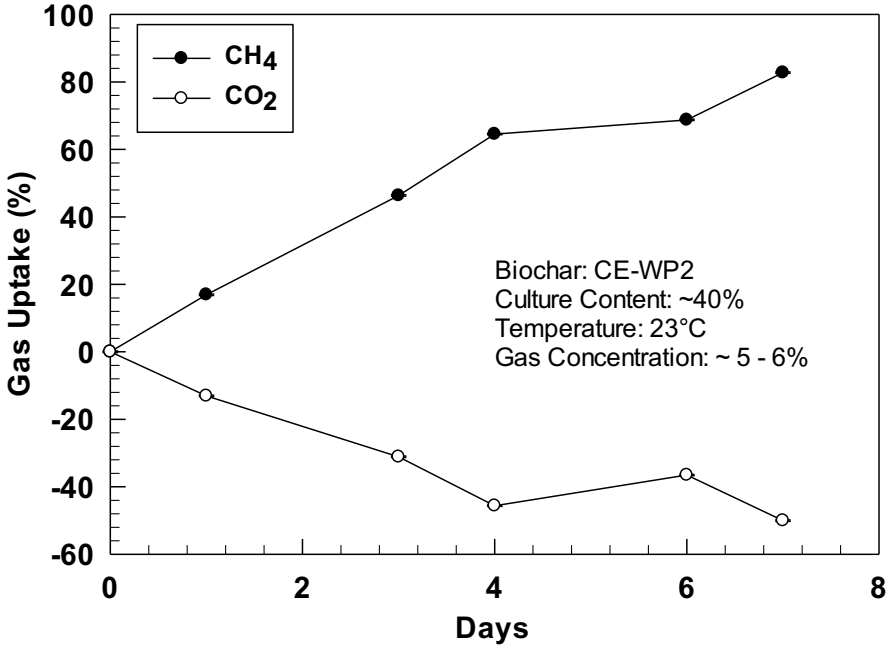

Figure 3-5. Methane and carbon dioxide uptake in methanotrophic activated biochar

3.4 CONCLUSIONS

Laboratory investigation on the landfill cover materials was conducted to evaluate the CH_4 and CO_2 uptake in order to determine their use in the newly proposed biogeochemical landfill cover system. The materials tested included: landfill cover soil, non-activated biochar, methanotrophic activated biochar, and BOF-slag. The results demonstrated that the landfill cover soil was dominated by the CH_4 oxidizing bacteria and were responsible for CH_4 oxidation. The non-activated biochar showed no CH_4 oxidation but showed low adsorption of CH_4 (9.6%) and CO_2 (5.3%). However, the methanotrophic activated biochar displayed substantial potential for

mitigating CH_4, suggesting the use of biochar as a habitat for microbial community thus improving CH_4 oxidation. Furthermore, the BOF slag showed minimal uptake of CH_4 (3.6%) but demonstrated significant removal of CO_2 (100%), suggesting its use in the landfill cover system for CO_2 sequestration. Finally, the use of BOF slag in conjunction with soil, biochar-amended soil or methanotrophic activated biochar-amended soil, is under detailed investigation in order to develop a cover profile that best suited for effective CH_4 oxidation and simultaneous CO_2 sequestration.

3.5 CITED REFERENCES

Andreas, L., Herrmann, I., Lidström-Larsson, M., and Lagerkvist, A. (2005, October). "Physical properties of steel slag to be reused in a landfill cover". *Proc. Sardinia*.

Bender, M., and Conrad, R. (1994). "Methane oxidation activity in various soils and freshwater sediments: occurrence, characteristics, vertical profiles, and distribution on grain size fractions". *Journal of Geophysical Research: Atmospheres*, 99(D8), 16531-16540.

Boeckx, P., Van Cleemput, O., and Villaralvo, I. D. A. (1996). "Methane emission from a landfill and the methane oxidising capacity of its covering soil". *Soil Biology and Biochemistry*, 28(10-11), 1397-1405.

Bonenfant, D., Kharoune, L., Sauve, S., Hausler, R., Niquette, P., Mimeault, M., and Kharoune, M. (2008). "CO_2 sequestration potential of steel slags at ambient pressure and temperature." *Industrial & Engineering Chemistry Research*, 47(20), 7610-7616.

Börjesson, G., Sundh, I., Tunlid, A., Frostegård, Å., and Svensson, B. H. (1998a). "Microbial oxidation of CH_4 at high partial pressures in an organic landfill cover soil under different moisture regimes". *FEMS Microbiology Ecology*, 26(3), 207-217.

Börjesson, G., Sundh, I., Tunlid, A., and Svensson, B. H. (1998b). "Methane oxidation in landfill cover soils, as revealed by potential oxidation measurements and phospholipid fatty acid analyses". *Soil Biology and Biochemistry*, 30(10-11), 1423-1433.

Diener, S., Andreas, L., Herrmann, I., Ecke, H., and Lagerkvist, A. (2010). Accelerated carbonation of steel slags in a landfill cover construction. *Waste Management*, 30(1), 132-139.

Huijgen, W. J., Witkamp, G. J., and Comans, R. N. (2005). "Mineral CO_2 sequestration by steel slag carbonation". *Environmental Science & Technology*, 39(24), 9676-9682.

Rai, R. K., Chetri, J. K., Green, S. J., and Reddy, K. R. (2018). "Identifying active methanotrophs and mitigation of CH_4 emissions in landfill cover soil". *Proc. International Congress on Environmental Geotechnics* (pp. 308-316). Springer, Singapore.

Reddy, K. R., Yargicoglu, E. N., Yue, D., and Yaghoubi, P. (2014). "Enhanced microbial methane oxidation in landfill cover soil amended with biochar". *Journal of Geotechnical and Geoenvironmental Engineering*, 140(9), 04014047.

Reddy, K. R., Grubb, D. G., and Kumar, G. (2018a). "Innovative biogeochemical soil cover to mitigate landfill gas emissions". *Proc. International Conference on Protection and Restoration of the Environment XIV*, Thessaloniki, Greece.

Reddy, K. R., Kumar, G., Gopakumar, A., Rai, R. K., and Grubb, D. G. (2018b). "CO_2 sequestration using BOF slag: Application in landfill cover". *Proc. International Conference on Protection and Restoration of the Environment XIV*, Thessaloniki, Greece.

Reddy, K. R., Gopakumar, A., and Chetri, J. K., (2019). "Critical review of applications of iron and steel slags for carbon sequestration and environmental remediation". *Reviews in Environmental Science and Bio/Technology*, DOI: 10.1007/s11157-018-09490-w.

Sadasivam, B. Y., and Reddy, K. R. (2014). “Landfill methane oxidation in soil and bio-based cover systems: A review”. *Reviews in Environmental Science and Biotechnology*, 13(1), 79-107.

Sadasivam, B. Y., and Reddy, K. R. (2015). “Adsorption and transport of methane in biochars derived from waste wood”. *Waste Management*, 43, 218-229.

Scheutz, C., and Kjeldsen, P. (2004). “Environmental factors influencing attenuation of methane and hydrochlorofluorocarbons in landfill cover soils”. *Journal of Environmental Quality*, 33(1), 72-79.

Scheutz, C., Kjeldsen, P., Bogner, J. E., De Visscher, A., Gebert, J., Hilger, H. A., and Spokas, K. (2009). “Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions”. *Waste Management & Research*, 27(5), 409-455.

Scheutz, C., Fredenslund, A. M., Chanton, J., Pedersen, G. B., and Kjeldsen, P. (2011). “Mitigation of methane emission from Fakse landfill using a biowindow system”. *Waste Management*, 31(5), 1018-1028.

Sethupathi, S., Zhang, M., Rajapaksha, A. U., Lee, S. R., Mohamad Nor, N., Mohamed, A. R., and Y. S. (2017). “Biochars as potential adsorbers of CH_4, CO_2 and H_2S”. *Sustainability*, 9(1), 121.

Stern, J. C., Chanton, J., Abichou, T., Powelson, D., Yuan, L., Escoriza, S., and Bogner, J. (2007). “Use of a biologically active cover to reduce landfill methane emissions and enhance methane oxidation”. *Waste Management*, 27(9), 1248-1258.

Su, T. H., Yang, H. J., Shau, Y. H., Takazawa, E., and Lee, Y. C. (2016). “CO_2 sequestration utilizing basic-oxygen furnace slag: Controlling factors, reaction mechanisms and V–Cr concerns”. *Journal of Environmental Sciences*, 41, 99-111.

Yargicoglu, E. N., Sadasivam, B. Y., Reddy, K. R., and Spokas, K. (2015). "Physical and chemical characterization of waste wood derived biochars". *Waste Management*, 36, 256-268.

Yargicoglu, E. N., and Reddy, K. R. (2017a). Effects of biochar and wood pellets amendments added to landfill cover soil on microbial methane oxidation: A laboratory column study. *Journal of Environmental Management*, 193, 19-31.

Yargicoglu, E. N., and Reddy, K. R. (2017b). Biochar-amended soil cover for microbial methane oxidation: Effect of biochar amendment ratio and cover profile. Journal of Geotechnical and Geoenvironmental Engineering, 144(3), 040

CHAPTER 4 - METHANOTROPHIC METHANE OXIDATION IN NEW BIOGEOCHEMICAL LANDFILL COVER SYSTEM

4.1 INTRODUCTION

The content of this chapter has been previously published by Rai and Reddy (2019) during the author's master's thesis work [Previously published as Rai, R. K., and Reddy, K. R. (2019) Methanotrophic methane oxidation in new biogeochemical landfill cover system, Proc. 34th International Conference on Solid Waste Technology and Management, Annapolis, MD, USA, March 31st – April 3rd, 2019.]

Landfills are the third largest source of anthropogenic CH_4 emissions in the United States. The landfill gas (LFG) typically comprises of 50% CH_4 and 50% CO_2, both of which are greenhouse gases impacting global climate change. Mitigation of CH_4 emissions has received greater attention for a long time, and many researchers evaluated reducing CH_4 emissions by studying the potential of CH_4 oxidizing bacteria present in the cover soil to convert CH_4 into CO_2 (Whalen et al. 1990; Kightley et al. 1995; Boeckx et al. 1996, Cao et al. 2011). In recent years, many researchers have focused on the use of biocovers that support microbial proliferation and enhance CH_4 oxidation. These biocovers typically employ organic-rich materials such as garden waste compost, yard waste, sewage sludge, peat, and biochar (Hummer and Lechner, 1999; Stern et al. 2007; Pedersen, 2011; Scheutz et al. 2011; Yargicoglu and Reddy, 2017) to enhance microbial CH_4 oxidation when placed alone or in amendments with soil.

In addition, an interest in utilizing industrial waste materials as components of landfill cover has also come into the limelight, due to their favorable physicochemical properties. Some of the industrial wastes that have shown potential landfill cover materials include paper mill sludge (Kovačić, 1996), coal fly ash (Nhan et al. 1996), bottom ash (Kim et al. 2016), and steel slag

(Herrmann et al. 2010, Andreas et al. 2014) and they have been investigated as barrier or drainage layer depending on their hydraulic and geotechnical properties.

Steel slag, a byproduct from steel mills, has gained a significant attention in recent years, especially in the construction industry as an aggregate material and in environmental applications as media for contaminant adsorption and carbon dioxide sequestration. It is investigated in treating heavy metals from groundwater (Smith, 2003), phosphate removal from wastewater (Lu et al. 2008), heavy metals from acid mine drainage (Sheridan, 2014), and fertilizer/soil modifier in agriculture (Zhang et al. 2003; Kimio, 2015). Recently, Reddy et al. (2018) investigated use of BOF slag for sequestration of carbon dioxide from landfill gas emissions (Reddy et al. 2018).

An innovative, sustainable and practical biogeochemical cover system consisting of soil, biochar and BOF slag is being investigated to achieve zero emissions from the landfills (Reddy et al. 2018). The alkalinity and the presence of alkaline metals in BOF slag is conducive in sequestering CO_2 (Reddy et al. 2019 a). However, it could be challenging for the microbial community in soil or biochar-amended soil to thrive under extreme alkaline condition induced by slag. The objective of this study is to investigate the effect of three landfill cover materials both on methanotrophic CH_4 oxidation and CO_2 sequestration.

4.1.1 Biogeochemical Cover Concept

Biogeochemical cover is an innovative, low-cost landfill cover system consisting of steel slag in combination with soil and biochar (Reddy et al. 2018). Steel slag is a co-product of steel making process and basic oxygen furnace (BOF) slag is a type of steel slag, which is rich in alkaline minerals such as CaO, MgO, etc. The alkaline metal oxides present in the slag react with CO_2 forming stable carbonates. Many studies have explored the carbonation potential of steel slag for

the mineral CO_2 sequestration. Past studies (Reddy et al. 2014; Yargicoglu and Reddy 2017) have shown promising potential in biochar-amended soil to mitigate CH_4 emissions by the enhanced methanotrophic oxidation of CH_4.

The biogeochemical cover aims to combine the carbonation potential of BOF slag with the methanotrophic CH_4 oxidation potential of biochar-amended soil to mitigate both the CH_4 and CO_2 emissions from the MSW landfills and ultimately leading to "Zero Emissions Landfill Cover". **Fig. 4-1** shows the schematic of the steel slag and biochar amended-soil biogeochemical cover system. The proposed biogeochemical cover also has the potential to sequester hydrogen sulfide (H_2S) if present in the LFG as shown in **Fig.4-1**. The use of proposed biogeochemical cover in landfills will not only reduce the environmental concerns associated with the fugitive LFG emissions but also opens up a door for the sustainable management of steel slags which are generally stockpiled in the steel industry or landfilled.

Although the proposed biogeochemical cover offers wide range of environmental as well as economic benefits, it is of utmost importance to analyze various factors, which are crucial to the functioning of the coupled biogeochemical processes. A comprehensive laboratory testing program consisting of multiple tasks is undertaken for this purpose; this study presents the results from one of these tasks.

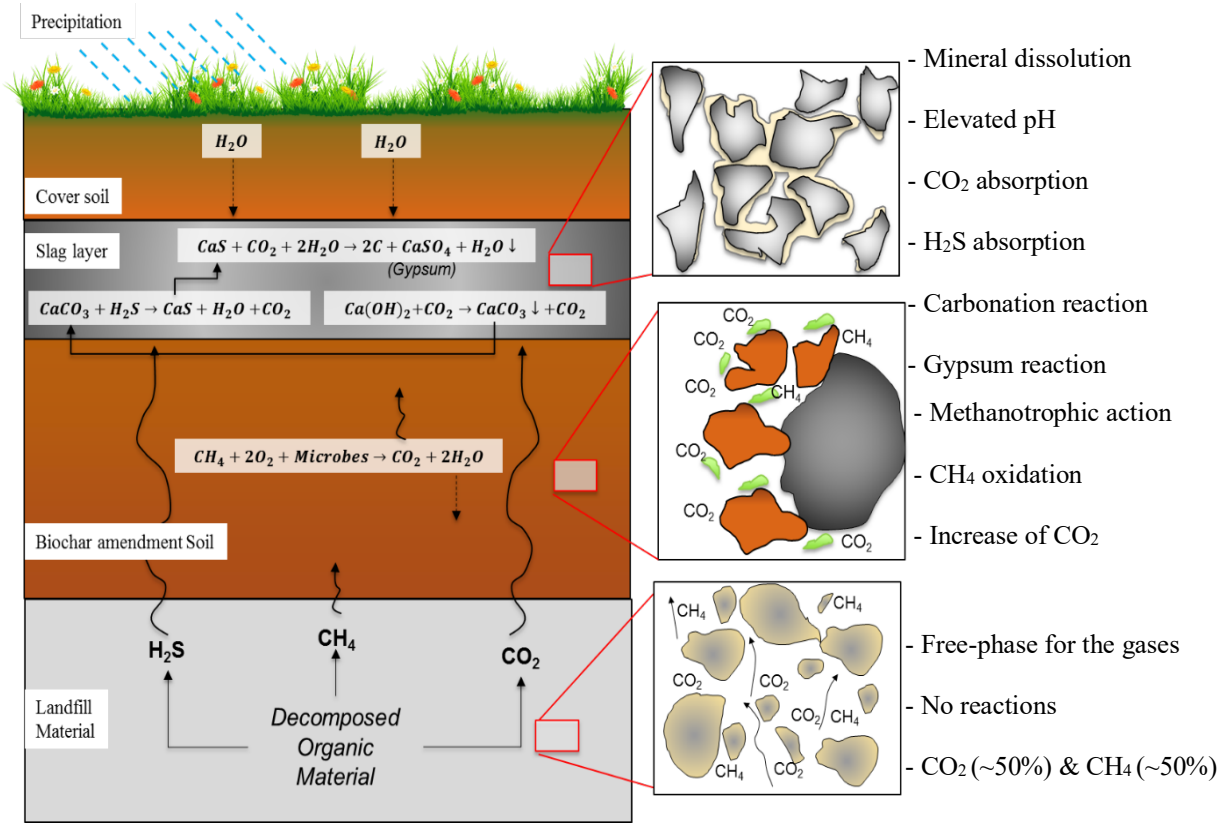

Fig.4-1. Schematic of biogeochemical cover system for zero emissions (Reddy et al. 2018)

4.2 MATERIALS AND METHODS

4.2.1 Soil

Soil was collected from Zion landfill site, located in Greater Chicago area, Illinois, USA. Soil samples were collected from an interim cover layer at a depth of ~1 to 2 feet and were shipped to the Geotechnical and Geoenvironmental Engineering Laboratory at the University of Illinois at Chicago (UIC) where it was stored at room temperature (23 ±2°C). Soil samples were air dried (moisture content <0.5%), pulverized and screened through a 2 mm sieve prior to conducting the experiments.

4.2.2 Biochar (CE-WP2)

Biochar was obtained from a commercial vendor in Illinois, USA. The biochar used in this study was produced from pinewood pellets subjected to gasification at a high temperature of ~520°C. In this study, biochar in pellet form was used- fines were sieved and discarded. The biochar was oven-dried at 105°C to remove any moisture content before conducting the experiments.

4.2.3 BOF Slag

The BOF slag used in this experiment was obtained from Indiana Harbor East of Arcelor Mittal steel industry, located in Indiana, USA. All the tests were performed using the slag as obtained from the plant. The steel slag was also oven-dried at 105°C prior to conducting the experiments.

4.2.4 Properties Testing

The three landfill cover materials were tested for specific gravity as per ASTM D854, grain size distribution following ASTM D422, while Atterberg limits of soil was determined as per ASTM D4318. Hydraulic conductivity was determined according to the ASTM D2434 (for biochar and slag) and ASTM 5084 for soil using a flexible wall triaxial set up. The water holding capacity (WHC) of all the materials were determined using procedure as described in Yargicoglu et al. (2015). Each material under investigation (10 g) was soaked in 0.01 M $CaCl_2$ solution (L/S of 1:1) for 2 hours prior to measuring pH, ORP and electrical conductivity as per ASTM D4972. The pH meter was calibrated using standard buffers (4, 7 and 10) prior to testing. The organic content of the materials was analyzed following the ASTM D2974.

4.2.5 Batch Incubation Tests

4.2.5.1 Mixed Systems

10 g of the total material (soil, biochar, and BOF slag) individually and in different proportions (**Table 4-2**) was placed in 125 mL-serum vials and adjusted to a moisture content of 20% (w/w) using deionized water. The vials were sealed airtight using butyl rubber septa followed by crimp cap. 20 mL of air from the headspace of each vial was replaced with equal volume of synthetic LFG comprising of 50% (v/v) CH_4 and 50% (v/v) CO_2 to achieve a headspace concentration of ~5-6% (v/v) CH_4 and ~5-6% (v/v) CO_2 balanced in air (~88-90%). The change in the headspace concentration was determined by collecting and analyzing the gas samples on a regular basis using gas chromatography (GC) until the headspace concentration dropped to less than 1%. All the experiments were conducted in duplicate/triplicate along with the controls (gas with no material). The controls using only soil (sterilized for 2 hours using Napco Model 8000-DSE autoclave) were also tested to discern any microbial activity in the soil. The CH_4 oxidation rates were calculated from the linear regression analysis of CH_4 concentration versus elapsed time, based on the zero-order kinetics.

4.2.5.2 Slag Isolated Systems

Separate series of incubation experiments were conducted in which soil and biochar-amended soil were not mixed with slag, but slag existed separated in a cage. A steel cage of size 2" x 2" x 2” was used to contain the steel slag (10% of the total material) and placed inside the serum vial using nylon thread to isolate slag from the soil and biochar-amended soil. The material was adjusted to the desired moisture content of 20%. The vial was sealed airtight using butyl rubber septa followed

by crimp cap. Similar procedure as mixed system was followed in the isolated system to achieve the headspace concentration of ~5-6% (v/v) CH_4 and ~5-6% (v/v) CO_2 balanced in air (~88-90%).

4.2.6 Gas Analysis

The gas samples were analyzed at regular time intervals and analyzed for CH_4, CO_2 and O_2 concentrations using an SRI 9300 GC equipped with a thermal conductivity detector (TCD) and CTR-1 column that separates N_2 and O_2 for simultaneous analysis of CO_2, CH_4, O_2 and N_2 as previously described (Yargicoglu and Reddy, 2017). Gas samples were withdrawn using 1 mL syringe where 0.5 mL of the sample was discarded and remaining 0.5 mL was injected into the GC to reduce any pressure effects due to sampling. A calibration curve for a minimum of three points was established using high purity standard gas mixtures ranging from 1% to 50% CH_4 and CO_2.

4.3 RESULTS AND DISCUSSION

Table 4-1 summarizes the physical and chemical properties of BOF slag, soil and biochar used in this study. The BOF slag consisted of ~74% sand-sized particles and was classified as SP (poorly graded sand) as per the Unified Soil Classification System (USCS). The specific gravity of the BOF slag, soil and biochar were determined as 3.5, 2.57 and 0.6, respectively (Reddy et al. 2019 b). The hydraulic conductivity of BOF slag and biochar were both approximately 10^{-3} - 10^{-4} cm/s. The soil was highly impermeable with a hydraulic conductivity in the order of 10^{-8} cm/s. The WHC of soil, BOF slag, and biochar were 43%, 20% and 52% (w/w), respectively. BOF slag was observed to be highly alkaline with pH 12.4. The ORP of all three materials were negative, demonstrating higher reducing capacity.

Both mixed and slag isolated systems were investigated for pH at the beginning of the experiments. Soil, biochar and BOF slag had pH of 7.6, 6.7 and 12.4, respectively (**Table 4-2**).

Many studies on landfill cover soil have shown pH ranging from 4.3 to 9 (Gebert et al. 2009; Chi et al. 2015) with most of them at near neutral pH. The biochar is reported to have a wide range of pH, highly dependent on the type of feedstock used. Yargicoglu et al. (2015) showed pH ranging from 6.24 to 8.86 for five different types of biochar, produced from coconut charcoal, pinewood, aged oak, pinewood pellets, and 90% pine with 10% fir wood. The BOF slag was highly alkaline in nature due to the presence of basic oxides like CaO and MgO (Reddy et al. 2018; Bonenfant et al. 2008; Navarro et al. 2010). The amendment of BOF slag at 10% (total weight) in the mixed systems (slag-amended soil-biochar and slag-amended soil) decreased the pH by 1-1.4 units, keeping the overall pH at 11-11.5 and 11-11.3, respectively. This change in the pH was mainly due to high buffering capacity of the soil. However, the biochar-amended soil had the pH (7.4) close to the pH of the soil (7.6), indicating biochar had no major effect on the overall pH of the mixed materials.

Table 4-1: Physical and chemical characteristics of BOF slag, cover soil and biochar

Properties	ASTM Method	BOF Slag	Soil	Biochar
Specific Gravity	D854	3.5	2.57	0.6
Grain Size Distribution:	D422			
Gravel (%)		20.8	3.7	45
Sand (%)		74.2	14.7	54
Fines (%)		4.9	81.9	1
D_{50} (mm)			0.009	4.3
C_c		0.7	-	0.82
C_u		18	-	2.42
Atterberg Limits:	D4318			
Liquid Limit (%)		Non-	39	Non-Plastic
Plastic Limit (%)		Plastic	22	
Plasticity Index (%)			17	
USCS Classification	D2487	SP-SM	CL	SP
Water Holding Capacity (w/w)		20	43	51.6
Dry Density (g/cm^3)		1.72	1.8	1.15
Hydraulic Conductivity (cm/s)	D2434	1.1×10^{-3}	5.4×10^{-8}	2×10^{-4}
Loss of Ignition (%)	D2974	1.6	5.8	96.71
pH (1:1)	D4972	12.4	7.6	6.5
Electrical Conductivity (mS/cm)	D4972	13.3	0.55	0.8
Redox Potential (mV)	D4972	-313.3	-53.8	-6.3

C_c=Coefficient of curvature; C_u=Coefficient of uniformity

Table 4-2: pH of the mixed and slag isolated systems

Cover Materials	pH
Soil (100%)	7.6
Soil (90%) + Biochar (10%)	7.4
Soil (90%) + Slag (10%)	11-11.3
Soil (80%) + Biochar (10%) + Slag (10%)	11-11.5
Soil (90%) & Slag in Basket (10%)	7.6 (Soil); 12.4 (Slag)
Soil (80%) + Biochar (10%) & Slag in Basket (10%)	7.4 (Soil + Biochar); 12.4 (Slag)
Biochar (90%) & Slag in Basket (10%)	6.7; 12.4 (Slag)
Slag in Basket (100%)	12.4

Figures 4-2(a) and **4-2(b)** show trends in CH_4 consumption, carbon dioxide production and oxygen consumption with time for soil and biochar-amended soil, respectively. An initial lag phase of 24-72 hours was observed, which could be mainly due to time needed for the adaptation of the microbial population to their environment. Thereafter, a gradual decrease in CH_4 concentration, increase in the CO_2 levels and decrease in the oxygen levels were observed, confirming CH_4 oxidation by the CH_4 oxidizing bacteria in both the systems. This was further confirmed using the controls (only sterilized soil and LFG) that showed no major change in the headspace gas concentration (not shown) confirming CH_4 oxidation by the naturally existing CH_4 oxidizing bacteria in the soil. The CH_4 oxidation rates calculated based on the zero-order kinetics were 89.2 μg CH_4 $g^{-1}d^{-1}$ and 79 μg CH_4 $g^{-1}d^{-1}$, respectively, for soil and biochar-amended soil. The results from **Fig. 4-2(b)** also suggests that the biochar had no major effect on the CH_4 oxidation process when amended with soil. Previous study from our research laboratory demonstrated promising results of biochar amendment in enhancing CH_4 oxidation rates in the long term, as the microbes take time for colonizing and acclimatizing (Yargicoglu and Reddy, 2017). The reason for negligible effect of biochar amendment in CH_4 oxidation in the current study could be attributed to the shorter duration of testing.

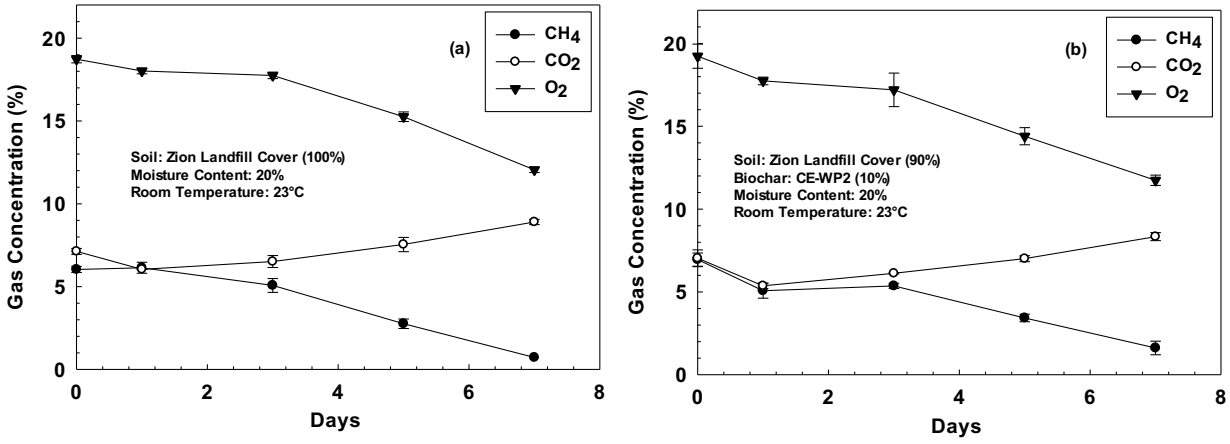

Fig.4-2. Removal of methane with: (a) Soil and (b) Biochar-amended soil

Figures 4-3(a) and **4-3(b)** show changes in the gas concentrations with time for slag-amended soil and slag isolated soil. The slag-amended soil showed complete removal of CO_2 within the first few hours of the experiment but showed negligible change in the CH_4 concentration throughout the course of experiment suggesting inhibition of CH_4 oxidation activity of the CH_4 oxidizing bacteria, which could possibly be due to the high pH (11-11.3) of the system and the presence of heavy metals that is in direct contact with the soil bacteria. However, in the slag isolated soil system, the slag was placed inside the steel mesh not in contact with the soil, wherein CH_4 oxidation by the soil and simultaneous CO_2 sequestration by the BOF slag was observed. The rate of CO_2 removal in the isolated system was slower when compared to the mixed system, which could likely be attributed to the diffusion limitations posed by the steel mesh. The CH_4 oxidation rates were 0.74 µg CH_4 $g^{-1}d^{-1}$ and 85.5 µg CH_4 $g^{-1}d^{-1}$, respectively, for slag-amended soil and slag isolated soil.

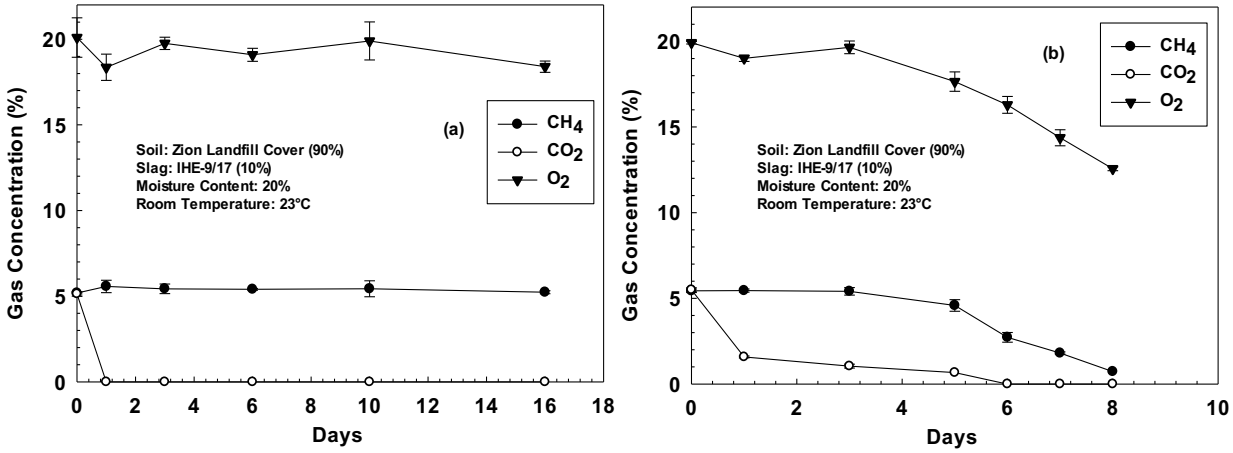

Fig. 4-3. Removal of carbon dioxide and methane with: (a) Slag-amended soil (b) Slag isolated soil

Figures 4-4(a) and **4-4(b)** show changes in the gas concentrations with time for slag-amended soil-biochar and slag isolated biochar-amended soil. No change in the CH_4 concentration was observed in the slag-amended soil-biochar similar to the slag-amended soil (Appendix A, **Fig. S3-1**), but complete removal of CO_2 in the presence of BOF slag was noted. However, in the slag isolated biochar-amended soil, change in CH_4 concentration with time was observed showing CH_4 oxidation along with a prolonged removal of CO_2. It is known that CH_4 oxidizing bacteria grow at a pH ranging from 5.5 to 8.5 in soils and sediments of different ecosystem (Dunfield 1993; Hutsch 1994; Scheutz and Kjeldsen 2004; Sherry et al. 2016; Han et al. 2016), although few methanotrophs growing in extreme environments such as soda lake and marine environments at pH 9-11 requiring NaCl for their growth (Kalyuzhnaya et al. 2008, Sorokin et al. 2000, Khmelenina et al. 1997) have been identified. Therefore, for an effective CH_4 oxidation to occur, slag isolated from the soil or overlain the soil is recommended so that methanotrophic activity is not inhibited by the high pH or metal content of slag in the system. The CH_4 oxidation rate for these systems were calculated to be 0.98 µg CH_4 $g^{-1}d^{-1}$ (slag-amended soil-biochar) and 80 µg CH_4 $g^{-1}d^{-1}$ (slag isolated biochar-amended soil), respectively.

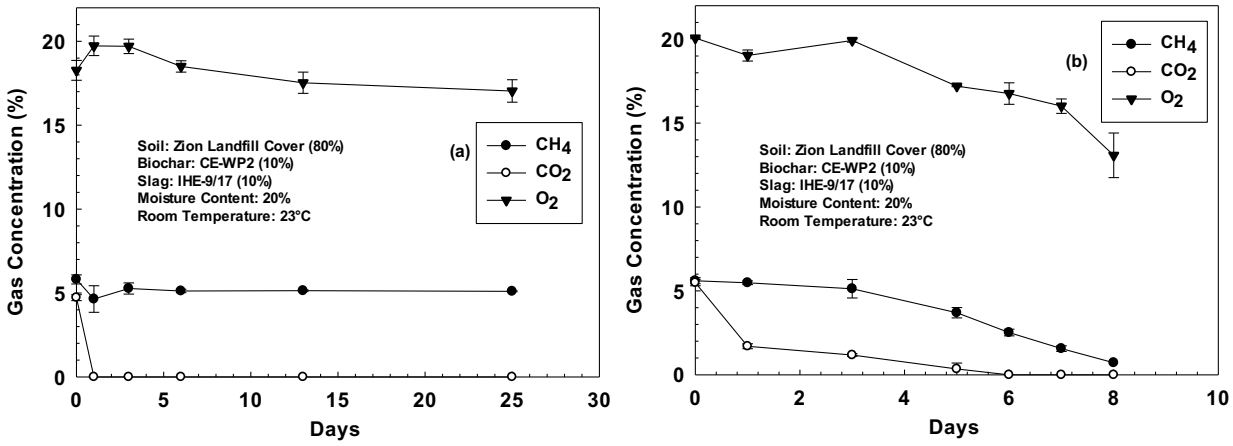

Fig. 4-4. Removal of carbon dioxide and methane with: (a) Slag-amended soil-biochar (b) Slag isolated biochar-amended soil

Fig. 4-5 shows trend in both CH_4 and CO_2 with time for BOF slag alone. The results show negligible removal of CH_4 and significant removal of CO_2 suggesting the BOF slag to be an invaluable material for CO_2 sequestration. Overall, the three landfill cover materials studied demonstrated an effective CH_4 oxidation in the soil, slag isolated biochar-amended soil, slag isolated soil, and biochar-amended soil. It is important to note that negligible CH_4 oxidation in slag-amended soil-biochar and slag-amended soil was observed. Furthermore, BOF slag demonstrated significant potential in sequestering CO_2 in both mixed as well as slag isolated systems.

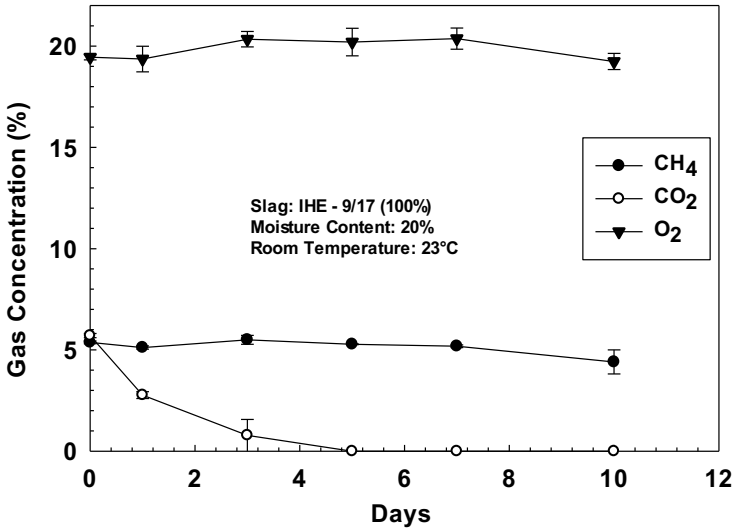

Fig.4-5 Removal of carbon dioxide using BOF Slag

4.4 CONCLUSIONS

Preliminary investigation on the landfill cover materials was conducted to study the effect of cover materials (soil, biochar-amended soil, and BOF slag) on the CH_4 oxidation and CO_2 sequestration. Our results demonstrated that the landfill cover soil was dominated by the CH_4 oxidizing bacteria and were responsible for the CH_4 oxidation. No negative impact on the CH_4 oxidation was observed when the soil was amended with biochar, but a negative effect was observed when the BOF slag was mixed with soil or biochar-amended soil. Nevertheless, BOF slag showed effective CO_2 sequestration in both mixed and slag isolated systems. Therefore, our preliminary results propose the use of slag-isolated soil or slag isolated biochar-amended soil systems for an effective CH_4 oxidation and simultaneous CO_2 sequestration in the biogeochemical landfill cover system. Further, column studies and field scale evaluation of slag isolated soil and biochar-amended soil cover systems are being conducted in order to better understand the effect of BOF slag on microbial CH_4 oxidation in the long-term field applications.

4.5 CITED REFERENCES

Andreas, L., Diener, S., and Lagerkvist, A. (2014). "Steel slags in a landfill top cover–Experiences from a full-scale experiment". Waste Management, 34(3), 692-701.

Boeckx, P., Van Cleemput, O., and Villaralvo, I. D. A. (1996). "Methane emission from a landfill and the methane oxidizing capacity of its covering soil". *Soil Biology and Biochemistry*, 28(10-11), 1397-1405.

Bonenfant, D., Kharoune, L., Sauve, S., Hausler, R., Niquette, P., Mimeault, M., and Kharoune, M. (2008). "CO2 sequestration potential of steel slags at ambient pressure and temperature". *Industrial & Engineering Chemistry Research*, 47(20), 7610-7616.

Cao, Y., and Staszewska, E. (2011). "Methane emission mitigation from landfill by microbial oxidation in landfill cover". *International conference on environmental and agriculture engineering IPCBEE* (Vol. 15, p. 57).

Chi, Z. F., Lu, W. J., & Wang, H. T. (2015). Spatial patterns of methane oxidation and methanotrophic diversity in landfill cover soils of Southern China. J. Microbiol. Biotechnol, 25(4), 423-430.

Dunfield, P., Dumont, R., and Moore, T. R. (1993). "Methane production and consumption in temperate and subarctic peat soils: response to temperature and pH". *Soil Biology and Biochemistry*, 25(3), 321-326.

Gebert, J., Singh, B. K., Pan, Y., and Bodrossy, L. (2009). "Activity and structure of methanotrophic communities in landfill cover soils". *Environmental Microbiology Reports*, 1(5), 414-423.

Han, J. S., Mahanty, B., Yoon, S. U., and Kim, C. G. (2016). "Activity of a methanotrophic consortium isolated from landfill cover soil: Response to temperature, pH, CO2, and porous adsorbent". *Geomicrobiology Journal*, 33(10), 878-885.

Herrmann, I., Andreas, L., Diener, S., and Lind, L. (2010). "Steel slag used in landfill cover liners: laboratory and field tests". *Waste Management & Research*, 28(12), 1114-1121.

Kalyuzhnaya, M. G., Khmelenina, V., Eshinimaev, B., Sorokin, D., Fuse, H., Lidstrom, M., and Trotsenko, Y. (2008). "Classification of halo (alkali)philic and halo (alkali) tolerant methanotrophs provisionally assigned to the genera Methylomicrobium and Methylobacter and emended description of the genus Methylomicrobium". *International Journal of Systematic and Evolutionary Microbiology*, 58(3), 591-596.

Khmelenina VN, Kalyuzhnaya MG, Starostina NG, Suzina NE & Trotsenko YA (1997). "Isolation and characterization of halotolerant alkaliphilic methanotrophic bacteria from Tuva soda lakes". *Microbiology*, 35: 257–261.

Kightley, D., Nedwell, D. B., and Cooper, M. (1995). "Capacity for methane oxidation in landfill cover soils measured in laboratory-scale soil microcosms". *Applied and Environmental Microbiology*, 61(2), 592-601.

Kim, G. W., Ho, A., Kim, P. J., and Kim, S. Y. (2016). "Stimulation of methane oxidation potential and effects on vegetation growth by bottom ash addition in a landfill final evapotranspiration cover". *Waste Management,* 55, 306-312.

Kimio, I. T. O. (2015). "Steelmaking slag for fertilizer usage." Nippon Steel Sumitomo Metal Tech Rep, 109, 130-136.

Kovacic, D. (1996). “Materials for the final cover of sanitary landfills”. *International Journal of Rock Mechanics and Mining Sciences and Geomechanics Abstracts* (Vol. 1, No. 33, p. 40A).

Lu, S. G., Bai, S.Q., and Shan, H. D. (2008). “Mechanisms of phosphate removal from aqueous solution by blast furnace slag and steel furnace slag.” *J. Zhejiang Univ. Sci.*1066 A, 9(1), 125-132.

Navarro, C., Díaz, M., and Villa-García, M. A. (2010). “Physico-chemical characterization of steel slag. Study of its behavior under simulated environmental conditions”. *Environmental Science & Technology*, 44(14), 5383-5388.

Nhan, C. T., Graydon, J. W., and Kirk, D. W. (1996). “Utilizing coal fly ash as a landfill barrier material”. *Waste Management*, 16(7), 587-595.

Pedersen, G. B., Scheutz, C., and Kjeldsen, P. (2011). “Availability and properties of materials for the Fakse Landfill biocover”. *Waste Management*, 31(5), 884-894.

Reddy, K. R., Yargicoglu, E. N., Yue, D., and Yaghoubi, P. (2014). “Enhanced microbial methane oxidation in landfill cover soil amended with biochar.” *Journal of Geotechnical and Geoenvironmental Engineering*, 140(9), 04014047.

Reddy, K.R., Grubb, D.G. and Kumar, G. (2018). "Innovative biogeochemical soil cover to mitigate landfill gas emissions," Proceedings of the *International Conference on Protection and Restoration of the Environment XIV*, Thessaloniki, Greece.

Reddy, K. R., Gopakumar, A., and Chetri, J. K. (2019 a). “Critical review of applications of iron and steel slags for carbon sequestration and environmental remediation”. *Reviews in Environmental Science and Bio/Technology*, 18(1), 127-152.

Reddy, K. R., Gopakumar, A., Chetri, J. K., Kumar, G., & Grubb, D. G. (2019 b). Sequestration of landfill gas emissions using basic oxygen furnace slag: Effects of moisture content and humid gas flow conditions. Journal of Environmental Engineering, 145(7), 04019033.

Scheutz, C., and Kjeldsen, P. (2004). "Environmental factors influencing attenuation of methane and hydrochlorofluorocarbons in landfill cover soils". *Journal of Environmental Quality*, 33(1), 72-79.

Scheutz, C., Fredenslund, A. M., Chanton, J., Pedersen, G. B., and Kjeldsen, P. (2011). "Mitigation of methane emission from Fakse landfill using a biowindow system". *Waste Management*, 31(5), 1018-1028.

Sheridan, C. (2014). "Remediation of acid mine drainage using metallurgical slags." *Miner. Eng*., 64, 15-22.

Sherry, A., Osborne, K. A., Sidgwick, F. R., Gray, N. D., and Talbot, H. M. (2016). "A temperate river estuary is a sink for methanotrophs adapted to extremes of pH, temperature and salinity". *Environmental Microbiology Reports*, 8(1), 122-131.

Smith, J. S. (2003). "Method for purifying contaminated groundwater using steel slag". U.S.1117 Patent No. 6,602,421.

Sorokin, D. Y., Jones, B. E., and Kuenen, J. G. (2000). "An obligate methylotrophic, methane-oxidizing Methylomicrobium species from a highly alkaline environment". *Extremophiles,* 4(3), 145-155.

Stern, J. C., Chanton, J., Abichou, T., Powelson, D., Yuan, L., Escoriza, S., and Bogner, J. (2007). "Use of a biologically active cover to reduce landfill methane emissions and enhance methane oxidation". *Waste Management*, 27(9), 1248-1258.

Su, T. H., Yang, H. J., Shau, Y. H., Takazawa, E., and Lee, Y. C. (2016). "CO_2 sequestration utilizing basic-oxygen furnace slag: Controlling factors, reaction mechanisms and V–Cr concerns". *Journal of Environmental Sciences*, 41, 99-111.

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.

Xie, T., Sadasivam, B. Y., Reddy, K. R., Wang, C., and Spokas, K. (2015). "Review of the effects of biochar amendment on soil properties and carbon sequestration". *Journal of Hazardous, Toxic, and Radioactive Waste*, 20(1), 04015013.

Yargicoglu, E. N., Sadasivam, B. Y., Reddy, K. R., and Spokas, K. (2015). "Physical and chemical characterization of waste wood derived biochars". *Waste Management*, 36, 256-268.

Yargicoglu, E. N., and Reddy, K. R. (2017). "Biochar-amended soil cover for microbial methane oxidation: Effect of biochar amendment ratio and cover profile". *Journal of Geotechnical and Geoenvironmental Engineering*, 144(3), 04017123.

Zhang, Y. L., Liu, M. D., Wang, Y. J., and Du, L. D. (2003). "Effects of slag application on Si, Fe and Mn in paddy soil and rice plant." *Chinese J. Soil Sci*., 4, 016.

CHAPTER 5 – EFFECT OF pH ON METHANE OXIDATION AND COMMUNITY COMPOSITION IN LANDFILL COVER SOIL

5.1 INTRODUCTION

In the USA, landfills are estimated to be the third largest anthropogenic source of CH_4 emissions making up 14.1% of the total CH_4 emissions in 2016 (USEPA 2019). Although significant amount of CH_4 is emitted from landfills, it is estimated that between 10% and 90% of CH_4 produced is being consumed by methane oxidizing bacteria (MOBs) before it enters the atmosphere (Semrau et al. 2010; De Visscher et al. 2007). Methanotrophs or MOBs, are a subset of a larger microbial community called methylotrophs. Methanotrophs utilize CH_4 as a sole source of carbon and energy, whereas methylotrophs use a broader range of C1-compounds as their source of carbon and energy (Hanson and Hanson 1996). Methanotrophs within the phylum Proteobacteria have been classified into three phylogenetically distinct groups: Type I, Type II and Type X methanotrophs, where Type I and Type X are grouped in the class Gamma proteobacteria and Type II within the class Alpha proteobacteria (Hanson and Hanson 1996; Semrau et al. 2010). Type I, Type II and Type X methanotrophs are ubiquitous in nature and are usually active where CH_4 and O_2 are present.

Landfill cover soils are typically dominated by Type I or Type II methanotrophs depending upon environmental factors such as moisture content, pH, temperature, substrate concentration, soil texture and nutrients (Su et al. 2014; Borjesson et al. 2004; Urmann et al. 2009; Scheutz et al. 2009). Studies have shown the greatest abundance of Type I methanotrophs is in landfill cover soils with pH ranging from 6.7-8.2 (Chi et al. 2015; Yargicoglu and Reddy 2017a) and Type II methanotrophs at pH range of 5-6.5 (Wise et al. 1999; Cebron et al. 2007; Su et al.2014). Methanotrophs are known to adapt to in-situ pH levels in the landfill cover soil (Scheutz and

Kjeldsen 2004), though environmental selection can lead to a shift in microbial community structure (Su et al.2014). But, in recent years, many studies have shown the resiliency of methanotrophs to extreme acidic and alkaline conditions in the forest soil, peat soil, sediments, mine impoundments and soda lakes (Dunfield 2009; Baesman et al. 2015; Kalyuzhnaya et al. 2008). These studies have identified methanotroph clades in acidic environments that belong to the families Methylocystaceae, Beijerinckiaceae and Methylococcaceae; typically identified as acidophilic or acid-tolerant methanotrophic bacteria (Nguyen et al. 2018). Further, studies have shown a broad diversity of methanotrophs in alkaline environments. For example, an alkaliphilic methanotroph was first isolated from Tuva soda lakes (Kalyuzhnaya et al. 2008; Khmelenina et al.1997). Due to difficulties in cultivating these microbes in laboratory, cultivation-independent molecular tools have been adopted to detect methanotrophs by employing targeted amplification and sequencing of functional or structural genes, or through selection -free shotgun sequencing approaches. PCR-based amplification protocols typically target structural genes such as microbial small subunit ribosomal RNA genes (16S rRNA gene), as well as methane monooxygenase (MMO) genes and genes involved in C1 compound oxidation.

Several studies have been conducted to investigate the effects of pH on CH_4 oxidation and microbial community composition using molecular techniques in different ecosystems and is summarized in **Table 5-1**. For example, Amaral et al. (1998) studied the effects of pH (4 to 8) on atmospheric CH_4 consumption by forest soil microbial communities using soil slurries and culture consortia. They observed differences in optimal pH of the soil methylotrophs using slurry (4-6.5) or culture consortia (5.8) even though the bacteria were extracted from the same soil, and that cultivation conditions lead to selection for different taxa. They concluded that the consumers of ambient CH_4 were physiologically different from the known methanotrophs. Dunfield et al. (1993)

tested CH_4 consumption by peat soil obtained from different locations to study the effect of pH ranging from 3.5-8. They observed that maximum CH_4 consumption was observed at 0-1 units above the native acidic pH of the peat samples (Saari et al. 2004) and concluded that the microflora involved in CH_4 metabolism was not well adapted to low pH conditions. Baesman et al. (2015) studied the CH_4 oxidation potential and characterized microbial community in a mercury mine impoundment using slurry enrichments. Their studies showed maximum oxidation activity at pH range of 4.5-7, with minimal or no activity at pH 3, 9 and 11 and soils contained Type I and Type II Methanotrophs as well as Methylotrophs based on *pmoA* and 16S rRNA gene amplicon analysis.

Relatively few studies have focused on analyzing the effect of pH on CH_4 oxidation and microbial communities in landfill cover soils (Scheutz and Kjeldsen, 2004; Han et al. 2016; Su et al. 2014). Scheutz and Kjeldsen (2004) studied the effect of environmental factors on CH_4 oxidation in the landfill cover soil. Their results showed optimum oxidation at pH (6.5-7.5), concluding that pH was within the range of pH values at the site (6.9) and not one of the important factors controlling CH_4 oxidation. Furthermore, due to the natural buffering capacity of soil, landfill cover soils frequently have circumneutral pH and facilitates CH_4 oxidation. Su et al. (2014) studied various factors affecting the diversity of methanotrophs in the landfills with and without landfill gas (LFG) recovery systems. Their studies concluded that pH was the most dominant factor influencing the methanotrophic diversity in the landfill cover soil, followed by water content and organic content.

Our study focuses in understanding the broader aspect of effect of pH on both CH_4 oxidation and microbial community structure in a newly proposed biogeochemical cover for landfill, where a layer of highly alkaline BOF slag (pH > 12) will be overlain or mixed with biochar-amended soil. Previously, the behavior and activity of CH_4-oxidizing bacteria was

characterized in a series of highly alkaline biogeochemical cover wherein steel slag was amended with soil and biochar-amended soil (Rai and Reddy, 2019). It was noted that steel slag when amended with soil or biochar-amended soil inhibited CH_4 oxidation process. It is hypothesized that the high pH of these mixtures (>11) could have affected the enzymes of MOBs, thereby inhibiting CH_4 oxidation. To further confirm this hypothesis the goal of this study was to investigate the effect of pH on CH_4 oxidation and the microbial community structure, by employing an experimental framework of characterizing enrichment cultures and soil suspension derived from the landfill cover soil. The results from this study will be used in designing the cover profiles for column experiments.

5.2 MATERIALS AND METHODS

5.2.1 Soil Enrichment

Soil was collected from the Zion landfill, located in Zion, Illinois, USA. Soil samples were collected from an interim cover at a depth of ~1 to 2 feet and were shipped to the Geotechnical and Geoenvironmental Engineering Laboratory at the University of Illinois at Chicago (UIC), where they were stored at room temperature (23 ± 2°C). Soil samples were air dried (moisture content < 0.5%), pulverized and screened through a 2 mm sieve prior to conducting experiments. To obtain methanotrophic-enriched consortia, approximately 5 g of sieved soil was mixed with 100 mL of modified (Nitrate Mineral Salts) NMS media (Whittenbury et al. 1970) in a 500 mL-serum vial and stoppered using long sleeved rubber septa. Approximately 80 mL of air from the headspace was replaced with equal volume of mix gas CH_4 /CO_2 to achieve a headspace concentration of 7% CH_4 (v/v) and 7% CO_2 (v/v) balanced in air (86%) and was incubated for 15 days at 23°C. To determine the activity of methanotrophs and CH_4 oxidation rates, gas samples

were analyzed on a regular basis using Gas Chromatography (GC) until the CH_4 headspace concentration dropped below 1%. To enrich the methanotrophic culture, the mix gas (CH_4/CO_2) was replenished twice.

5.2.2 Enrichment culture batch tests

Serum vials, rubber septa and pipettes were sterilized using a Napco Model 8000-DSE autoclave operated at >120°C for a minimum of 60 minutes to ensure sterilization prior to experiments with enrichment culture. The supernatant from soil enrichments (above) was collected for use in batch tests. Briefly, 5 mL of enrichment culture supernatant was mixed 5 mL of sterile modified NMS medium (without phosphate buffer) and placed in a 125 mL-serum vial and sealed using butyl rubber septa followed by crimp cap. Approximately 20 mL of air from the headspace was replaced with an equal volume of synthetic landfill gas composed of 50% (v/v) CH_4 and 50% (v/v) CO_2 to achieve a headspace concentration of ~5 to 6% CH_4 (v/v) and 5 to 6% CO_2 (v/v) balanced in air (89-90%). The pH of enrichment cultures was adjusted from 2 to 12 using either 1M H_2SO_4 or 1M NaOH. Headspace samples were analyzed using gas chromatography (described below). Headspace CH_4 concentrations were monitored until CH_4 concentrations dropped below 1%. In addition, the pH of the liquid medium in each sample was tested periodically with a pH meter (Orion 720A model). Rates of CH_4 oxidation were determined from linear regression analysis of CH_4 concentration with respect to time based on zero-order kinetics. At the end of the incubation, microbial cells in the enrichment media were pelleted by centrifugation and frozen for later DNA extraction and microbial community analysis. Experiments were conducted in duplicates, along with the controls containing only sterile media.

5.2.3 Soil suspension batch tests

10 g of sieved soil was mixed with 10 mL of sterile distilled water (1:1 ratio), placed in 125 mL serum vials and sealed air tight using butyl rubber septa followed by crimp cap. The vials were spiked with 1M H_2SO_4 to achieve an acidic pH of 4 and 1M NaOH to achieve an alkaline pH of 9, 10 and 12. Bottles with an initial pH of 2 were difficult to maintain at pH, and these samples were not included in downstream analyses in maintaining the targeted pH. As described above, synthetic landfill gas was added to each bottle to achieve a starting headspace concentration of ~5 to 6% CH_4 (v/v) and ~5 to 6% CO_2 (v/v) balanced in air (89-90%). The initial pH value was measured after 60 minutes of addition of acid or alkali, and final pH values measured at the end of the experiment (22 days). At the end of the incubation, the soil suspension samples were centrifuged at 12,500 RPM for 15 min, and the supernatant decanted. The residual soil material was frozen at -20°C for later DNA extraction and microbial community analysis. All experiments were conducted in replicates along with the controls (LFG gas without biological material).

5.2.4 Gas Analysis

Gas samples were collected at regular time intervals and analyzed for CH_4 and CO_2 concentrations using an SRI 9300 Gas Chromatography (GC) equipped with thermal conductivity detector (TCD) as previously described (Yargicoglu and Reddy, 2017). Gas samples were withdrawn using 1-mL syringe, and 0.5 mL of gas sample was injected into a GC equipped with TCD. A calibration curve with a minimum of three points was established using high purity standard gas mixtures ranging from 1% to 50% CH_4 (v:v) and 5% to 50% CO_2 (v:v).

5.2.5 Analysis of microbial community structure

Genomic DNA (gDNA) was extracted from soil samples and from cell pellets using a DNeasy PowerSoil Kit (Qiagen) based on manufacturer's instructions with a slight modification. Samples were heated at 65°C for 10 min before homogenizing with FastPrep-24 5G bead-beating device (MP Biomedicals) at 6 m/s for 40 sec. After homogenization, extraction protocols were automated on a QIAcube instrument (Qiagen), according to the manufacturer's instructions. Genomic DNA was processed for microbial community analysis using 16S rRNA gene amplicon sequencing. 16S rRNA gene amplicon sequencing was performed using a two-stage amplification protocol as described previously (Green et al. 2015; Naqib et al. 2018). The primer set 515F-806R was used to amplify the V4 variable region of the microbial 16S rRNA gene, and sequencing was performed on an Illumina MiniSeq instrument, employing paired-end 2x153 base reads. Raw sequence data were initially processed by merging forward and reverse reads using the software package PEAR (Zhang et al. 2013). Merged reads were trimmed to remove ambiguous nucleotides, primer sequences, and trimmed based on quality scores. Chimeric sequences were identified and removed using the USEARCH algorithm with a comparison to Silva 132 reference sequence database [Edgar 2010; Glöckner et al. 2017; Quast et al.2012). The standard QIIME pipeline (Caporaso et al. 2012) was modified to generate taxonomic summaries using sub-OTU resolution of the sequence dataset (Tikhonov et al. 2015). Taxonomic annotations were assigned using the USEARCH algorithm with the Silva 132 reference database (Edgar 2010), and data were output as biological observation matrices (BIOMs; (MacDonald et al.2012)) at multiple taxonomic levels. Generation of BIOMs was performed by the Research Informatics Core (RIC) at UIC.

5.2.6 Data archive

Raw sequence data files were submitted in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) and are available under the BioProject identifier PRJNA545136.

5.2.7 Statistical analysis

Statistical analysis of batch test results was performed using one-way (ANOVA) and t-tests (equivalency of sample means) using Microsoft Excel-2018. A significance level, alpha = 0.05 was used to assess statistical significance in all tests. Microbial community abundance data were analyzed within the software package Primer7 (Clarke and Gorley, 2015) to calculate alpha-diversity indices and generate ordination plots (*i.e.*, multidimensional scaling (MDS) plots). Significant differences in community structure between experimental conditions were assessed using analysis of similarity (ANOSIM).

5.3 RESULTS

5.3.1 Effect of pH on methane oxidation in soil-suspension and enrichment culture

The enrichment culture and soil-suspension samples were exposed to a wide range of pH conditions to investigate the effect of pH on CH_4 oxidation potential and community composition. **Fig 5-1.** shows the CH_4 consumption with time in enrichment culture (starting pH 2-12) and soil-suspension samples (starting pH 4 -12), along with the corresponding pH measurement. An initial lag phase of 3 days was observed in enrichment cultures at starting pH 2 as shown in **Fig 5-1a.** after which a gradual decrease in CH_4 concentration was observed from day 3 to 13. Thereafter, no change in CH_4 concentration was observed until day 29. The pH values were measured from

1.9 - 2.3 throughout the course of the incubation. **Fig 5-1b.** shows CH_4 headspace concentration with time at a starting pH of 4 in enrichment culture and soil-suspension. An initial lag phase of two days was observed in enrichment culture after which a gradual decrease in CH_4 headspace concentration was measured from day 3 to day 13. No change in CH_4 headspace was measured by the end of the experiment (day 22). In contrast, soil-suspension samples showed no change in CH_4 concentration throughout the course of the incubation. pH values in the enrichment culture fluctuated from 4.2 at the beginning of the experiment to 5.1 at the end of the experiment and increased from 4.2 to 6 in soil-suspension reactor samples. **Fig 5-1c.** shows CH_4 headspace concentration and corresponding pH measurements as a function of time in reactors with a starting pH of 7. An initial lag phase of one and three days was observed in enrichment culture and soil-suspension, respectively, after which a rapid decrease in CH_4 headspace concentration was observed in enrichment cultures and a more gradual decrease in soil-suspension reactors. pH values remained nearly constant throughout the incubation with a decrease of 0.3 - 0.4 units in enrichment cultures and 0.2 units in soil-suspension reactors by the end of the experiment. **Figs 5-1d** and **5-1e** show CH_4 headspace concentration and the corresponding pH measurement as a function of time for reactors with starting pH values of 9 and 10, respectively. An initial lag phase of three days was noted in enrichment culture reactors with starting pH values of 9 and, after which a rapid decrease in CH_4 headspace concentration was observed. By the end of the experiment, reactor pH values had dropped to approximately 7.6-7.8, despite intermittent pH adjustment through NaOH addition. In soil-suspension reactors an initial lag phase of 2 days was observed together with a gradual decrease in CH_4 headspace concentration. Consistent with the enrichment culture reactors, elevated pH levels were not maintained, and average pH values at the end of the experiment were 7.6 and 7.4 for pH 9 and pH 10 reactors, respectively. No major change in CH_4

headspace concentration of reactors was observed throughout the course of the incubation in enrichment culture and soil-suspension reactors with starting pH values of 12. In enrichment culture reactors, pH values dropped from 12.3 to 10.4 by day 4 and were adjusted by adding of NaOH. Thereafter, average pH values fluctuated between 11.8 and 12.3. In soil-suspension reactors, pH remained nearly constant throughout the incubation (12.2-12.1). A comparison of all reactors is shown in **Fig 5-2** and demonstrates similar rates of methane oxidation in enrichment culture reactors with starting pH values of 7, 9 and 10, and minimal or no methane oxidation in reactors with starting pH values of 2,4 and 12. Similarly, soil suspension reactors with start pH values of 7.6 and 10 had similar rates of methane oxidation, and no methane oxidation was observed in reactors with starting pH values of 4 and 12.

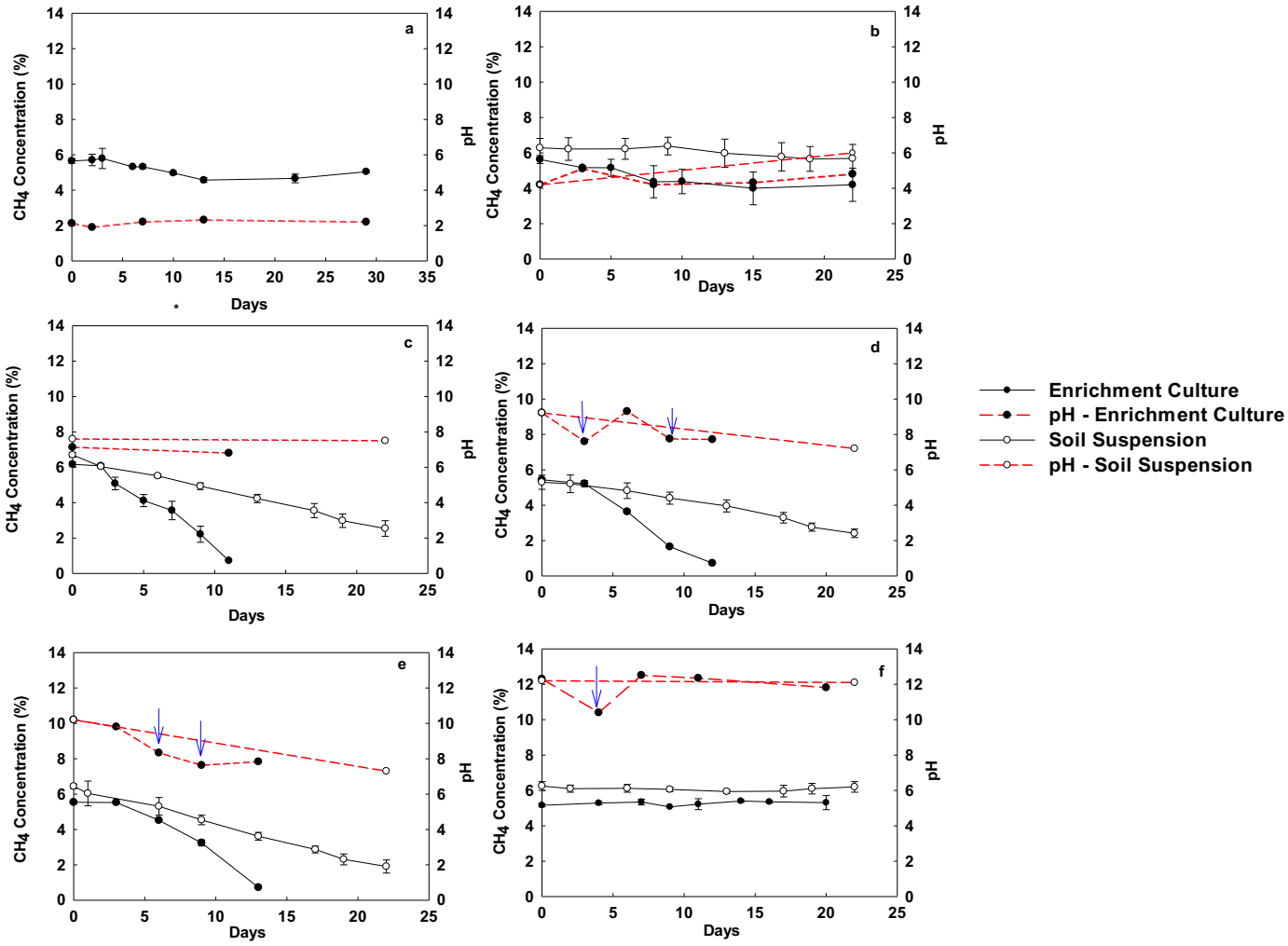

Figure 5-1. Methane concentration over time as a function of pH in enrichment culture and soil-suspension reactors with starting values of (a) pH 2, (b) pH 4, (c) pH 7/7.6, (d) pH 9, (e) pH 10 (f), and pH 12. (All pH values are ± 0.1 - 0.3 units). The blue arrows indicate addition of NaOH in enrichment cultures

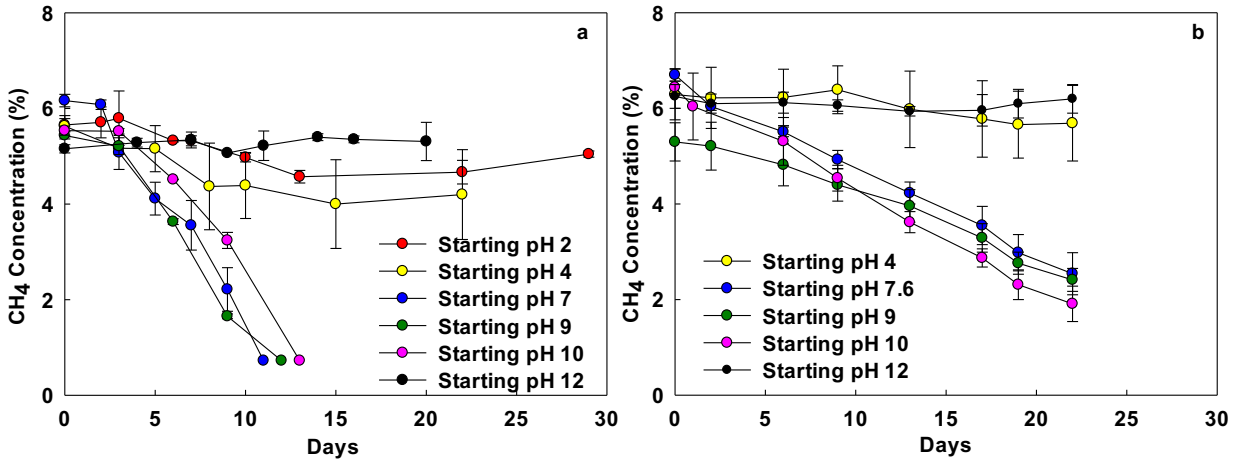

Figure 5-2. Methane consumption in (a) enrichment culture reactors and (b) soil suspension reactors with starting pH values ranging from 2 to 12

Fig 5-3. shows average CH_4 oxidation rates in soil-suspension and enrichment culture reactors incubated at starting pH values ranging from 2 to 12. The difference in the oxidation rates between soil-suspension and enrichment culture reactors may be attributed to substrate diffusion limitations in aqueous phase (Park et al. 2005; Sadasivam and Reddy 2014). Maximum CH_4 oxidation rates were observed in reactors with starting pH values of 7-10 (42.9 - 47.5 µg CH_4 $mL^{-1}d^{-1}$) in enrichment culture and at starting pH values of 10 (20 µg CH_4 $g^{-1}d^{-1}$) in soil-suspension reactors. CH_4 oxidation rates in reactors with starting pH values of 7, 9 and 10 were not significantly different from each other in enrichment culture (ANOVA, $p = 0.3325$), whereas rates of CH_4 oxidation were significantly different in reactors with starting pH values of 7.6, 9 and 10 (ANOVA, $p=0.0147$) in soil-suspension reactors. Low rates of CH_4 oxidation were observed in enrichment culture reactors with starting pH values of 2 and 4 (2.4 and 7.3 µg CH_4 $mL^{-1}d^{-1}$, respectively). No methane oxidation was observed in enrichment culture reactors with a starting pH of 12 and in soil suspension reactors with starting pH values of 4 and 12.

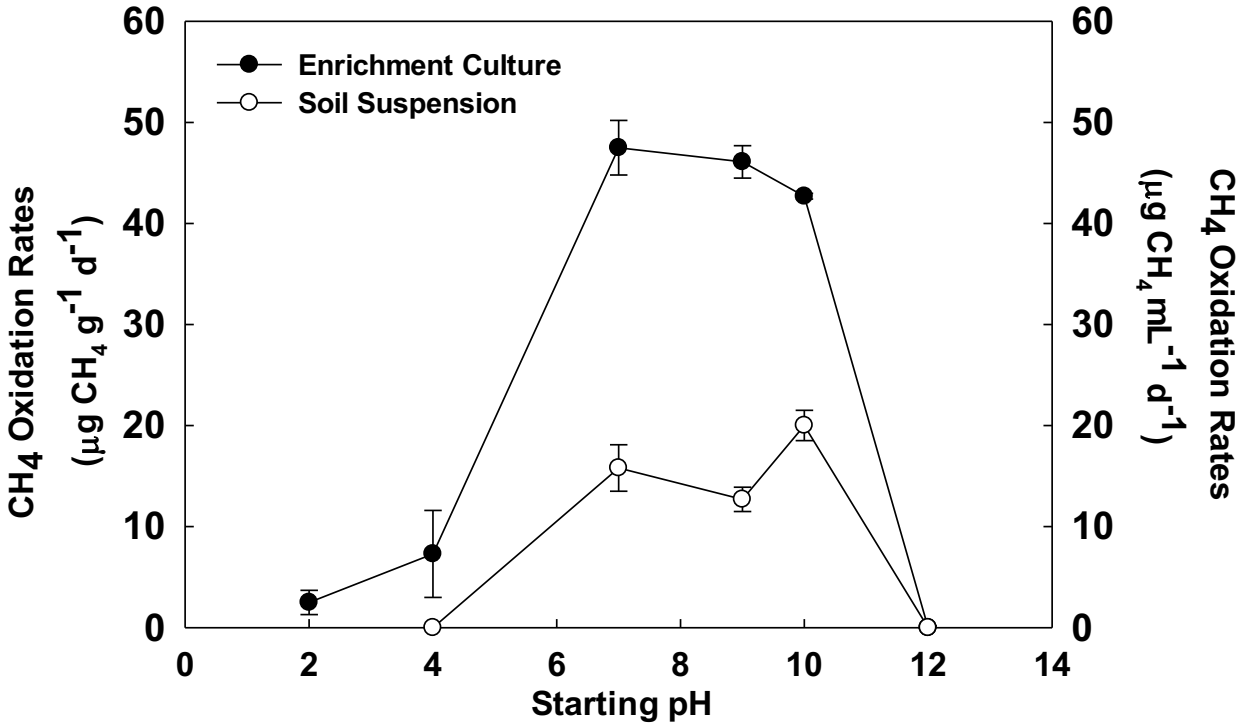

Figure 5-3. Methane oxidation rates in soil-suspension and enrichment culture reactors with starting pH values ranging from 2 to 12

5.3.2 Methylotroph microbial community structure in enrichment cultures incubated across a pH gradient

Microbial community structure in the enrichment cultures reactors was analyzed using deep sequencing of microbial 16S rRNA gene amplicons (**Fig 5-4**). The average relative abundance of 16S rRNA gene amplicon sequences from methylotrophs relative to the total microbial community ranged from 26% (starting pH 2) to 74% (starting pH 12). Microbial community structure varied by starting pH (**Fig 5-4c**), and the methanotrophic communities were dominated by bacteria from the genera *Methylobacter, Methylovorus, Methylocystis, Methylomicrobium, Methylosinus* and *Mehyloversatilis*. *Methylobacter,* a Type I methanotroph, was found in abundance across all pH conditions examined. More specifically, *Methylobacter marinus* A45 was the most abundant

methanotrophic species detected in the enrichment reactors, and sequences from this taxon constituted 78, 22, 36, 56 and 78% of the total methylotrophic 16S rRNA sequences identified at starting pH values of 2, 4, 9, 10, and 12, respectively. Type II methanotrophs were also detected, including bacteria from the genera *Methylocystis*. *Methylocystis* were abundant in reactors with starting pH values in the range of 2-7, but negligible in reactors with higher pH values. In reactors with low and high starting pH values (*i.e.*, pH of 2 and 12), microbial communities were dominated (>90% relative abundance) by methanotrophs from the genus *Methylobacter*. This similarity likely represents low or no microbial activity in these reactors, leading to an observed microbial community derived from the same source enrichment. At other starting pH values, microbial growth led to shifts in observed microbial community structure.

5.3.3 Methylotroph microbial community structure in soil-suspensions incubated across a pH gradient

Microbial community structure in soil-suspensions reactors with starting pH values ranging from 7.6 to 12 were analyzed using 16S rRNA gene amplicon sequencing (**Fig 5-4b)**. The average relative abundance of 16S rRNA gene amplicon sequences from methanotrophs relative to sequences from the total microbial community ranged from 3% (starting pH 12) to 32% (starting pH 9 and 10). Four taxa dominated the active methanotrophic microbial communities in reactors with starting pH values of 7.6, 9 and 10, including bacteria from the genera *Methylobacter* and *Methylomicrobium* and bacteria from the family Methylophilaceae and Methylomonaceae. The bacteria from members of the family Methylomonaceae was found in abundance with a relative abundance of 22.5%, 32% and 29%.at starting pH 7.6, 9 and 10, respectively, followed by Methylophilaceae with a relative abundance of 28.7%, 11.6% and 22.7%. However, at starting pH

12, only 3% of the total sequences that belong to the methylotrophic community were identified, indicating high alkaline conditions (starting pH 12) did not favor the growth of methylotrophic community. Further, due to sequencing difficulties at starting pH 4, microbial community was not detected from these sample sets.

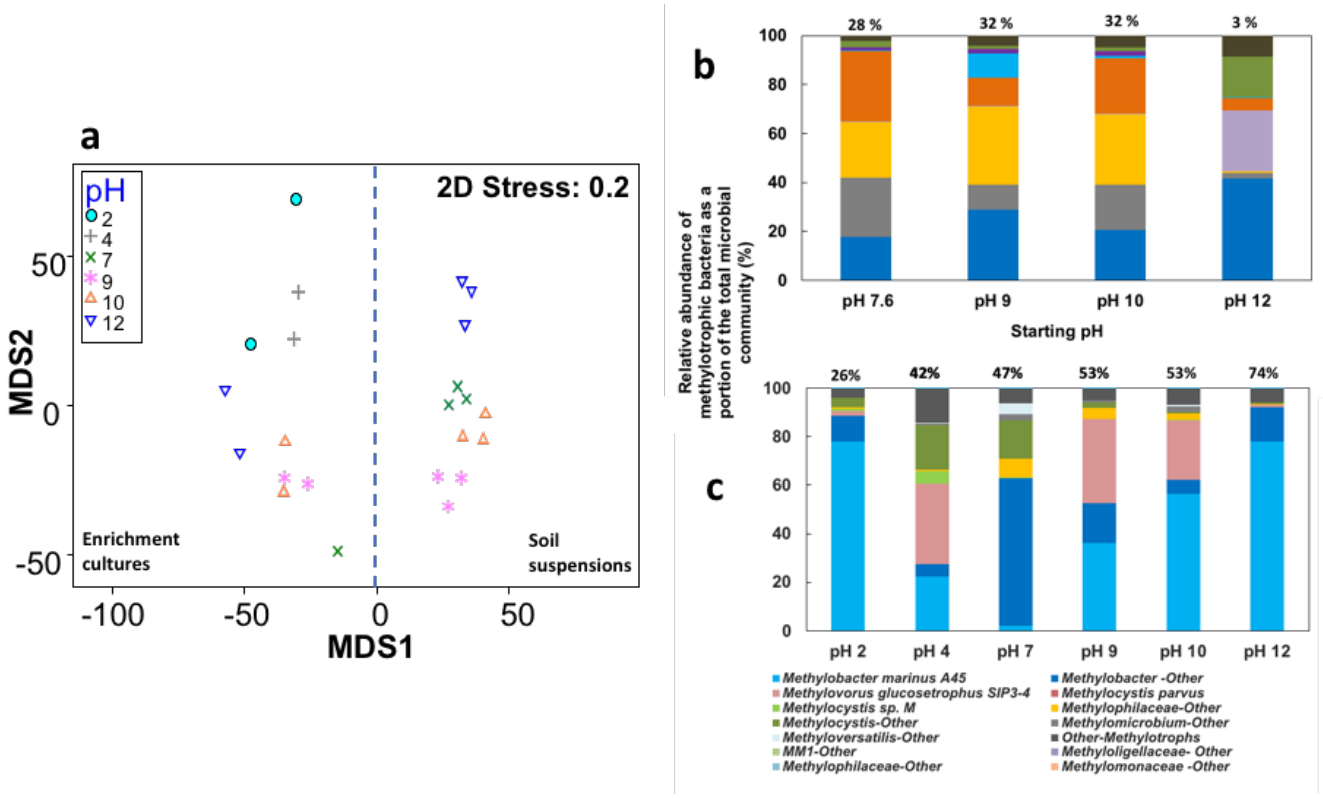

Figure 5-4. Microbial community structure in soil and enrichment culture microcosms as assessed by DNA-based 16S rRNA gene amplicon sequence analysis. (a) Metric multi-dimensional scaling plot of total microbial community structure by pH. (b) Bar chart of the average methylotrophic bacteria communities in soil suspension microcosms across a range of incubation pH values. The relative abundance of methylotrophic bacteria as a portion of the total microbial community is shown above each condition. (c) Bar chart of the average methylotrophic bacteria in enrichment culture microcosms across a range of incubation pH values. The relative abundance of methylotrophic bacteria as a portion of the total microbial community is shown above each condition

5.4 DISCUSSION

This study evaluated the effect of pH on CH_4 oxidation potential and microbial community structure in landfill cover soil and in methanotrophic enrichment cultures. The highest CH_4 oxidation rates were observed at circumneutral starting pH values of 6.7-7.1 (enrichment culture reactors) and 7.4-7.6 (soil-suspension reactors), consistent with prior studies on landfill covers (Scheutz and Kjeldsen, 2004, Han et al. 2016, Wang et al. 2011). This finding is also consistent with the *in-situ* pH of the landfill cover soil (pH 7.6), and the long-term stability of the soil pH could limit the available microbial diversity. The highest CH_4 oxidation rates by pure cultures of methanotrophs have been shown at pH values of 6.6-6.8 (Whittenbury et al. 1970, Hanson and Hanson 1996). For environmental methanotrophic communities, the highest rates of CH_4 oxidation have been shown to be at pH values between 5.5-8.5 in soils and sediments from a variety of different ecosystems (Dunfield 1993, Hutsch 1994; Scheutz and Kjeldsen 2004; Sherry et al. 2016; Han et al. 2016). Our results show a slight, but not significant, decrease in CH_4 oxidation rates with increasing pH in enrichment cultures with starting pH values between 7 and 10. At starting pH values of 9 and 10, the small decrease in the oxidation rates could be a result of the initial alkaline pH. By day 6, despite addition of NaOH, the pH was measured to be 9.3 and 8.3, and corresponding decreases in CH_4 headspace concentrations were also observed. Thereafter, the pH decreased to 7.7-7.8, with continuing oxidation of CH_4. This drop in the pH could be a result of bacterial growth releasing metabolites, acids and/or production of CO_2 during CH_4 oxidation process. Similar process was observed in soil-suspension experiments, where the pH dropped to neutral pH by the end of the experiment, likely due to the metabolic activity of methanotrophic and heterotrophic bacteria in the reactors. With decreasing pH values, rates of methane oxidation increased. At an extreme alkaline starting pH 12, methanotrophic and heterotrophic activity were

restricted, and pH values did not decrease substantially, with the exception of a drop in pH after 4 days in the enrichment culture reactors. After pH adjustment, no further significant change in pH levels were observed. This may be in part due to buffering by the carbonate system (pKa of 6.4 and 10.3). Since the targeted pH of 12 was maintained throughout the incubation in both enrichments and soil-suspension, no CH_4 oxidation was observed, confirming inhibition of activity of MOBs at such pH values. Currently, no studies have shown the oxidation of CH_4 at an alkaline pH >12, although alkaliphilic methanotrophs have been isolated from extreme alkaline lakes and marine environments, and these organisms can grow at pHs of 9-11 in the presence of NaCl (Kalyuzhnaya et al. 2001, Sorokin et al. 2000, Khmelenina et al. 1997). These halophilic or alkaliphilic methanotrophs are genotypically and phenotypically different from the habitants in freshwater and has been identified as new species within the genera *Methylobacter* and *Methylomicrobium* (Khmelenina et al. 2009). Their specific biochemical properties such as synthesis of osmoprotectants, formation of glycoprotein S-layers on the outer surface of the cell walls and the ability to modify chemical composition of cell membranes help them to adapt to highly alkaline habitats (Trotsenko and Khmelenina, 2002).

At acidic starting pH of 2 and 4, pH varied between 1.9-2.3 and 4.2-5.1 in enrichment cultures. Marginal oxidation of CH_4 occurred in enrichment culture reactors with starting pH 4 in contrast to soil-suspension reactors that showed no oxidation at starting pH 4. This could be due to differing microbial communities in the soil suspension reactors and enrichment culture reactors. Methanotrophs capable of oxidizing CH_4 in acidic soils (pH 4-6) have been discovered previously (Wise et al.1999; Cebron et al. 2007; Kong et al. 2014; Benstead and King, 2001; Amaral et al. 1998). However, this study suggests that the landfill cover soil methanotrophic microbial

community was not adapted to lower pH conditions even though the enrichments did respond to acidic pH with marginal CH_4 consumption.

It was observed that methanotrophic microbial community structure varied by pH in soil and enrichment reactors. Bacteria most closely related to *Methylobacter marinus* A45*,* a Type I methanotroph, were identified in abundance across all pH conditions tested in enrichment cultures, with lower relative abundance at circumneutral pH (2.1%). *Methylobacter marinus* A45 are aerobic methanotrophs, typically found in coastal and hydrothermal vent marine ecosystems (Flynn et al. 2016) and are halophilic or alkali-tolerant methanotrophic bacteria that require NaCl or Na ions for growth (Bowman et al. 1993; Kalyuzhnaya et al. 2008). Not all species of *Methylobacter* require NaCl for growth, however (Bowman et al. 1993), and most species are considered to be non-halophilic (Osudar et. al 2017). Our studies detected this species at both acidic and alkaline pHs, but the DNA-based data can be difficult to interpret with regards to activity or CH_4 oxidation at these pHs.

In reactors with starting pH values of 7, 9, and 10, bacteria from the family Methylophilaceae (soil suspension), Methylomonaceae (soil suspension) and genera *Methylobacter* (enrichment culture) and *Methylomicrobium* (soil suspension) were abundant. Prior studies have identified the presence these taxa in landfill cover soil (Gebert et al. 2009; Su et al. 2014; Wise et al. 1999; Chi et al. 2015; Xing et al. 2017). Bacteria from the genus *Methylobacter* have been shown to grow at pH ranging from 5-9 with an optimal growth at 6.5-7 (Bowman 1993), consistent with this study.

Methanotrophs from the genus *Methylocystis,* Type II methanotrophs, were identified in samples from enrichment culture reactors with starting pH values 2, 4 and 7. These microbes were also identified in multiple studies of acidic (pH 4.8 & 6.2) and neutral (pH 7.6) landfill cover soils

(Wise et al. (1999), Cebron et al. (2007), Su et al. (2014), and Kong et al. (2014)). The relative abundance of bacteria from the genus *Methylocystis* in soil-suspensions was low at pH values of 7.6 and above, possibly due to competition with Type I methanotrophs. Type I methanotrophs have been shown to outcompete Type II methanotrophs at higher O_2 and lower CH_4 concentrations (Amaral and Knowles 1995; Henckel et al. 2000). Our incubation conditions have likely limited the growth of Type II methanotrophs in this study. Bacteria from the genus *Methylocystis* have been shown to grow within a pH range from 4.5-9 (Bowman et al.1993). They possess diverse systems of membrane transporters that ensures pH homeostasis (Nguyen et al. 2018).

Bacteria from the genus *Methylovorus* were also identified in enrichment cultures at starting pH values of 4, 9 and 10. *Methylovorus glucosetrophus SIP3-4,* of the family *Methylophilaceae*, are obligate methylotrophs that utilize C1 compounds as a source of carbon and energy for growth (Lapidus et al. 2011). This organism was first isolated from sediments of Lake Washington, growing at a pH 4.2-8 (optimum at 6.5) and temperature 9-37°C (Kalyuzhnaya et al. 2012). These organisms may have obtained their carbon from methanol produced during oxidation of CH_4 by the enzyme MMO (Cebron et al. 2007). Kallistova et al. (2005) and Han et al. (2016) detected *Methylovorus glucosetrophus* in the landfill cover soil (pH 6-8) and were successful in cultivating them in laboratory, suggesting that their occurrence in landfill cover soils is not unusual and is consistent with the community composition found in the current study.

5.5 CONCLUSIONS

In the present study, enrichment cultures and soil suspensions derived from landfill cover were studied to evaluate the effect of pH on CH_4 oxidation and microbial community structure. The results demonstrate that the CH_4 oxidation was highest in reactors with pH 7-7.6, negligible at pH

2 (enrichment culture) and completely inhibited at pH 12 (enrichment culture and soil-suspension). Analysis of microbial community structure in the enrichment culture reactors demonstrated shifts in the microbial communities with Type I, Type II methanotrophs and methylotrophs identified in reactors with starting pH values of 4, 7, 9 and 10. However, in soil-suspension no strong shift in methylotrophic community was observed at starting pH 7.6, 9, and 10 but were significantly different from each other and were generally dominated by Type I methanotrophs and methylotrophs. Overall, this study shows CH_4 oxidation at pH range of 4-10 in enrichment culture, 7.6–10 in soil-suspension and no oxidation at an extreme alkaline pH 12 (enrichment culture and soil-suspension) in the landfill cover soil. This study indicates that the high pH of slag (pH >12) could inhibit methanotrophic activities in soil if the slag comes in direct contact of the soil. However, the study also shows that the microbes can adapt to a wide range of pH conditions (pH 4-10) suggesting feasibility of the layered system of slag and soil in biogeochemical cover. Further study is underway which analyzes the microbial activity in various profiles of biogeochemical cover system under dynamic environmental conditions.

5.6 CITED REFERENCES

Amaral, J. A., Ren, T., and Knowles, R. (1998). "Atmospheric methane consumption by forest soils and extracted bacteria at different pH values". *Applied and Environmental Microbiology*, 64(7), 2397-2402.

Baesman, S. M., Miller, L. G., Wei, J. H., Cho, Y., Matys, E. D., Summons, R. E., and Oremland, R. S. (2015). "Methane oxidation and molecular characterization of methanotrophs from a former mercury mine impoundment". *Microorganisms*, 3(2), 290-309.

Bender, M., and Conrad, R. (1995). Effect of CH_4 concentrations and soil conditions on the induction of CH4 oxidation activity. *Soil Biology and Biochemistry*, 27(12), 1517-1527.

Benstead, J., and King, G. M. (2001). "The effect of soil acidification on atmospheric methane uptake by a Maine forest soil". *FEMS Microbiology Ecology*, 34(3), 207-212.

Belova, S. E., Kulichevskaya, I. S., Bodelier, P. L., and Dedysh, S. N. (2013). "Methylocystis bryophila sp. nov., a facultatively methanotrophic bacterium from acidic Sphagnum peat, and emended description of the genus Methylocystis (ex Whittenbury et al. 1970) Bowman et al. 1993". *International Journal of Systematic and Evolutionary Microbiology*, 63(3), 1096-1104.

Börjesson, G., Sundh, I., and Svensson, B. (2004). "Microbial oxidation of CH4 at different temperatures in landfill cover soils". *FEMS Microbiology Ecology*, 48(3), 305-312.

Bowman, J. P., Sly, L. I., Nichols, P. D., and Hayward, 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 and Evolutionary Microbiology*, 43(4), 735-753.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., and Huttley, G. A. (2010). "QIIME allows analysis of high-throughput community sequencing data". *Nature Methods*, 7(5), 335.

Cébron, A., Bodrossy, L., Chen, Y., Singer, A. C., Thompson, I. P., Prosser, J. I., and Murrell, J. C. (2007). "Identity of active methanotrophs in landfill cover soil as revealed by DNA-stable isotope probing". *FEMS Microbiology Ecology*, 62(1), 12-23.

Chi, Z. F., Lu, W. J., and Wang, H. T. (2015). "Spatial patterns of methane oxidation and methanotrophic diversity in landfill cover soils of Southern China". *J. Microbiol. Biotechnol*, 25(4), 423-430.

Clarke, K. R., and Gorley, R. N. (2015). "Getting started with PRIMER v7. PRIMER-E: Plymouth", Plymouth Marine Laboratory.

De Visscher, A., Boeckx, P., and Van Cleemput, O. (2007). "Artificial Methane Sinks". *Greenhouse Gas Sinks*, 184.

Dunfield, P., Dumont, R., and Moore, T. R. (1993). "Methane production and consumption in temperate and subarctic peat soils: response to temperature and pH". *Soil Biology and Biochemistry*, 25(3), 321-326.

Dunfield, P. F. (2001). "Methanotrophy in extreme environments". e LS.

Dunfield, P. F., Yuryev, A., Senin, P., Smirnova, A. V., Stott, M. B., Hou, S., and Wang, J. (2007). "Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia". Nature, 450(7171), 879.

Edgar, R. C. (2010). "Search and clustering orders of magnitude faster than BLAST". *Bioinformatics*, 26(19), 2460-2461.

Flynn, J. D., Hirayama, H., Sakai, Y., Dunfield, P. F., Klotz, M. G., Knief, C., and Murrell, J. C. (2016). "Draft genome sequences of gammaproteobacterial methanotrophs isolated from marine ecosystems". Genome Announcements, 4(1), e01629-15.

Gagliano, A. L., D'Alessandro, W., Tagliavia, M., Parello, F., and Quatrini, P. (2014). "Methanotrophic activity and diversity of methanotrophs in volcanic geothermal soils at Pantelleria (Italy)". *Biogeosciences* (BG).

Gebert, J., Singh, B. K., Pan, Y., and Bodrossy, L. (2009). "Activity and structure of methanotrophic communities in landfill cover soils". *Environmental Microbiology Reports*, 1(5), 414-423.

Glöckner FO, Yilmaz P, Quast C, Gerken J, Beccati A, Ciuprina A, Bruns G, Yarza P, Peplies J, Westram R, Ludwig W (2017). "25 years of serving the community with ribosomal RNA gene reference databases and tools". *J Biotechnol,* 261, pp.169-176

Green SJ, Venkatramanan R, Naqib A (2015). "Deconstructing the polymerase chain reaction: understanding and correcting bias associated with primer degeneracies and primer-template mismatches". *PLoS ONE* 10(5), e0128122.

Han, B., Chen, Y., Abell, G., Jiang, H., Bodrossy, L., Zhao, J., and Xing, X. H. (2009). "Diversity and activity of methanotrophs in alkaline soil from a Chinese coal mine". *FEMS Microbiology Ecology*, 70(2), 196-207.

Han, J. S., Mahanty, B., Yoon, S. U., and Kim, C. G. (2016). "Activity of a Methanotrophic Consortium Isolated from Landfill Cover Soil: Response to Temperature, pH, CO2, and Porous Adsorbent". *Geomicrobiology Journal*, 33(10), 878-885.

Hanson, R. S., and Hanson, T. E. (1996). "Methanotrophic bacteria". *Microbiological Reviews*, 60(2), 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". *Environmental Microbiology*, 2(6), 666-679.

Hou, S., Makarova, K. S., Saw, J. H., Senin, P., Ly, B. V., Zhou, Z., and Wolf, Y. I. (2008). "Complete genome sequence of the extremely acidophilic methanotroph isolate V4,

Methylacidiphilum infernorum, a representative of the bacterial phylum Verrucomicrobia". *Biology Direct*, 3(1), 26.

Hütsch, B. W., Webster, C. P., and Powlson, D. S. (1994). "Methane oxidation in soil as affected by land use, soil pH and N fertilization". *Soil Biology and Biochemistry*, 26(12), 1613-1622.

Kallistova, A. Y., Kevbrina, M. V., Nekrasova, V. K., Glagolev, M. V., Serebryanaya, M. I., and Nozhevnikova, A. N. (2005). "Methane oxidation in landfill cover soil". *Microbiology*, 74(5), 608-614.

Kaluzhnaya, M., Khmelenina, V., Eshinimaev, B., Suzina, N., Nikitin, D., Solonin, A., and Trotsenko, Y. (2001). "Taxonomic characterization of new alkaliphilic and alkalitolerant methanotrophs from soda lakes of the Southeastern Transbaikal region and description of Methylomicrobium buryatense sp. Nov". *Systematic and Applied Microbiology*, 24(2), 166-176.

Kalyuzhnaya, M. G., Khmelenina, V., Eshinimaev, B., Sorokin, D., Fuse, H., Lidstrom, M., and Trotsenko, Y. (2008). "Classification of halo (alkali)philic and halo (alkali) tolerant methanotrophs provisionally assigned to the genera Methylomicrobium and Methylobacter and emended description of the genus Methylomicrobium". *International Journal of Systematic and Evolutionary Microbiology*, 58(3), 591-596.

Kalyuzhnaya, M. G., Beck, D. A., Vorobev, A., Smalley, N., Kunkel, D. D., Lidstrom, M. E., and Chistoserdova, L. (2012). "Novel methylotrophic isolates from lake sediment, description of Methylotenera versatilis sp. nov. and emended description of the genus Methylotenera". *International Journal of Systematic and Evolutionary Microbiology*, 62(1), 106-111.

Khmelenina VN, Kalyuzhnaya MG, Starostina NG, Suzina NE & Trotsenko YA (1997). "Isolation and characterization of halotolerant alkaliphilic methanotrophic bacteria from Tuva soda lakes". *Microbiology*, 35: 257–261.

Khmelenina, V. N., Shchukin, V. N., Reshetnikov, A. S., Mustakhimov, I. I., Suzina, N. E., Eshinimaev, B. T., and Trotsenko, Y. A. (2010). "Structural and functional features of methanotrophs from hypersaline and alkaline lakes". *Microbiology*, 79(4), 472-482.

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.

Kong, J. Y., Bai, Y., Su, Y., Yao, Y., and He, R. (2014). "Effects of trichloroethylene on community structure and activity of methanotrophs in landfill cover soils". *Soil Biology and Biochemistry*, 78, 118-127.

Kim, I.T., Ahn, K., Yoo, Y.S., and Kim, K.S. (2017). "Effect of Ammonium Chloride on the Methane-Oxidizing Bacterial Community" (not peer reviewed)

McDonald D, Clemente JC, Kuczynski J, Rideout JR, Stombaugh J, Wendel D, Wilke A, Huse S, Hufnagle J, Meyer F, Knight R (2012). "The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome". Gigascience, 1(1), p.7.

Moonsamy, P. V., Williams, T., Bonella, P., Holcomb, C. L., Höglund, B. N., Hillman, G., and Simen, B. B. (2013). "High throughput HLA genotyping using 454 sequencing and the Fluidigm Access Array™ system for simplified amplicon library preparation". *HLA*, 81(3), 141-149.

Naqib, A., Poggi, S., Wang, W., Hyde, M., Kunstman, K. and Green, S.J. (2018). "Making and sequencing heavily multiplexed, high-throughput 16S ribosomal RNA gene amplicon

libraries using a flexible, two-stage PCR protocol". *In Gene Expression Analysis* (pp. 149-169). Humana Press, New York, NY.

Nguyen, N. L., Yu, W. J., Gwak, J. H., Kim, S. J., Park, S. J., Herbold, C. W., and Rhee, S. K. (2018). "Genomic Insights into the Acid Adaptation of Novel Methanotrophs Enriched from Acidic Forest Soils". *Frontiers in Microbiology*, 9.

Osudar, R., Klings, K. W., Wagner, D., and Bussmann, I. (2017). "Effect of salinity on microbial methane oxidation in freshwater and marine environments". *Aquatic Microbial Ecology*, 80(2), 181-192.

Park, J. R., Moon, S., Ahn, Y. M., Kim, J. Y., and Nam, K. (2005). "Determination of environmental factors influencing methane oxidation in a sandy landfill cover soil". *Environmental Technology*, 26(1), 93-102.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Rese 41(D1), pp. D590-D596.

Rai, R. K., and Reddy, K. R. (2019). "Methanotrophic methane oxidation in new biogeochemical landfill cover system." *Proc. 34th International Conference on Solid Waste Technology and Management*, Annapolis, MD, USA, March 31st – April 3rd, 2019.

Saari, A., Rinnan, R., and Martikainen, P. J. (2004). "Methane oxidation in boreal forest soils: kinetics and sensitivity to pH and ammonium". *Soil Biology and Biochemistry*, 36(7), 1037-1046.

Sadasivam, B. Y., and Reddy, K. R. (2014). "Landfill methane oxidation in soil and bio-based cover systems: a review". *Reviews in Environmental Science and Bio/Technology*, 13(1), 79-107.

Scheutz, C., and Kjeldsen, P. (2004). "Environmental factors influencing attenuation of methane and hydrochlorofluorocarbons in landfill cover soils". *Journal of Environmental Quality*, 33(1), 72-79.

Scheutz, C., Kjeldsen, P., Bogner, J. E., De Visscher, A., Gebert, J., Hilger, H. A., and Spokas, K. (2009). "Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions". *Waste Management & Research*, 27(5), 409-455.

Semrau, J. D., DiSpirito, A. A., and Yoon, S. (2010). "Methanotrophs and copper". *FEMS Microbiology Reviews*, 34(4), 496-531.

Sherry, A., Osborne, K. A., Sidgwick, F. R., Gray, N. D., and Talbot, H. M. (2016). "A temperate river estuary is a sink for methanotrophs adapted to extremes of pH, temperature and salinity". *Environmental Microbiology Reports*, 8(1), 122-131.

Sorokin, D. Y., Jones, B. E., and Kuenen, J. G. (2000). "An obligate methylotrophic, methane-oxidizing Methylomicrobium species from a highly alkaline environment". *Extremophiles,* 4(3), 145-155.

Su, Y., Zhang, X., Xia, F. F., Zhang, Q. Q., Kong, J. Y., Wang, J., and He, R. (2014). "Diversity and activity of methanotrophs in landfill cover soils with and without landfill gas recovery systems". *Systematic and Applied Microbiology*, 37(3), 200-207.

Trotsenko, Y. A., and Khmelenina, V. N. (2002). "The biology and osmoadaptation of haloalkaliphilic methanotrophs". *Microbiology*, 71(2), 123-132.

Tikhonov M, Leach RW, Wingreen NS (2015). "Interpreting 16S metagenomic data without clustering to achieve sub-OTU resolution". *ISME J* 9(1), p.68.

United States Environmental Protection Agency (USEPA) (2019) Landfill methane outreach program: Basic information about landfill gas. Available at:

https://www.epa.gov/lmop/basic-information-about-landfill-gas (accessed on 19th April, 2019)

Urmann, K., Lazzaro, A., Gandolfi, I., Schroth, M. H., and Zeyer, J. (2009). "Response of methanotrophic activity and community structure to temperature changes in a diffusive CH4/O2 counter gradient in an unsaturated porous medium". *FEMS Microbiology Ecology*, 69(2), 202-212.

Wang, J., Xia, F. F., Bai, Y., Fang, C. R., Shen, D. S., and He, R. (2011). "Methane oxidation in landfill waste biocover soil: kinetics and sensitivity to ambient conditions". *Waste Management,* 31(5), 864-870.

Wang, X., Cao, A., Zhao, G., Zhou, C., and Xu, R. (2017). "Microbial community structure and diversity in a municipal solid waste landfill". *Waste Management*, 66, 79-87.

Whittenbury, R., Phillips, K. C., and Wilkinson, J. F. (1970). "Enrichment, isolation and some properties of methane-utilizing bacteria". *Microbiology*, 61(2), 205-218.

Wise, M. G., McArthur, J. V., and Shimkets, L. J. (1999). "Methanotroph diversity in landfill soil: isolation of novel type I and type II methanotrophs whose presence was suggested by culture-independent 16S ribosomal DNA analysis". *Applied and Environmental Microbiology*, 65(11), 4887-4897.

Xing, Z. L., Zhao, T. T., Gao, Y. H., Yang, X., Liu, S., and Peng, X. Y. (2017). "Methane oxidation in a landfill cover soil reactor: Changing of kinetic parameters and microorganism community structure". *Journal of Environmental Science and Health*, Part A, 52(3), 254-264.

Yargicoglu, E. N., and Reddy, K. R. (2017, a). “Microbial abundance and activity in biochar-amended landfill cover soils: evidence from large-scale column and field experiments”. *Journal of Environmental Engineering*, 143(9), 04017058.

Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. (2013). “PEAR: a fast and accurate Illumina Paired-End read merger”. *Bioinformatics*, 30(5), 614-620

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Table 5-1. Effect of pH on CH_4 oxidation and community composition in different ecosystem

pH Range	Optimal/ Maximum pH	CH_4 concentration	Ecosystem	Molecular Biomarker	Genus/ Species/ Type of Methanotrophs	References
4 - 9	4 - 6.5 (Soil)	5%	Forest soil	-	-	Amaral et al. 1998
	5 - 8 (Culture)	20%		-	-	
3.5 - 8	-	0.1%	Peat soil	-	-	Dunfield et al. 1993
4.3 - 8	-	<1%	Upland soils	*pmoA* PLFA	Type I Methanotrophs (*Methylocaldum*) Type II Methanotrophs (*Methylocystis, Methylosinus*)	Knief et al. 2003
3 - 10	6.5 – 7.5	15%	Landfill cover	-	-	Scheutz and Kjeldsen, 2004
9.4	-	-	Coal mine	16S rRNA *pmoA* microarray RFLP DGGE	Type I Methanotrophs (*Methylobacter, Methylosoma, Methylococcus*) Type II Methanotrophs (*Methylocystis, Methylosinus*)	Han et al. 2009
4.7 - 8.1	-	5%	Landfill cover	*pmoA* TRFLP	Type I Methanotrophs (pH 6.8 – 8.1) Type II Methanotrophs (pH 4.7 – 5.35)	Su et al. 2014
4 - 9	6 – 8	5%	River estuary	*pmoA*	*Methylomonas, Methylosoma* (pH 4 - 5) *Methylomicrobium* (pH 9) *Methylobacter* (pH 6 – 9)	Sherry et al. 2016
3 - 11	4.5	1%	Mercury mine impoundment	*pmoA* 16S rRNA	Type I Methanotrophs and Type II Methanotrophs (pH 4.5)	Baesman et al. 2015
5 - 10	7	20%	Landfill cover	-	-	Han et al. 2016

CHAPTER 6 – EFFECT OF TEMPERATURE ON METHANE OXIDATION AND COMMUNITY COMPOSITION IN LANDFILL COVER SOIL

6.1 INTRODUCTION

MSW landfills are the third largest source of anthropogenic CH_4 emissions in the United States [46]. Landfill gas (LFG) is typically comprised of 50% CH_4 and 50% CO_2, both of which are potent greenhouse gases (GHG) contributing to global climate change. Mitigation of landfill CH_4 emissions has received a great deal of attention, and many researchers have evaluated CH_4 emissions mitigation measures, including the potential of MOB present in the landfill cover soil to convert CH_4 into CO_2 [4, 51]. MOBs, also known as methanotrophs, are a subset of a larger microbial community called methylotrophs. Methanotrophs utilize CH_4 as a sole source of carbon and energy, whereas methylotrophs use C1-compounds as their source of carbon and energy [23, 40]. These methanotrophs are widespread in nature and are usually found in abundance in environments across a broad range of of CH_4 concentrations. These organisms oxidize CH_4 using the enzyme methane monooxygenase (MMO) [23, 40]

The microbial CH_4 oxidation process is controlled by various environmental factors such as moisture content, pH, temperature, soil particle size, and nutrients [1,2,38]. As with all enzymatic processes, temperature is a critical factor regulating oxidative activity of MOB [39, 48]. Methanotrophs are generally mesophilic, growing at moderate temperatures of 25 - 35°C [38], and many laboratory incubation studies have reported maximum CH_4 oxidation rates at temperatures of 20 - 31°C in landfill cover soil [4, 35, 39, 48, 51], peat soil [16], wetland and forest soil [34], and boreal soil [52]. Whalen et al. (1990) studied temperature effects and CH_4 oxidation potential of landfill cover soil by exposing soil cores to temperatures ranging between 4°C and 46°C and

moisture contents ranging from 5% to 71%. According to their study, the optimum temperature and moisture content for CH_4 oxidation were 31°C and 11%, respectively. With a fixed moisture content of 11%, increasing CH_4 oxidation rates were observed with increasing temperature from 4 to 36°C, but a decline in oxidation rate was observed at elevated temperatures (> 46°C). CH_4 oxidation rates also dropped significantly when the moisture content was < 5% or > 11% [51]. Conversely, Boeckx et al. (1996) showed moisture content to be the dominant factor in controlling CH_4 oxidation rates when compared to temperature with an optimum moisture content of 15.6 - 18.8% and optimum temperature 20 - 30°C. Their study showed a decrease in optimum temperature with increases in moisture content and deduced that temperature had minimal effects on CH_4 oxidation [4]. Scheutz and Kjeldsen (2004) also studied the effect of various factors on CH_4 oxidation in the landfill cover soil. Their studies concluded that moisture content, temperature and gas concentration were the most important factors affecting CH_4 oxidation, with an optimum moisture content of 25% facilitating gas transport for microbial activity and an optimal temperature of 30°C [39].

While rates of microbial CH_4 oxidation are influenced by various environmental factors, many studies have also shown the effect of these factors on change in methanotrophic community structure and diversity. Soil texture, pH, gas concentration and moisture content are important factors controlling community structure in landfills [42, 45], but relatively few studies have also shown the effect of temperature on the microbial diversity using molecular techniques in various ecosystems [6, 25-26, 32] (**Table 6-1**). A study by Börjesson et al. (2004) showed temperature to be an important factor affecting community composition in the landfill cover soil using Phospholipid Fatty Acids (PLFAs) as biomarkers. Their studies determined growth of Type-I methanotrophs at lower temperatures (5 - 10°C) and Type-II methanotrophs at higher temperatures

(20°C) [6], and this finding was supported by other studies [26, 47]. In contrast, Mohanty et al. (2007) reported differences in the relative abundance of methanotrophs in two different soils (Rice field and Forest soil), by assessing Terminal Restriction Fragment Length Polymorphism (T-RFLP) of particulate methane monooxygenase genes (*pmoA)*. The study by Mohanty et al. (2007) described temperature dependence and existence of both Type-I and Type-II methanotrophs in low and high temperatures studied (5 - 45°C) in soils [32].

Few studies have examined the effects of temperature on CH_4 oxidation and microbial diversity together in landfill cover soil. Previously the use of biochar-amended soil with steel slag (biogeochemical cover) was examined to mitigate CH_4 and CO_2 emissions from the MSW landfills [37]. Here, this study sought to systematically examine the effect of temperature on CH_4 oxidation potential, microbial activity, and microbial community structure. Such studies are needed to evaluate the overall performance of biogeochemical cover under dynamic meteorological conditions. As a part of this study, batch scale experiments were performed with soil microcosms and methanotrophic enrichment cultures derived from a landfill cover soil, under a temperature gradient consistent with temperature ranges observed at landfill sites.

6.2 MATERIALS AND METHODOLOGY

6.2.1 Soil enrichment

Soil was collected from the Zion landfill site, located in Greater Chicago area, Illinois, USA. Soil samples were collected from an interim cover at a depth of ~1 to 2 feet and were shipped to the Geotechnical and Geoenvironmental Engineering Laboratory at the University of Illinois at Chicago (UIC), where they were stored at room temperature (23 ± 2°C). Soil samples were air dried (moisture content < 0.5%), pulverized and screened through a 2 mm sieve prior to conducting

experiments. To obtain methanotrophic-enriched consortia, approximately 5 g of sieved soil was mixed with 100 mL of modified NMS medium [53] in a 500 mL-serum vial and stoppered using long sleeved rubber septa. Approximately 80 mL of air from the headspace was replaced with equal volume of mix gas CH_4 /CO_2 to achieve a headspace concentration of 7% CH_4 (v/v) and 7% CO_2 (v/v) balanced in air (86%) and was incubated for 15 days at 23°C. To determine the activity of methanotrophs and CH_4 oxidation rates, gas samples were analyzed on a regular basis using Gas Chromatography (GC) until the headspace concentration dropped to less than 1%. To enrich the methanotrophic culture, the mix gas (CH_4/CO_2) was replenished twice.

6.2.2 Soil microcosm batch tests

For batch tests, 10 g of sieved soil was placed in 125 mL-serum vials and adjusted to a moisture content of 20% (w/w) using deionized water. The moisture content of 20% (w/w) was selected based on literature reporting an optimal moisture content of 10 - 25% for CH_4 oxidation [1, 38]. The vials were sealed using butyl rubber septa and secured by a crimp cap. 20 mL of air from the headspace of each vial was replaced with equal volume of synthetic LFG comprising of 50% (v/v) CH_4 and 50% (v/v) CO_2 to achieve a headspace concentration of ~5 - 6% (v/v) CH_4 and ~5 - 6% (v/v) CO_2 balanced in air (~88 - 90%). The soil microcosms were then incubated at different temperatures (30°C, 40°C, 50°C and 70°C) in Heratherm incubators (Thermo Fisher Scientific) except samples at 6°C (refrigerator) and 23°C (lab room temperature). The change in the headspace concentration of CH_4 and CO_2 was determined by collecting and analyzing the gas samples at regular intervals using GC until the headspace concentration dropped to less than 1%. All experiments were conducted with replicates, together with the controls (LFG gas without biological material). CH_4 oxidation rates were determined from the linear slope of CH_4 versus time

following zero-order kinetics. At the end of each temperature experiment (6°C – 70°C), soil samples were frozen immediately at -20°C prior to nucleic acid extraction and molecular analysis.

6.2.3 Enrichment culture batch tests

Prior to experiments with enrichment cultures, serum vials, rubber septa and pipettes were sterilized using a Napco Model 8000-DSE autoclave operated at >120°C for a minimum of 60 minutes to ensure complete sterilization. Soil from the field site was incubated at room temperature for 15 days in the presence of the modified NMS medium and the artificial landfill gas headspace as discussed above. The supernatant from this incubation was then used as an enrichment culture to seed the enrichment culture batch microcosms. Specifically, for each microcosms, 5 mL of the enrichment supernatant was combined with 5 mL of modified NMS medium ($MgSO_4$: 0.2g; $CaCl_2$: 0.02g; KNO_3: 1g; KH_2PO_4: 0.7g; $Na_2.HPO_4.5H_2O$:1.5g; Trace element solution: 1mL; 1L distilled water).10 mL of this mixture was placed in 125 mL-serum vials and sealed air tight using butyl rubber septa followed by crimp cap. Similar procedure as soil microcosm test was followed in the culture tests to achieve the headspace concentration of ~5 - 6% (v/v) CH_4 and ~5 - 6% (v/v) CO_2 balanced in air (~88 - 90%). The enrichment cultures (pH 6.7 – 7.1) were then incubated at different temperatures (6°C, 23°C, 30°C, 40°C, 50°C and 70°C) as described earlier. All the experiments were conducted in replicates, along with controls (media - NMS). At the end of each temperature experiment (6°C – 70°C), the cells were pelletized in 2 mL micro-centrifuge tubes by centrifuging at 12500 RPM for 15 min, decanting the supernatant and freezing at -20°C for DNA extraction and molecular analysis.

6.2.4 Microbial community structure analysis

Genomic DNA (gDNA) was extracted from soil samples and from cell pellets using a DNeasy PowerSoil Kit (Qiagen) based on manufacturer's instructions with a slight modification. Samples were heated at 65°C for 10 min before homogenizing with FastPrep-24 5G bead-beating device (MP Biomedicals) at 6 m/s for 40 sec. After homogenization, extraction protocols were automated on a QIAcube instrument (Qiagen), according to the manufacturer's instructions. Genomic DNA was processed for microbial community analysis using 16S rRNA gene amplicon sequencing, and for some samples using shotgun metagenome sequencing (SMS). 16S rRNA gene amplicon sequencing was performed using a two-stage amplification protocol as described previously [22, 33]. The primer set 515F-806R was used to amplify the V4 variable region of the microbial 16S rRNA gene, and sequencing was performed on an Illumina MiniSeq instrument, employing paired-end 2x153 base reads. Raw sequence data were initially processed by merging forward and reverse reads using the software package PEAR [56]. Merged reads were trimmed to remove ambiguous nucleotides, primer sequences, and trimmed based on quality scores. Chimeric sequences were identified and removed using the USEARCH algorithm with a comparison to Silva 132 reference sequence database [17, 21, 36]. The standard QIIME pipeline [9] was modified to generate taxonomic summaries using sub-OTU resolution of the sequence dataset [43]. Taxonomic annotations were assigned using the USEARCH algorithm with the Silva 132 reference database [17], and data were output as biological observation matrices (BIOMs; [30]) at multiple taxonomic levels. Generation of BIOMs was performed by the Research Informatics Core (RIC) at UIC.

Selected samples were processed for shotgun metagenome sequencing using a Nextera XT library preparation kit (Illumina, San Diego, CA) according to the manufacturer's instructions. Libraries were pooled and sequenced on a NextSeq500 instrument, employing a 300 cycle (2x150

paired end reads) mid-output kit. Sequence data were analyzed using the online annotation server from OneCodex, as described previously [31]. DNA extraction, library preparation and sequencing were performed at the University of Illinois at Chicago Sequencing Core (UICSQC).

6.2.5 Data archive

Raw sequence data files were submitted in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) and are available under the BioProject identifier PRJNA540303.

6.2.6 Gas analysis

Gas samples were analyzed at regular time intervals for CH_4, CO_2 and O_2 concentrations using an SRI 9300 Gas Chromatograph (GC) (SRI Instruments, Torrance, California) equipped with a thermal conductivity detector (TCD) and CTR-1 column that separates N_2 and O_2 for simultaneous analysis of CO_2, CH_4, O_2 and N_2 using helium as a carrier gas. Chromatograms were plotted and analyzed using *PeakSimple 3.29* software as previously described [55]. Gas samples were withdrawn using 1 mL syringe where 0.5 mL of the sample was discarded and remaining 0.5 mL was injected into the GC to reduce any pressure effects due to sampling. A calibration curve with minimum of three points was established using high purity standard gas mixtures (Praxair Distribution, Chicago, Illinois) ranging from 1% to 50% CH_4 and 5% to 50% CO_2.

6.2.7 Statistical analysis

Statistical analysis of batch test results was tested using one-way (ANOVA) and t-tests (equivalency of sample means) using Microsoft Excel-2018. A significance level of alpha = 0.05

was used to assess statistical significance in all tests. Microbial community sequence relative abundance data were analyzed within the software package Primer7 [14] to calculate alpha-diversity indices to generate visualizations of microbial community (i.e., multidimensional scaling (MDS) plots), and to test for significant differences in microbial community by temperature (analysis of similarity, ANOSIM).Analyses were performed across all observed taxa, as well as restricted to known methylotrophic taxa.

6.3 RESULTS AND DISCUSSION

6.3.1 Temperature effect on CH_4 oxidation in soil and enrichment cultures

Temperature is known to be one of the prime factors influencing CH_4 oxidation rates in landfill cover soil [5, 11, 39, 48]. This study evaluated the effect of temperature on CH_4 oxidation potential and the microbial community in landfill cover soil microcosms and methanotrophic enrichments. **Figures 6-1 and 6-2** show CH_4 consumption with time in enrichment cultures and in soil samples at temperatures from 6° to 70°C. A gradual decrease in CH_4 headspace concentration (40 days) was observed at 6°C in soil and enrichment cultures (**Fig. 6-1a**), indicating reduced microbial activity [19, 49] or restriction in bacterial enzymatic processes [28] . At 23°C, soil and enrichment cultures showed an initial lag phase of 24 hours followed by decline in CH_4 concentration with time (**Fig. 6-1b**). A similar trend was observed in samples incubated at 30°C (**Fig. 6-1c**), where the soil samples demonstrated significant CH_4 consumption in 72 hours and enrichments showed gradual decrease of CH_4 with time (day 5), showing rapid CH_4 oxidation at 30°C than at lower temperatures (6 and 23°C). It is known that most methanotrophs are mesophylls that grow at temperature range of 25°C - 30°C [4, 35, 39, 41, 48], and consistent with this, it was observed that the relative abundance of MOB was the highest in soil microcosms at 23-30°C.

At 40°C (**Fig. 6-1d**) and 50°C (**Fig. 6-1e**), the CH_4 consumption in soil microcosms and enrichment samples responded differently. In soil microcosms, rapid CH_4 uptake was observed at 40°C and 50°C after an initial lag phase of 24 and 72 hours, respectively. Conversely, the enrichment cultures showed longer lag phase (5 days) at 40°C followed by rapid consumption of CH_4 which could be attributed to shift in the microbial population to higher temperatures. However, this phenomenon was not observed in the enrichment cultures at 50°C across 33 days of monitoring, indicative of no CH_4 oxidation. Furthermore, at 70°C, no CH_4 consumption was observed in either soil or enrichment samples throughout the course of the experiment, likely a result of thermal denaturing of enzymes or other proteins [7] and is consistent with studies by Spokas and Bogner (2011) on landfill cover soils [41]. A combined plot showing trend in CH_4 consumption at different temperatures in soil microcosms (**Fig. 6-2 a, b**) and enrichment cultures (**Fig. 6-2 c, d**) is shown in Fig. 2 for two-time scales (0–11 days) and (0-40 days).

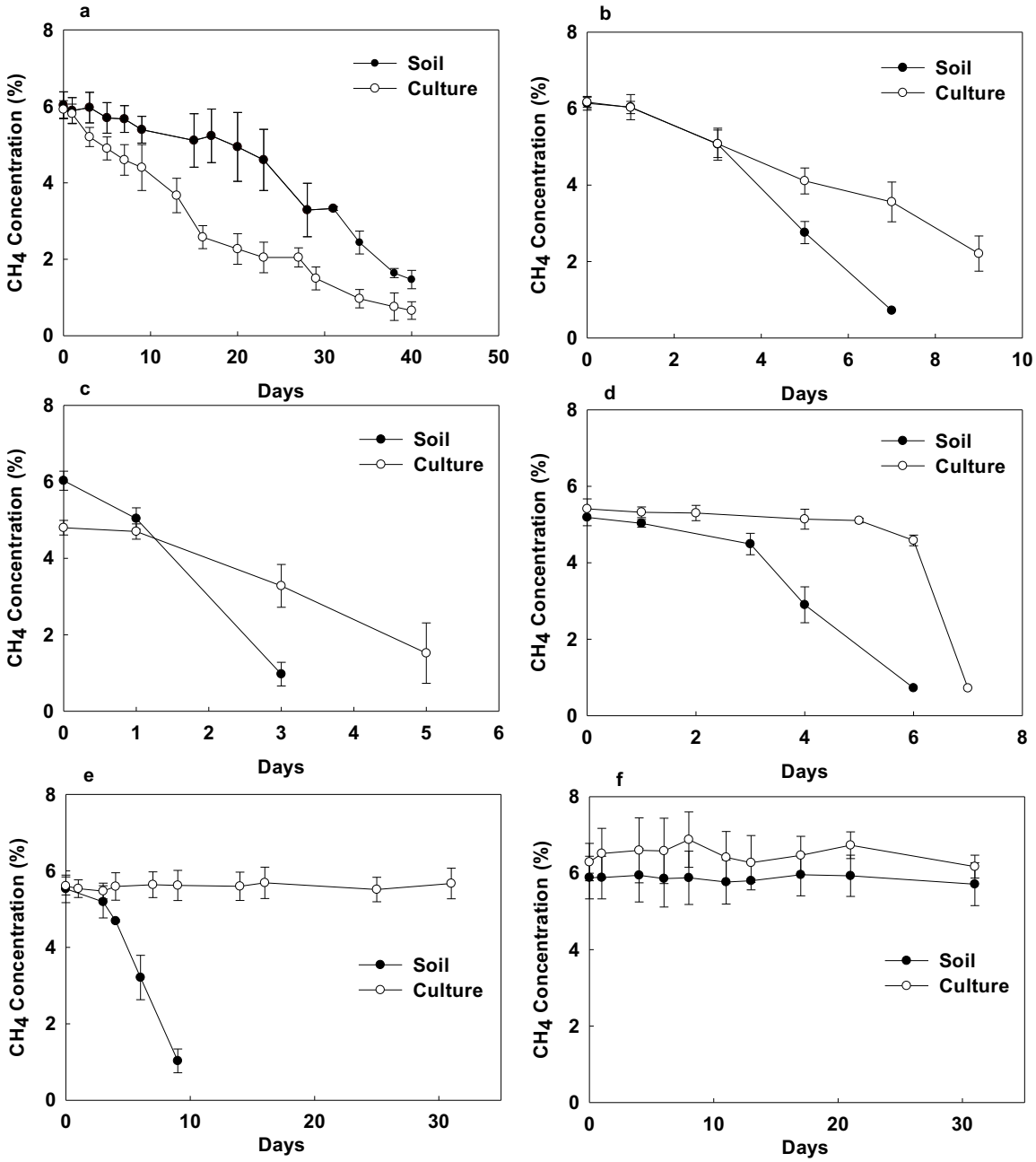


Fig. 6-1. Methane consumption with time in soil microcosms at 20% moisture content (w/w) and enrichment culture microcosms incubated at temperatures (a) 6°C; (b) 23°C; (c) 30°C;(d) 40°C; (e) 50°C; and (f) 70°C

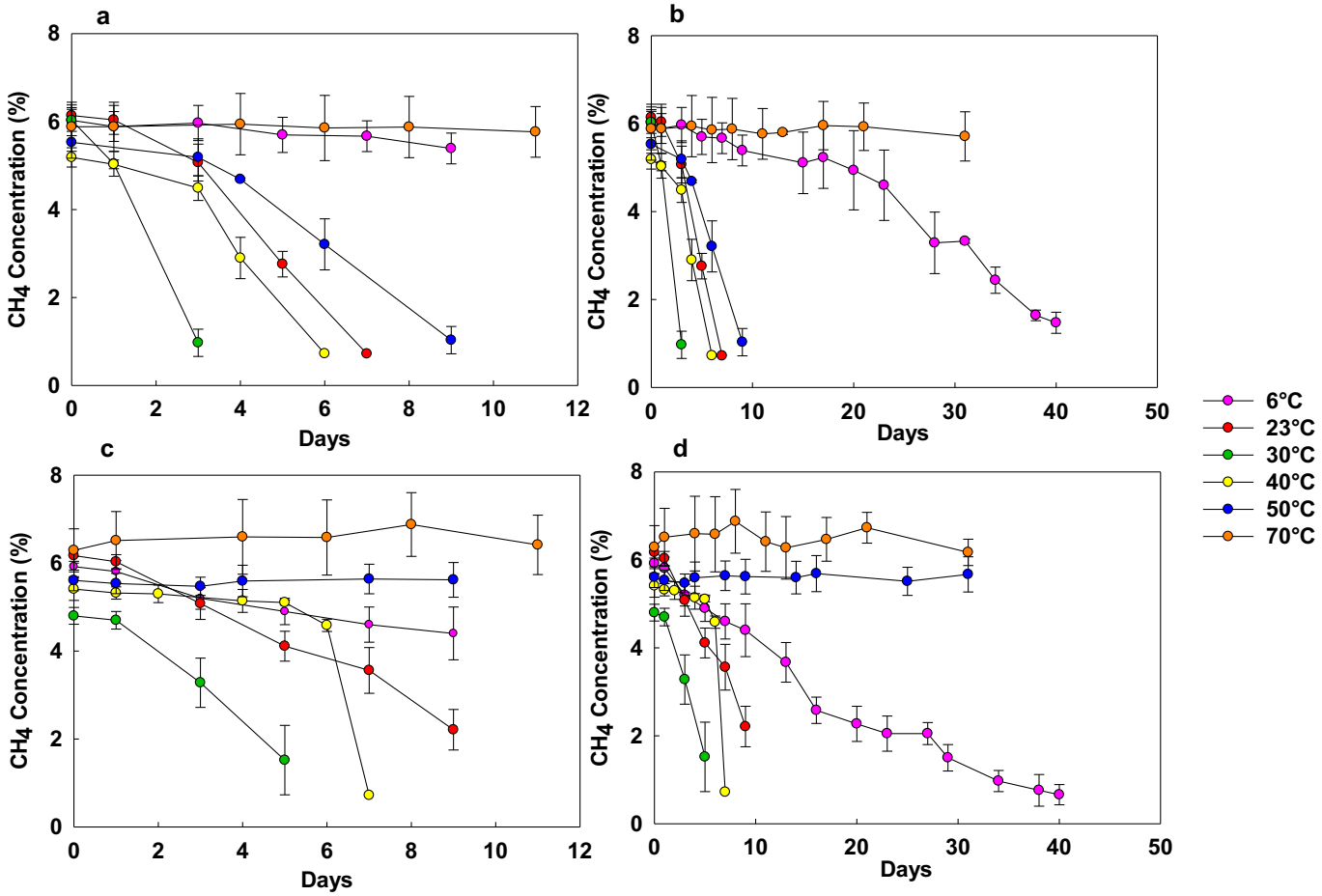


Fig. 6-2. Combined plot of methane consumption by temperature in soil microcosms (a, b) and enrichment cultures (c, d) at time scales 0-11 days (a, c) and 0-40 days (b, d)

Fig. 6-3 shows average CH_4 oxidation rates in soil and enrichment cultures incubated at temperatures ranging from 6 to 70°C, and these rates were significantly affected by temperature in soil samples (ANOVA, p= 0.0000076) and enrichment cultures (ANOVA, p=0.0044). Significantly lower CH_4 oxidation rates were observed in enrichments relative to soil microcosms at temperatures from 23°C to 50°C (Student's t- test, $p < 0.005$), and this difference is likely due to limitations of CH_4 diffusion in an aqueous phase [35, 38, 51]. These differences were not significant at 6°C ($p = 0.798$) due to low overall microbial oxidation activity. Due to slower

diffusion relative to gas-phase, MOB can become CH_4 and oxygen limited, thereby decreasing substrate utilization rates [35]. The CH_4 oxidation rates increased with increases in temperature from 6°C (13.6 µg CH_4 g^{-1} d^{-1} and 14.6 µg CH_4 mL^{-1} d^{-1}) to 30°C (195.3 µg CH_4 g^{-1} d^{-1} and 61.6 µg CH_4 mL^{-1} d^{-1}) in both soil and enrichment cultures; thereafter, rates declined at 40°C (77.5 µg CH_4 g^{-1} d^{-1} and 39 µg CH_4 mL^{-1} d^{-1}), with no oxidation at 50°C (enrichment cultures) and 70°C (soil and enrichment cultures). In soil microcosms, lower oxidation rates at higher temperature (> 40°C) may be attributed to substrate (CH_4) limitations. Prior studies on landfill cover soils have shown potential CH_4 oxidation at temperatures ranging from 2°C to 50°C [6, 13, 15, 41, 49, 51], and this is consistent with findings from this study. Little CH_4 oxidation was observed above 50°C in enrichment cultures as previously described by Kallistova et al. (2013) [27], though modest rates of oxidation were observed at this temperature in soil incubations. In this study, the maximum CH_4 oxidation rates were observed at 30°C in both soil microcosms and in enrichment cultures. However, CH_4 oxidation rates in enrichments at 23°C (47.6 µg CH_4 mL^{-1} d^{-1}) and 30°C were not significantly different from each other (p= 0.19, Student's t-test). Studies have reported a wide range of oxidation rates at 30°C in landfill cover soil ranging from as low as 73 µg CH_4 g^{-1} d^{-1} [51] to as high as 2496 µg CH_4 g^{-1} d^{-1}[39] and our data fit within this range. This difference in oxidation rates were mainly due to differences in their experimental conditions and other environmental factors influencing CH_4 oxidation. Studies have also reported moisture content to be one of the important factors affecting CH_4 oxidation along with temperature [18]. For example, an optimum moisture content of 10 - 20% (w/w) has been recommended [38] as moisture contents less than 5% causes microbial water stress and greater than 35% causes gas diffusion limitation [39].

The effect of temperature on the methanotrophic activity can also be expressed in terms of Q_{10} (temperature coefficient), which shows the change in microbial activity for every 10°C rise in

temperature. In this study, Q_{10} was calculated for CH_4 oxidation rates over the temperature range of 6-23°C and 23-30°C in both the soil and enrichment samples. In soil microcosms, Q_{10} was 2.99 and 3.27 at temperature range of 6-23°C and 23-30°C, respectively. These values (i.e., >2) indicate no substrate diffusion limitation. Conversely, in enrichment cultures, the Q_{10} values were 2 and 1.44 for the corresponding temperatures indicating limited supply of substrate for CH_4 oxidation [40], which also correlates to the lower oxidation rates observed in enrichment cultures when compared to soil microcosms. The lower Q_{10} values attained in enrichment cultures is a result of substrate diffusion in aqueous phase which is usually low when compared to diffusion in gaseous phase [38]. Low Q_{10} values were also observed in other studies [4, 16, 28, 51] and were mainly attributed to substrate limitations. Comparable results were reported by many other studies on landfill cover soil. Börjesson et al. (2004) reported Q_{10} value of 3.17-4.14 for temperatures ranging from 5-20°C [6]. Park et al. (2005) reported 2.57-2.69 for temperatures 10-30°C [35]. De Visscher et al. (2001) reported 2.8 for temperature range of 5-35°C [15]. However, higher Q_{10} values of 10.6 at temperature range 4-30°C was reported by Wang et al. (2011) [49] with Einola et al. (2008) reporting 6.5-8.4 for temperature range of 1-19°C [19].

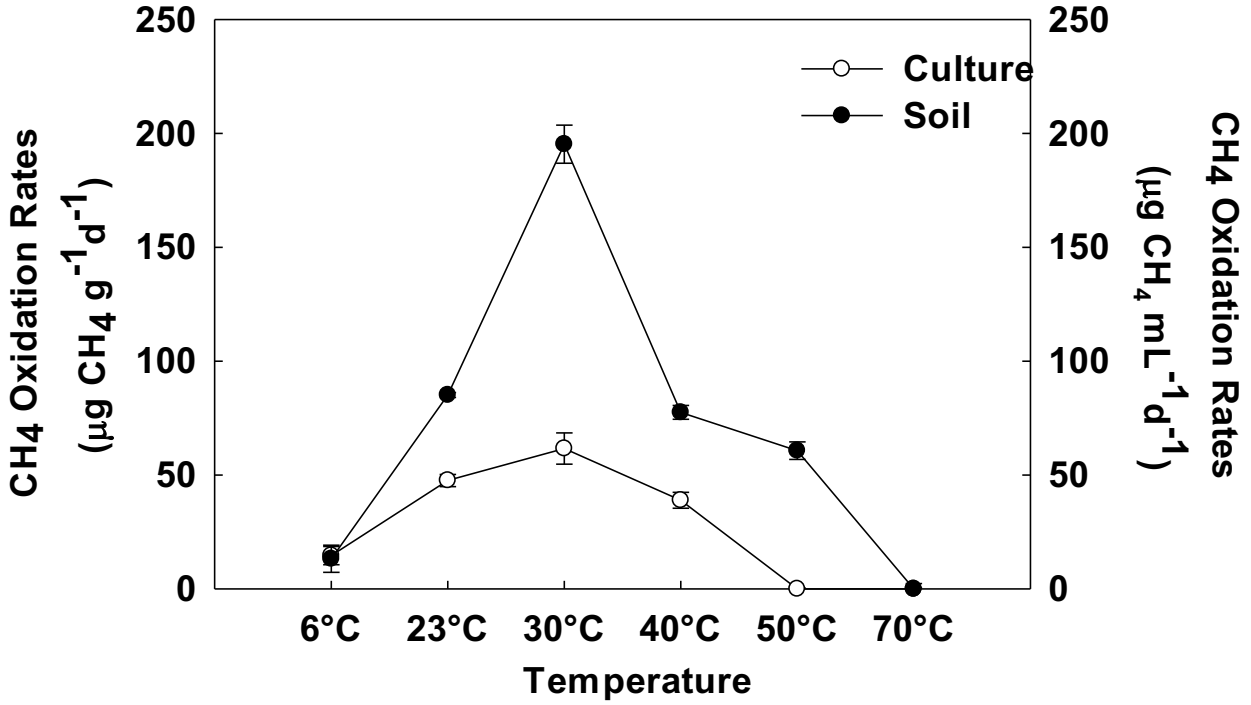


Fig. 6-3. Methane oxidation rates in soil and enrichment culture microcosms incubated at temperatures ranging from 6°C to 70°C.

6.3.2 Methylotroph microbial community structure in soil incubated across a temperature gradient

Soil microcosms were incubated in batch serum vials across a temperature gradient from 6°C to 70°C. Microbial genomic DNA was profiled using 16S rRNA gene amplicon sequencing (**Fig. 6-4**) and shotgun metagenome sequencing (**Fig. 6-5**). The two methods largely revealed similar results, with shotgun metagenome sequencing providing taxonomic resolution at the species level. In addition, a higher percentage of annotated sequence data was identified as being derived from methylotrophs in shotgun metagenome sequence data relative to 16S rRNA gene amplicon data (**Fig. 6-4b and 6-5b**), but the two methods provided broadly similar relative abundance of methylotrophs across the different temperatures. At temperatures below 40°C,

microbial communities were most similar, and were dominated by methylotrophs of the genus *Methylobacter* (**Fig. 6-4),** and of the species *Methylobacter luteus* (**Fig. 6-5b**). Sequences derived from *Methylobacter* accounted for 52%, 71% and 69% of all methylotroph 16S rRNA gene amplicon sequences at temperatures of 6°C, 23°C and 30°C, respectively, and at these temperatures, methylotroph sequences consisted of 15-27% of all 16S rRNA gene sequences, and 6-34% of all annotated shotgun metagenome sequences. Although *Methylobacter* dominated temperatures from 6-30°C, sequences from both *Methylobacter luteus* and *Methylobacter tundripaludum* were detected at 6°C, while sequences from *Methylobacter tundripaludum* were negligible at higher incubation temperatures. *Methylobacter*, a Type-I methanotroph, is one of the major abundant methanotrophs reported in studies of landfill cover soil [55, 12]. The majority of these species are mesophilic [8], though some strains within the genus are psychrophilic, with the capability to grow between 1°C-20°C [44]. In this study, although psychrophilic methanotrophic species were not identified using 16S rRNA gene amplicon sequencing, shotgun metagenome sequencing identified *Methylobacter tundripaludum* (abundant in soil microcosms) at 6°C. *Methylobacter tundripaludum* is a psychrotolerant aerobic MOB, first isolated from arctic wetland soil with CH_4 as the only source of carbon and energy, with minimal to no growth on methanol [50]. They are typically known to grow at temperatures between 5 and 23°C, with an optimal growth at 23°C. These organisms are phenotypically and genotypically different from other *Methylobacter* species [50]. Kallistova et al. (2013) also detected growth of this species in the enrichments from landfill cover soil at 10°C showing shift in the community at lower temperatures and the ability to oxidize CH_4 in winter [27]. Under the soil microcosm conditions in this study, *Methylobacter tundripaludum* was only observed at elevated relative abundance at 6°C, and this is consistent with field detection at lower temperatures in landfill cover soil. On the other hand,

Methylobacter luteus, conversely, was found at high relative abundance in soil microcosms at 23-30°C, consistent with other studies with temperatures between 20-30°C in landfill cover soil [27, 54].

The structure of soil microcosms MOB communities at 40°C and 50°C was markedly different than that of microcosms identified at temperatures from 6°C – 30°C (**Fig 6-4**). In these microcosms, bacteria from the thermophilic genus *Methylocaldum* (Type I methanotrophs) were dominant, consistent within the known growth range for the genus [3, 20]. For example, at 50°C, *Methylocaldum szegediense* constituted approximately 89% of the total methanotrophic community as determined by both 16S rRNA sequencing and shotgun genome sequencing. These are thermophilic bacteria, that alter their cell morphology at elevated temperatures [20] and were first isolated from the effluent of an underground hot spring, with at an optimal temperature of 55°C [3]. In soil microcosms, at 40°C, the relative abundance of *Methylocaldum szegediense* was very low (Fig. 5b) and other species of *Methylocaldum* were most abundant (e.g., *Methylocaldum* sp. SAD2 and *Methylocaldum* sp.14B), and is likely due to different temperature optima for different species within the genus *Methylocaldum*.

At 70°C, sequences derived from MOB represented less than 1% of all sequence reads, and the most abundant taxon was *Methylobacter luteus*. This likely represents the near total absence of growth by methanotrophs at 70°C, resulting in an observed methylotroph microbial community derived from inactive organisms, and therefore more similar to that of the source soil community.

Across all temperatures in the soil incubations, additional methanotrophs/methylotrophs were also detected in the soil, including *Methylocystis, Methyloversatilis* and *Crenothrix*. Some sequences could not be annotated at the taxonomic level of genus but were still identified as putative methylotrophs. These sequences were derived from members of the families

Methylophilaceae, Methylomonaceae, Methylococcaceae and Methylogelaceae. Overall, type-I methanotrophs were the most abundant MOB observed in this study. Prior studies on landfills have shown that Type-I methanotrophs dominate such environments under nutrient-rich conditions as well as in elevated oxygen concentrations and low CH_4 concentrations [54, 24, 10, 29, 12, 55] consistent with this study.

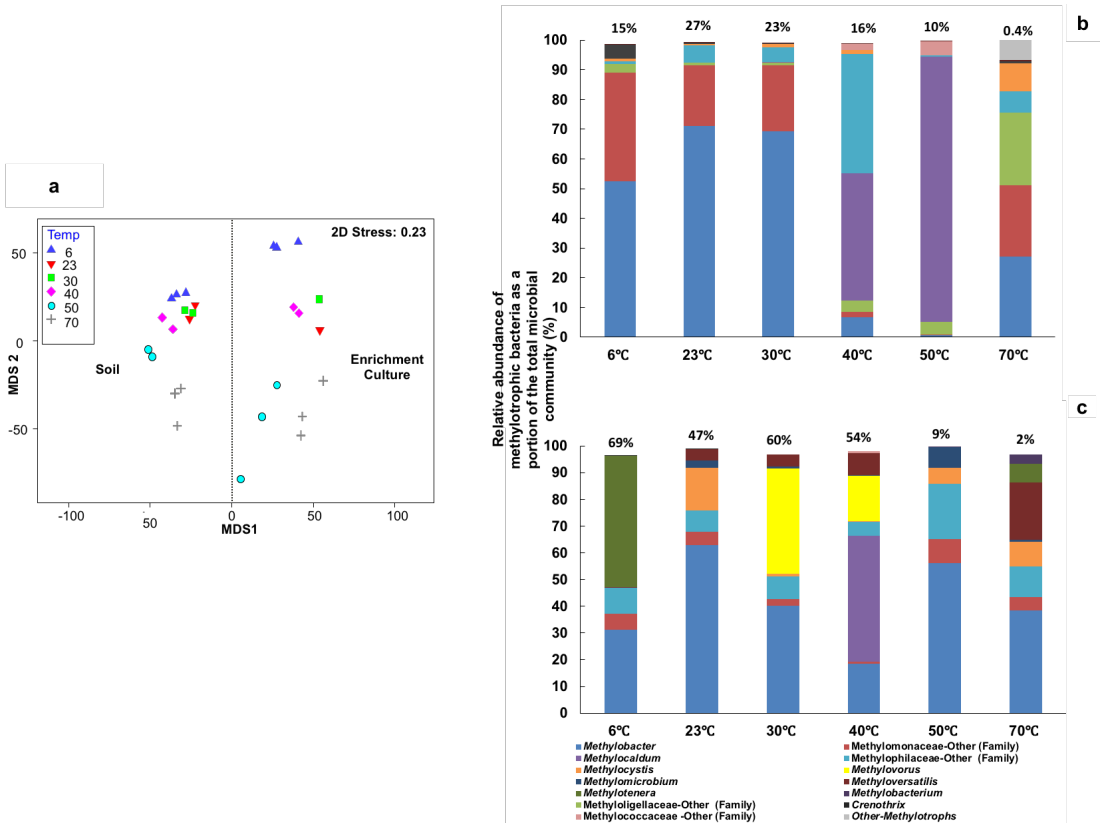


Fig.6-4. Microbial community structure in soil and enrichment culture microcosms as assessed by DNA-based 16S rRNA gene amplicon sequence analysis. (a) Metric multi-dimensional scaling plot of total microbial community structure by temperature. Soil and enrichment culture microcosm communities were significantly different (ANOSIM, R=0.761, p= 0.001, 999 permutations). Enrichment temperature (°C) for each sample is color coded. (b) Bar chart of methylotrophic bacteria in soil microcosms across a range of incubation temperatures. The relative abundance of methylotrophic bacteria as a portion of the total microbial community is shown above each temperature. (c) Bar chart of methylotrophic bacteria in enrichment culture microcosms across a range of incubation temperatures. The relative abundance of methylotrophic bacteria as a portion of the total microbial community is shown above each temperature.

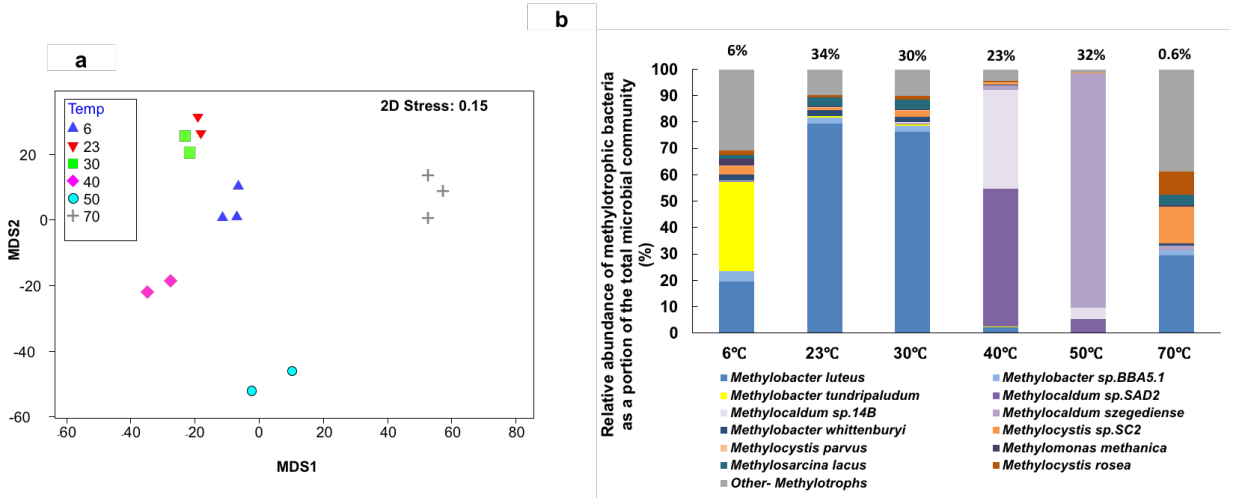


Fig. 6-5 Microbial community structure in soil microcosms as assessed by DNA-based shotgun metagenome sequence analysis. (a) Metric multi-dimensional scaling plot of total microbial community structure by temperature. Enrichment temperature (°C) for each sample is color coded. (b) Bar chart of methylotrophic bacteria in soil microcosms across a range of incubation temperatures. The relative abundance of methylotrophic bacteria as a portion of the total microbial community is shown above each temperature.

6.3.3 Methylotroph microbial community structure in enrichment cultures incubated across a temperature gradient

The microbial community in the enrichment cultures incubated at temperatures from 6°C to 70°C were analyzed using 16S rRNA gene amplicon sequencing (**Fig. 6-4**). The microbial community structure of the enrichment microcosms was significantly different than those of the soil microcosms (Analysis of similarity, ANOSIM; R=0.761, p= 0.001).The relative abundance of 16S rRNA gene amplicon sequences from MOB relative to the total microbial community ranged from 2% (70°C) to 69% (6°C) of the community and was significantly greater than that of soil microcosms incubated at the same temperatures, with the exception of 50°C (Student's t-test, p=0.696). Bacteria from the genus *Methylobacter* were present in incubations at all temperatures, with the relative abundance of this genus ranging from 18% to 61%. No CH_4 oxidation was observed at incubation temperatures of 50°C and 70°C; thus, observed MOB communities likely

represent dormant organisms derived from the source soil material. Although *Methylobacter* were abundant at all temperatures, secondary methylotrophic taxa were observed at high abundance, and these taxa varied by temperature. At 6°C, low CH_4 oxidation rates were observed, and the observed community was composed of bacteria from the genera *Methylobacter* and *Methylotenera* and from the family Methylophilaceae. At 23°C, the MOB community was dominated by bacteria from the genera *Methylobacter* and *Methylocystis*. At 30°C, bacteria from the genus *Methylovorus* were most abundant, and at 40°C, *Methylocaldum* was dominant. Sequences from the bacteria of the genus *Methylocaldum* accounted for 47% of the total methanotrophic community identified at 40°C, and was negligible at other temperatures, including 50°C. At 50°C in soil microcosms the genus *Methylocaldum* was the most abundant methylotrophic taxon; thus, the low abundance of *Methylocaldum* at the same temperature in the enrichment cultures represents an effect of diffusion limitation limiting the growth of *Methylocaldum*, and thereby preventing active methanotrophy at this temperature.

Other methylotrophs identified in the enrichment culture analyses included bacteria from the genera *Methylomicrobium* and *Methyloversatilis* and the families Methylophilaceae and Methylomonaceae. Although Type-I methanotrophs were primarily identified, some Type-II methanotrophs were also observed. For example, *Methylocystis* was observed at relatively high relative abundance in enrichment cultures at 23°C but were negligible in soil microcosms. *Methylocystis* are Type- II methanotrophs that typically grow at higher temperatures between 20°C and 40°C [8]. Many studies have correlated the abundance of *Methylocystis* to acidic pH of the soil [10, 54], high CH_4 and low O_2 concentrations [23-24] and temperatures >20°C [6]. It was noticed that the only condition where *Methylocystis* was enriched was under enrichment conditions at 23°C where oxygen concentrations were limited due to diffusion.

6.4 CONCLUSIONS

The effect of temperature on CH_4 oxidation potential in both soil microcosms and methanotrophic enrichment cultures was analyzed. The CH_4 oxidation rate increased with increasing temperature from 6°C to 30°C but decreased at temperatures above 30°C. CH_4 oxidation rates were highest at 30°C in soil microcosms and 23°C - 30°C in enrichment cultures. Although active CH_4 oxidation was observed in soil microcosms at 50°C, enrichments showed no CH_4 oxidation potential at 50°C, indicative of diffusion limitations. A temperature-associated shift in the structure of the methanotrophic community was observed, and putative psychrophilic, mesophilic and thermophilic methanotrophic bacteria were detected in soil and enrichment cultures. Temperature is critical factor influencing the CH_4 oxidation, and changes in temperature influence microbial community structure, and are also mediated by community structure and diffusion.

6.5 CITED REFERENCES

1. Abushammala MF, Basri NEA, Irwan D, Younes MK (2014) Methane oxidation in landfill cover soils: a review. Asian J Atmos Environ 8(1), 1-14. https://doi.org/10.5572/ajae.2014.8.1.001
2. Bender M, Conrad R (1995) Effect of CH4 concentrations and soil conditions on the induction of CH4 oxidation activity. Soil Biol Biochem 27(12), 1517-1527. https://doi.org/10.1016/0038-0717(95)00104-M
3. Bodrossy L, Holmes EM, Holmes AJ, Kovács KL, Murrell JC (1997) Analysis of 16S rRNA and methane monooxygenase gene sequences reveals a novel group of

thermotolerant and thermophilic methanotrophs, Methylocaldum gen. nov. Archives of Microbiol 168(6), pp.493-503. https://doi.org/10.1007/s002030050527

4. Boeckx P, Van Cleemput O, Villaralvo IDA (1996) Methane emission from a landfill and the methane oxidising capacity of its covering soil. Soil Biol Biochem 28(10-11), 1397-1405. https://doi.org/10.1016/S0038-0717(96)00147-2
5. Börjesson G, Svensson BH (1997) Seasonal and diurnal methane emissions from a landfill and their regulation by methane oxidation. Waste Manag Res 15(1), 33-54. https://doi.org/10.1177/0734242X9701500104
6. Börjesson G, Sundh I, Svensson B (2004) Microbial oxidation of CH4 at different temperatures in landfill cover soils. FEMS Microbiol Ecol 48(3), 305-312. https://doi.org/10.1016/j.femsec.2004.02.006
7. Borowik A, Wyszkowska J (2016) Impact of temperature on the biological properties of soil. INT AGROPHYS 30(1), pp.1-8. https://doi.org/10.1515/intag-2015-0070
8. Bowman JP, Sly LI, Nichols PD, Hayward AC (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. Int J Syst Evol Microbiol 43(4), pp.735-753. 10.1099/00207713-43-4-735
9. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, and Huttley GA (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7(5), 335. https://doi.org/10.1038/nmeth.f.303

10. Cébron A, Bodrossy L, Chen Y, Singer AC, Thompson IP, Prosser JI, Murrell JC (2007) Identity of active methanotrophs in landfill cover soil as revealed by DNA-stable isotope probing. FEMS Microbiol Ecol 62(1), pp.12-23. https://doi.org/10.1111/j.1574-6941.2007.00368.x
11. Chanton J, Liptay K (2000) Seasonal variation in methane oxidation in a landfill cover soil as determined by an in situ stable isotope technique. Global Biogeochem Cycles 14(1), 51-60. https://doi.org/10.1029/1999GB900087
12. Chi ZF, Lu WJ, Wang HT (2015) Spatial patterns of methane oxidation and methanotrophic diversity in landfill cover soils of southern china. J. Microbiol. Biotechnol 25(4), pp.423-430. http://dx.doi.org/10.4014/jmb.1408.08055
13. Christophersen M, Linderød L, Jensen PE, Kjeldsen P (2000) Methane oxidation at low temperatures in soil exposed to landfill gas. J Environ Qual 29(6), 1989-1997. doi:10.2134/jeq2000.00472425002900060036x
14. Clarke KR, Gorley RN (2015) Getting started with PRIMER v7. PRIMER-E: Plymouth, Plymouth Marine Laboratory. http://updates.primer-e.com/primer7/manuals/Getting_started_with_PRIMER_7.pdf
15. 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(3), 231-237. https://doi.org/10.1007/s003740000313
16. Dunfield P, Dumont R, Moore TR (1993) Methane production and consumption in temperate and subarctic peat soils: response to temperature and pH. Soil Biol Biochem 25(3), 321-326. https://doi.org/10.1016/0038-0717(93)90130-4

17. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19), 2460-2461. https://doi.org/10.1093/bioinformatics/btq461
18. Einola JKM, Kettunen RH, Rintala JA (2007) Responses of methane oxidation to temperature and water content in cover soil of a boreal landfill. Soil Biol Biochem 39(5), pp.1156-1164. https://doi.org/10.1016/j.soilbio.2006.12.022
19. Einola JK, Sormunen KM, Rintala JA (2008) Methane oxidation in a boreal climate in an experimental landfill cover composed from mechanically–biologically treated waste. Sci Total Environ 407(1), 67-83. https://doi.org/10.1016/j.scitotenv.2008.08.016
20. Eshinimaev BT, Medvedkova KA, Khmelenina VN, Suzina NE, Osipov GA, Lysenko AM, Trotsenko YA (2004) New thermophilic methanotrophs of the genus Methylocaldum. Microbiol 73(4), pp.448-456. https://doi.org/10.1023/B:MICI.0000036991.31677.13
21. Glöckner FO, Yilmaz P, Quast C, Gerken J, Beccati A, Ciuprina A, Bruns G, Yarza P, Peplies J, Westram R, Ludwig W (2017) 25 years of serving the community with ribosomal RNA gene reference databases and tools. J Biotechnol 261, pp.169-176. 10.1016/j.jbiotec.2017.06.1198
22. Green SJ, Venkatramanan R, Naqib A (2015) Deconstructing the polymerase chain reaction: understanding and correcting bias associated with primer degeneracies and primer-template mismatches. PLoS ONE 10(5), e0128122. https://dx.doi.org/10.1371%2Fjournal.pone.0128122
23. Hanson RS, Hanson TE (1996) Methanotrophic Bacteria. Microbiol Rev 60(2), 439-471. https://mmbr.asm.org/content/60/2/439

24. Henckel T, Roslev P, Conrad R (2000) Effects of O2 and CH4 on presence and activity of the indigenous methanotrophic community in rice field soil. Environ Microbiol 2(6), pp.666-679. https://doi.org/10.1046/j.1462-2920.2000.00149.x

25. He R, Wooller MJ, Pohlman JW, Quensen J, Tiedje JM, Leigh MB (2012) Shifts in identity and activity of methanotrophs in arctic lake sediments in response to temperature changes. Appl Environ Microbiol pp.AEM-00853. 10.1128/AEM.00853-12

26. Horz HP, Rich V, Avrahami S, Bohannan BJ (2005) Methane-oxidizing bacteria in a California upland grassland soil: diversity and response to simulated global change. Appl Environ Microbiol 71(5), pp.2642-2652. 10.1128/AEM.71.5.2642-2652.2005

27. Kallistova AY, Montonen L, Jurgens G, Münster U, Kevbrina MV, Nozhevnikova AN (2013) Culturable psychrotolerant methanotrophic bacteria in landfill cover soil. Microbiol 82(6), 847-855. https://doi.org/10.1134/S0026261714010044

28. King GM, Adamsen APS (1992) Effects of temperature on methane consumption in a forest soil and in pure cultures of the methanotroph Methylomonas rubra. Appl Environ Microbiol 58(9), 2758-2763. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC183004/

29. Li H, Chi ZF, Lu WJ, Wang HT (2013) Mitigating CH4 emissions in semi-aerobic landfills: Impacts of operating conditions on abundance and community structure of methanotrophs in cover soils. J Microbiol Biotechnol 23(7), pp.993-1003. http://dx.doi.org/10.4014/jmb.1212.12010

30. McDonald D, Clemente JC, Kuczynski J, Rideout JR, Stombaugh J, Wendel D, Wilke A, Huse S, Hufnagle J, Meyer F, Knight R (2012) The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome. Gigascience, 1(1), p.7. https://doi.org/10.1186/2047-217X-1-7

31. Minot SS, Krumm N, Greenfield NB (2015) One codex: A sensitive and accurate data platform for genomic microbial identification. BioRxiv, p.027607. https://doi.org/10.1101/027607
32. Mohanty SR, Bodelier PL, Conrad R (2007) Effect of temperature on composition of the methanotrophic community in rice field and forest soil. FEMS Microbiol Ecol 62(1), 24-31. https://doi.org/10.1111/j.1574-6941.2007.00370.x
33. Naqib A, Poggi S, Wang W, Hyde M, Kunstman K, Green SJ (2018) Making and sequencing heavily multiplexed, high-throughput 16S ribosomal RNA gene amplicon libraries using a flexible, two-stage PCR protocol. Methods Mol Biol 1783, 149-169. https://doi.org/10.1007/978-1-4939-7834-2_7
34. Nesbit SP, Breitenbeck GA (1992) A laboratory study of factors influencing methane uptake by soils. Agric Ecosyst Environ 41(1), 39-54. https://doi.org/10.1016/0167-8809(92)90178-E
35. Park JR, Moon S, Ahn YM, Kim JY, Nam K (2005) Determination of environmental factors influencing methane oxidation in a sandy landfill cover soil. Environ Technol 26(1), 93-102. https://doi.org/10.1080/09593332608618586
36. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Rese 41(D1), pp. D590-D596. 10.1093/nar/gks1219
37. Reddy KR, Grubb DG, Kumar G (2018) Innovative biogeochemical soil cover to mitigate landfill gas emissions. Proc. in International Conference on Protection and Restoration of the Environment XIV, Thessaloniki, Greece.

38. Sadasivam BY, Reddy KR (2014) Landfill methane oxidation in soil and bio-based cover systems: a review. Rev Environ Sci Biotechnol 13(1), 79-107. https://doi.org/10.1007/s11157-013-9325-z

39. Scheutz C, Kjeldsen P (2004) Environmental factors influencing attenuation of methane and hydrochlorofluorocarbons in landfill cover soils. J Environ Qual 33(1), 72-79. doi:10.2134/jeq2004.7200

40. Semrau JD, Dispirito AA, Yoon S (2010) Methanotrophs and copper. FEMS Microbiol Rev 34(4), 496-531. https://doi.org/10.1111/j.1574-6976.2010.00212.x

41. Spokas KA, Bogner JE (2011) Limits and dynamics of methane oxidation in landfill cover soils. Waste Manag 31(5), 823-832. https://doi.org/10.1016/j.wasman.2009.12.018

42. Su Y, Zhang X, Xia FF, Zhang QQ, Kong JY, Wang J, He R (2014) Diversity and activity of methanotrophs in landfill cover soils with and without landfill gas recovery systems. Syst Appl Microbiol 37(3), pp.200-207. https://doi.org/10.1016/j.syapm.2013.10.005

43. Tikhonov M, Leach RW, Wingreen NS (2015) Interpreting 16S metagenomic data without clustering to achieve sub-OTU resolution. ISME J 9(1), p.68. 10.1038/ismej.2014.117

44. Trotsenko YA, Khmelenina VN (2005) Aerobic methanotrophic bacteria of cold ecosystems. FEMS Microbiol Ecol 53(1), pp.15-26. https://doi.org/10.1016/j.femsec.2005.02.010

45. Urmann K, Lazzaro A, Gandolfi I, Schroth MH, Zeyer J (2009) Response of methanotrophic activity and community structure to temperature changes in a diffusive CH4/O2 counter gradient in an unsaturated porous medium. FEMS Microbiol Ecol 69(2), pp.202-212. https://doi.org/10.1111/j.1574-6941.2009.00708.x

46. United States Environmental Protection Agency (USEPA) (2019) Landfill methane outreach program: Basic information about landfill gas. Available at: https://www.epa.gov/lmop/basic-information-about-landfill-gas (accessed on 25th February, 2019)
47. Vecherskaya MS, Galchenko VF, Sokolova EN, Samarkin VA (1993) Activity and species composition of aerobic methanotrophic communities in tundra soils. Curr Microbiol 27(3), pp.181-184. https://link.springer.com/article/10.1007/BF01576018.
48. Visvanathan C, Pokhrel D, Cheimchaisri W, Hettiaratchi JPA, Wu JS (1999) Methanotrophic activities in tropical landfill cover soils: effects of temperature, moisture content and methane concentration. Waste Manag Res 17(4), 313-323. https://doi.org/10.1177/0734242X9901700408
49. Wang J, Xia FF, Bai Y, Fang CR, Shen DS, He R (2011) Methane oxidation in landfill waste biocover soil: kinetics and sensitivity to ambient conditions. Waste Manag 31(5), 864-870. https://doi.org/10.1016/j.wasman.2011.01.026
50. Wartiainen I, Hestnes AG, McDonald IR, Svenning MM (2006) Methylobacter tundripaludum sp. nov., a methane-oxidizing bacterium from Arctic wetland soil on the Svalbard islands, Norway (78 N). Int J Syst Evol Microbiol 56(1), pp.109-113. 10.1099/ijs.0.63728-0
51. Whalen SC, Reeburgh WS, Sandbeck KA (1990) Rapid methane oxidation in a landfill cover soil. Appl Environ Microbiol 56(11), 3405-3411. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC184961/

52. Whalen SC, Reeburgh WS (1996) Moisture and temperature sensitivity of CH4 oxidation in boreal soils. Soil Biol Biochem 28 (10-11), 1271-1281. https://doi.org/10.1016/S0038-0717(96)00139-3

53. Whittenbury R, Phillips KC, Wilkinson JF (1970) Enrichment, isolation and some properties of methane-utilizing bacteria. Microbiol 61(2), 205-218. 10.1099/00221287-61-2-205

54. Wise MG, McArthur JV, Shimkets LJ (1999) Methanotroph diversity in landfill soil: isolation of novel type I and type II methanotrophs whose presence was suggested by culture-independent 16S ribosomal DNA analysis. Appl Environ Microbiol 65(11), pp.4887-4897. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC91658/

55. Yargicoglu EN, Reddy KR (2017) Microbial abundance and activity in biochar-amended landfill cover soils: Evidence from large-scale column and field experiments. J Environ Eng 143(9), 04017058. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001254

56. Zhang J, Kobert K, Flouri T, Stamatakis A (2013) PEAR: a fast and accurate illumina paired-end read merger. Bioinformatics 30(5), 614-620. https://dx.doi.org/10.1093%2Fbioinformatics%2Fbtt593

Table 6-1. Studies showing genus or species level Methanotrophs/ Methylotrophs identified in ecosystems at varied temperatures

Temperature Range, °C	Specific Temperature, °C	CH_4 concentration	Sampling Depth	Ecosystem	Molecular Biomarker	Genus/ Species/ Type of Methanotrophs	References
6 - 70	6 23 30 40 50	5%	0.3 – 0.6m	Landfill cover soil	Shotgun sequencing, 16S rRNA	*Methylobacter luteus, Methylobacter tundripaludum, Methylotenera* *Methylobacter luteus, Methylocystis,* *Methylobacter luteus, Methylovorus glucosetrophus* *Methylocaldum* sp. SAD2, *Methylocaldum* sp.14B *Methylocaldum szegediense*	This paper
5 - 45	5, 10, 15, 25, 35	4%	0.05 – 0.2m	Rice field and Forest soil	TRFLP-*pmoA* gene	*Methylobacter* *Methylococcus/Methylocaldum* *Methylocystis/ Methylosinus* *Methylomonas methanica*	[32]
3 - 20	5, 10 20	5%	0 - 0.3m	Landfill cover soil	PLFA	Type I Methanotrophs Type II Methanotrophs	[6]
4 - 21	4 10 21	10%	0 – 0.25m	Arctic Lake	DNA-SIP	*Methylophilus, Methylobacter* *Methylobacter, Methylomonas,Methylosoma* *Methylocystis, Methylophilus, Methylobacter, Methylomonas*	[25]
5 - 40	5	5-50%	0.2 – 0.4m	Landfill cover soil	16S rRNA gene analysis (DGGE)	*Methylotenera versatilis*	[27]
	10					*Methylobacter tundripaludum, Methylovorus glucosetrophus* *Methylocella tundrae, Methylobacter marinus, Methylosinus sporium*	
	20					*Methylobacter marinus, Methylobacter luteus, Methylobacter tundripaludum, Methylosinus trichosporium, Methylosinus sporium*	
	40					*Methylocaldum gracile*	
4 - 20	4	-			FISH,	*Methylococcaceae, Methylobacteraceae* sp.,	[45]

			-	Hydrocarbon contaminated aquifer	TRFLP-*pmoA* gene	*Methylomonas* sp.	
	12					*Methylococcaceae, Methylobacteraceae* sp.	
	20					*Methylocystis* sp., *Methylococcaceae, Methylobacteraceae* sp	
7.5 – 9.5	-	-	0 – 0.4m	Tundra bog soil	Immunofluorescence	*Methylomonas, Methylobacter, Methylococcus, Methylocystis, Methylosinus*	[47]

CHAPTER 7 – EFFECT OF BASIC OXYGEN FURNACE (BOF) SLAG LEACHATE ON METHANE OXIDATION AND COMMUNITY COMPOSITION IN BIOGEOCHEMICAL LANDFILL COVER SYSTEM

7.1 INTRODUCTION

Industrial waste disposal is currently a major environmental and societal issues. These wastes are either reused within industry or stock-pilled for disposal in landfills. Slag, a byproduct from steel mills, is generated in enormous quantities during iron making and steel making process. Iron and steel slags composition varies depending upon the type of production process or type of furnace used in the production process. Two main types of slag are generated by the steel industry: blast furnace (BF) slag, an iron making slag, and steel slag, a steel making slag. Steel slag is further classified by technology used, including basic oxygen furnace (BOF) slag, ladle furnace (LF) slag, and electric arc furnace (EAF) slag. Among the types of iron and steel slags, BF slag has found its application in civil and construction industry, while steel slags have limited applications due to the finer particle size and volumetric instability resulting in the stockpiling as well as landfilling of the steel slag (Reddy et al. 2019). The major concern regarding the disposal of the steel slag is its effect on terrestrial and aquatic environments. The most widespread impact is that of slag leachate on receiving water bodies by drastically increasing the pH of the water, depleting O_2 in the water bodies, and increasing salinity and metal concentrations. An alkaline pH of 8.5-10 has been reported to cause severe disturbance to fish life leading to death (Saha et al. 2002). Excess carbonate precipitation in Onondaga Lake, New York, USA has shown reduced diversity of zooplankton and growth of macrophytes (Effler and Matthews 2003). In contrast, the dumping of industrial slag since 1880s in the low-lying Lake Calumet area of southeast Chicago and north-

west Indiana, has shown diverse microbial communities that inhabits alkaliphiles, iron-reducing and sulfur reducing bacteria in extremely alkaline groundwater (Roadcap et al. 2006).

Recently, Reddy et al. 2018 proposed the concept of an innovative biogeochemical cover consisting of biochar-amended soil and BOF slag to mitigate fugitive CH_4 and CO_2 emissions from the landfills. The use of biochar amended soil has been studied previously in our laboratory showing promising results in mitigating CH_4 emissions from landfills due to its physico-chemical properties such as water holding capacity, high porosity and surface area for proliferation of microbes (Yargicoglu and Reddy, 2017a, b). In recent years, BOF slag has shown promising potential for CO_2 sequestration due to the presence of various calcium-containing minerals such as free lime (CaO), portlandite ($Ca(OH)_2$) and larnite (Ca_2SiO_4) (Huijgen et al. 2005; Reddy et al. 2019 a, b, c). Despite these compounds, the use of BOF slag as an alternative landfill cover material to mitigate fugitive CO_2 emissions had not been explored before. Use of BOF slag in conjunction with other materials such as soil and biochar in the biogeochemical cover system has been proposed to mitigate both CH_4 and CO_2 emissions. Since, BOF slag is highly alkaline in nature (pH ~ 12), it is crucial to understand the effect of BOF slag on the general microbial community, and specifically the methylotrophs which play a pivotal role in the oxidation of CH_4 in biochar-amended soil. Preliminary investigations from our laboratory has shown inhibition of CH_4 oxidation when BOF slag was mixed with soil or biochar-amended soil in contrast to an isolated or layered system (soil/biochar amended soil isolated from slag) that showed substantial CH_4 oxidation, affirming feasibility of the layered system in the biogeochemical landfill cover. To further investigate the effect of slag infiltrates or leachate on the microbial activity in the layered biogeochemical landfill cover this study aims in assessing the effect of BOF slag leachate on CH_4 oxidation and community composition in both soil and methanotrophic enrichment culture.

7.2 MATERIALS AND METHODS

7.2.1 Soil

Soil was collected from the Zion landfill, located in Zion, Illinois, USA. Soil samples were collected from an interim cover at a depth of ~1 to 2 feet and were shipped to the Geotechnical and Geoenvironmental Engineering Laboratory at the University of Illinois at Chicago (UIC), where they were stored at room temperature (23 ± 2°C). Soil samples were air dried (moisture content <0.5%), pulverized and screened through a 2 mm sieve prior to conducting the experiments.

7.2.2 BOF slag

The BOF slag used in this experiment was obtained from Indiana Harbor East (IHE) of Arcelor Mittal steel plant, located in East Chicago, Indiana, USA. The BOF slag, was sampled from the steel plant in September 2017, and is designated as IHE 9/17 (samples collected from Indiana Harbor East on Sep 17, 2017). All the tests were performed using the bulk slag sample as obtained from the plant. The steel slag was also oven-dried at 105°C prior to conducting the experiments.

7.2.3 Characteristics of BOF slag

Particle size distribution was determined as per American Society for Testing and Materials (ASTM) D422. The specific gravity, soil classification, and water holding capacity (WHC) were determined according to ASTM D584, D2487 and D2980, respectively. Loss on ignition (LOI) was determined as per ASTM D2974. Hydraulic conductivity was measured according to ASTM D2434 standard procedure using a rigid wall permeameter. The pH and oxidation-reduction

potential (ORP) were measured using an ORION Model 720A pH meter at a liquid to solid ratio of 1:1. The pH meter was pre-calibrated using standard buffer solutions of pH 4, 7 and 10 before use. Electrical conductivity (EC) was measured using a Corning 311 Conductivity Meter and pre-calibrated using standard solution of 12.9 mS/cm.

The total elemental content analysis of BOF slag was conducted through a combination of X-ray fluorescence (XRF) and solid phase acid digestion with chemical analysis by inductively coupled plasma optical emission spectrometry (ICP-OES). Leaching behavior of slag was analyzed by performing Toxicity Characteristic Leaching Procedure (TCLP) and Synthetic Precipitation Leaching Procedure (SPLP) tests as per EPA Method 1311 and 1312 standard procedures.

7.2.4 BOF slag leachate collection

An acrylic glass column of 30 cm height and 2.5 cm inner diameter was filled with BOF slag in three layers each of 5 cm with slight tamping to a total height of 15 cm. **Fig. 7- 1.** shows a picture of BOF slag leachate collection procedure. The glass column was secured with bed support mesh screen and screw cap at the bottom to prevent spillage of the fines. To simulate infiltration, the headspace of the column was filled with regular tap water, and was allowed to percolate through the slag, and leachate was collected at the bottom after passing through Ahlstrom grade 55 filter paper (**Fig. 7-1**). Around 20 pore volumes (PVs) of leachate (1 PV = 29. 23 mL) was collected for the experiments. The collected leachate samples were tested for pH and electrical conductivity. It was observed that for all the PVs tested, the pH and electrical conductivity remained nearly constant (12.32-12.38 and 8.30-8.60 mS/cm, respectively).

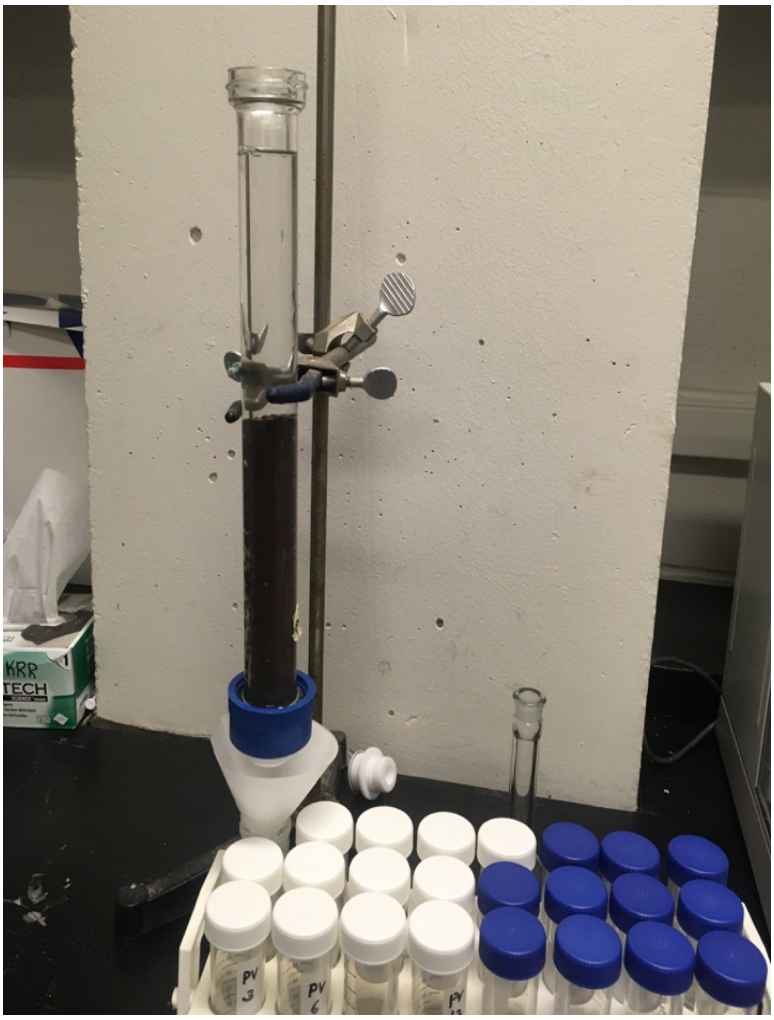


Fig. 7-1. BOF slag leachate collection

7.2.5 Soil microcosm batch tests

For batch testing, 10 g of soil was placed in 125 mL-serum vials and moisture content was adjusted to 20% (w/w) using BOF slag leachate and deionized water (DI) at different proportions: 0% (control - 2 mL DI water), 5% (0.1 mL slag leachate + 1.9 mL DI water), 20% (0.4 mL slag leachate + 1.6 mL DI water), 60% (1.2 mL slag leachate + 0.8 mL DI water), and 100% (2 mL slag leachate). The vials were sealed airtight using butyl rubber septa and secured using crimp cap. 20 mL of air from the headspace was replaced with equal volume of synthetic landfill gas (LFG) comprising of 50% (v/v) CH_4 and 50% (v/v) CO_2 to achieve a headspace concentration of 5-6%

CH_4 (v/v), 5-6% CO_2 (v/v) and a balance (~88–90%) of air. The change in the headspace concentrations was determined by collecting and analyzing the gas samples on a regular basis using gas chromatography (GC) until the headspace concentration of CH_4 dropped below 1%. All the experiments were conducted in triplicate along with the controls (with synthetic LFG without any material). The CH_4 oxidation rates were calculated from linear regression analysis of CH_4 concentration versus elapsed time, based on the zero-order kinetics.

7.2.6 Enrichment culture batch tests

Serum vials, rubber septa and pipettes were sterilized using a Napco Model 8000-DSE autoclave operated at >120°C for a minimum of 60 minutes to ensure sterilization. A mixed methanotrophic culture was cultivated in the laboratory by enriching soil in Nitrate Mineral Salts (NMS) media along with CH_4 and O_2 as previously described in Rai et al. (2018). The enrichment cultures from the above was used in the experiments and inoculated with BOF slag leachate at different proportions : 0%, 11% (1 mL slag leachate and 9 mL enrichment culture), 25% (2 mL slag leachate and 8 mL enrichment culture) and 100% (5 mL slag leachate and 5 mL enrichment culture). Similar procedure as microcosm batch tests were followed in the culture batch tests to achieve a headspace concentration of ~5-6% (v/v) CH_4, ~5-6% (v/v) CO_2 and a balance (~88-90%) of air. All the experiments were conducted in triplicate along with the controls (media-NMS). The pH of the samples was measured at the beginning and end of the experiment.

7.2.7 Gas analysis

Gas samples were analyzed at regular time intervals and analyzed for CH_4 and CO_2 concentrations using an SRI 9300 GC equipped with a thermal conductivity detector (TCD) as previously

described (Yargicoglu and Reddy, 2017). Gas samples were withdrawn using 1 mL syringe where 0.5 mL of the sample was discarded and remaining 0.5 mL was injected into the GC to reduce any pressure effects due to sampling. A calibration curve for a minimum of three points was established using high purity standard gas mixtures ranging from 1% to 50% CH_4 and 5% to 50% CO_2.

7.2.8 Microbial community structure analysis

Genomic DNA (gDNA) was extracted from soil samples and from cell pellets using a DNeasy PowerSoil Kit (Qiagen) based on manufacturer's instructions with a slight modification. Samples were heated at 65°C for 10 min before homogenizing with FastPrep-24 5G bead-beating device (MP Biomedicals) at 6 m/s for 40 sec. After homogenization, extraction protocols were automated on a QIAcube instrument (Qiagen), according to the manufacturer's instructions. Genomic DNA was processed for microbial community analysis using shotgun metagenome sequencing (SMS), using a Nextera XT library preparation kit (Illumina, San Diego, CA) according to the manufacturer's instructions. Libraries were pooled and sequenced on a NextSeq500 instrument, employing a 300 cycle (2x150 paired end reads) mid-output kit. Sequence data were analyzed using the online annotation server from OneCodex, as described previously (Minot et al. 2015). DNA extraction, library preparation and sequencing were performed at the University of Illinois at Chicago Sequencing Core (UICSQC).

7.2.9 Data archive

Raw sequence data files were submitted in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI). The BioProject identifiers of the SMS samples is PRJNA540300.

7.2.10 Statistical analysis

Statistical analysis of batch test results was tested using one-way (ANOVA) and t-tests (equivalency of sample means) using Microsoft Excel-2018. A significance level of alpha = 0.05 was used to assess statistical significance in all tests. Microbial community sequence data were analyzed within the software package Primer7 (Clarke and Gorley, 2015) to calculate alpha-diversity indices and multidimensional scaling (MDS) plots. Significant differences in community structure between experimental conditions were assessed using analysis of similarity (ANOSIM).

7.3 RESULTS AND DISCUSSION

7.3.1 BOF Slag Characterization

The physical and chemical properties of the BOF slag is summarized in **Table 7-1**. The specific gravity of the BOF slag (3.5) was relatively high and is attributed to high iron content (30.2%). The WHC of the BOF slag was 20%. The pH of the slag was 12.4, indicating slag to be highly alkaline in nature.

The Toxicity Characteristic Leaching Procedure (TCLP) and Synthetic Precipitation Leaching Procedure (SPLP) results for the constituents of concern (COC) metals are presented in **Table 7-2** and is consistent with the ranges reported by Proctor et al. (2000). The TCLP concentrations are observed to be below the Resource Conservation and Recovery Act (RCRA) limits, demonstrating BOF slag to be non-hazardous. BOF slag is a complex mixture with many insoluble silicates and strongly pH buffered minerals, and studies have shown low leaching of heavy metals from the BOF slag (Proctor et al. 2000; Motz and Geiseler; 2001; Chand et al. 2017; Reddy et al. 2019 b) indicating safe disposal of slag into the environment. In contrast, few studies have shown oxidation of Vanadium (oxidation state +3) to Vanadium (oxidation state +5) during leaching, that is highly mobile and toxic (Chaurand et al. 2007; De Windt et al. 2011). The leachate

obtained in this study shows low levels of heavy metals being leached as per SPLP results (**Table 7-2**) consistent with most of the studies mentioned above.

Table 7-1. Physical and chemical characteristics of BOF slag (IHE 9/17)

Properties	ASTM Method	BOF Slag (IHE 9/17)
Grain Size Distribution:	D422	
Gravel (%)		20.8
Sand (%)		74.2
Fines (%)		4.9
D_{50} (mm)		
C_c		0.7
C_u		18
Atterberg Limits:	D4318	
Liquid Limit (%)		Non-Plastic
Plastic Limit (%)		
Plasticity Index (%)		
USCS Classification	D2487	SP-SM equivalent
Specific Gravity	D854	3.5
Dry Density (g/cm^3)		1.72
Hydraulic Conductivity (cm/s)	D2434	1.1 x 10^{-3}
Loss-on-Ignition (%)	D2974	1.6
pH (1:1)	D4972	12.4
Electrical Conductivity (mS/cm)	D4972	13.3
Redox Potential (mV)	D4972	-313.3
Elemental Analysis	XRF	
Ca (%)		40.35
Fe (%)		30.25
Si (%)		9.55
Mg (%)		10.9
Mn (%)		2.2
Al (%)		3.95

C_c=Coefficient of curvature; C_u=Coefficient of uniformity

Table 7-2. Leaching properties of BOF slag (IHE 9/17)

Metals	RCRA Limit (mg/L)	TCLP (mg/L)	TCLP (mg/l) Proctor et al. 2000	SPLP (mg/L)	ASTM Water Leachate Test (mg/l), Proctor et al. 2000
Aluminum		<1.0	-	0.19	2.7
Antimony		< 0.015	ND	< 0.0060	ND
Arsenic	5	< 0.010	0.002	< 0.0040	0.003
Barium	100	0.13-0.15	0.41	0.078-0.083	0.11
Beryllium		< 0.0050	ND	<0.002	ND
Boron		< 0.23	-	0.08-0.084	-
Cadmium	1	< 0.0050	0.001	<0.002	ND
Calcium		2,200-2,600	-	880-1,000	-
Chromium	5	0.029-0.045	0.01	0.010-0.011	ND
Cobalt		<0.010	-	< 0.0040	-
Copper		<0.1	-	< 0.020	ND
Iron		7.8-9.4	-	3.6-3.8	ND
Lead	5	<0.005	0.004	< 0.0020	0.027
Magnesium		0.29-0.35	-	< 0.40	-
Manganese		<0.01	30.15	< 0.0040	0.0022
Mercury	0.2	<0.0002	0.0003	< 0.00020	ND
Nickel		0.025-0.03	0.012	0.0094-0.0098	ND
Potassium		0.87-1.0	-	0.48-0.56	-
Selenium	1	<0.01	ND	< 0.0040	ND
Silver	5	< 0.010	0.0064	< 0.0040	ND
Thallium		< 0.0050	ND	< 0.0020	ND
Tin		< 0.050	-	< 0.020	ND
Vanadium		0.01-0.015	-	< 0.0040	0.0087
Zinc		<0.05	0.07	< 0.020	ND

7.3.2 Methane Oxidation Potential in Soil and Enrichment Culture

The CH_4 oxidation rates in soil microcosms at different slag leachate proportions (0, 5, 20, 60, 100%) are shown in **Fig. 7-2(a)** and ranged between 97 to 116 μg CH_4 $g^{-1}d^{-1}$. A marginal decrease in the CH_4 oxidation rates with increased leachate proportion, which could be a result of the slight but significant higher pH of the soil-slag leachate mixture at higher slag leachate proportion or quantity. **Table 7-3** shows the pH of the soil mixed with slag leachate from 0% to 40%, (40%) is the water holding capacity (WHC) of the soil. At different proportions of slag leachate, the pH fluctuated within a narrow range of 7.87-8.04, which was ascribed to the high buffering capacity of the soil, thereby resisting change in the pH. Statistical analysis (Single factor- one-way ANOVA) showed that the CH_4 oxidation rates at different slag leachate proportions were not significantly different from each other ($p = 0.16$). This was further confirmed by the lower leachability of heavy metals from the BOF slag as per the SPLP results (**Table 7-2),** suggesting negligible effects of heavy metals on CH_4 oxidation rates. Prior studies have shown CH_4 oxidation in agricultural soils contaminated with lead, zinc and nickel at a concentration of 60, 120 and 35 μg g^{-1}, respectively (Walkiewicz et al. 2016). In contrary, Contin et al. (2012) showed higher inhibition rate in sewage sludge treated soil with zinc at a dose of 1500 μg g^{-1} when compared to sewage sludge treated soil with lead at a dose of 1000 mg g^{-1} in arable and grassland soils. Wnuk et al. (2016) showed considerable CH_4 oxidation in lead-contaminated mineral soils optimal at 13% (v/v) moisture content. Chromium significantly inhibited CH_4 oxidation in the alluvial rice soil at concentration > 20 μg g^{-1} and 60% WHC, while zinc inhibited CH_4 oxidation under flooded conditions in both alluvial and laterite rice soils (Mohanty et al. 1990). Bowman and Hayward (1990) reported few of the culturable methanotrophic species sensitive to mercury and cadmium at concentrations > 0.5 and 10 μM, respectively but relatively resistant to copper, chromium and

zinc. However, in this study, even though the elemental composition of the slag is high, the metal constituents in the slag leachate (TCLP and SPLP) were very low indicating no effect of heavy metals on the methanotrophic activity.

Fig.7-2(b) shows the CH_4 oxidation rates in enrichment culture inoculated with slag leachate at proportions of 0%, 11%, 25% and 100%, respectively. **Fig.7-2(b)** shows a significant decrease in the CH_4 oxidation rates with increase in slag leachate proportion, with rates ranged between 11 to 36 µg CH_4 $mL^{-1}d^{-1}$. The lower oxidation rates in enrichment culture as opposed to soil microcosms is a result of diffusion limitation in aqueous phase when compared to gaseous phase (Whalen et al. 1990; Park et al. 2005). CH_4 oxidation rates with different slag leachate proportions were significantly different from each other (ANOVA, $p = 0.003$). **Table 7-4** shows the pH of the cultures at different slag leachate content. The pH of the cultures varied from an initial pH of 8.2 at 11% leachate content to 11.3 at 100% slag leachate content. The higher pH at higher leachate content may be a result of calcium leaching from the slag matrix. Likely due to the metabolic activity (*i.e.*, aerobic respiration leading to CO_2 production) of heterotrophic microorganisms and atmospheric CO_2 in the system, a significant drop in the pH over the course of incubation was observed, with near neutral pH values measured at the end of the experiment (6.7-7.3). Despite an eventual decrease in pH, this study observed significantly lower rates of CH_4 oxidation rates in incubations with elevated slag leachate, and CH_4 oxidation rates were inversely correlated with initial pH levels. The microbial community composition was analyzed only at the end time point, and likely a shift in the methanotrophic microbial community accompanied the decreasing pH over the course of the experiment, as most methanotrophs favor circumneutral pH (Hanson and Hanson, 1996). However, some methanotrophs have been identified in highly acidic (Semrau et al. 2008; Baesman et al. 2015) or alkaline environments (Khmelenina et al. 1997;

Kalyuzhnaya et al. 2008). pH is thus the likely driver of observed community structure and CH_4 oxidation rate; as the BOF slag showed low metal leaching properties (**Table 7- 2**), the effect of heavy metals in the slag on the CH_4 oxidation is assumed to be minimal.

Table 7-3. pH of the soil at different slag leachate proportions

Slag Leachate (%)	Initial pH
Soil + 10% slag leachate	7.87 ± 0.03
Soil + 20% slag leachate	7.92 ± 0.01
Soil + 30% slag leachate	8.01 ± 0.01
Soil + 40% slag leachate	8.04 ± 0.02

Table 7-4. pH of the enrichment culture at different slag leachate proportions

Slag Leachate (%)	Initial pH	Final pH
0% slag leachate	7.6	7.6
11% slag leachate	8.2	6.83
25% slag leachate	10.52	6.88
100% slag leachate	11.35	7.36

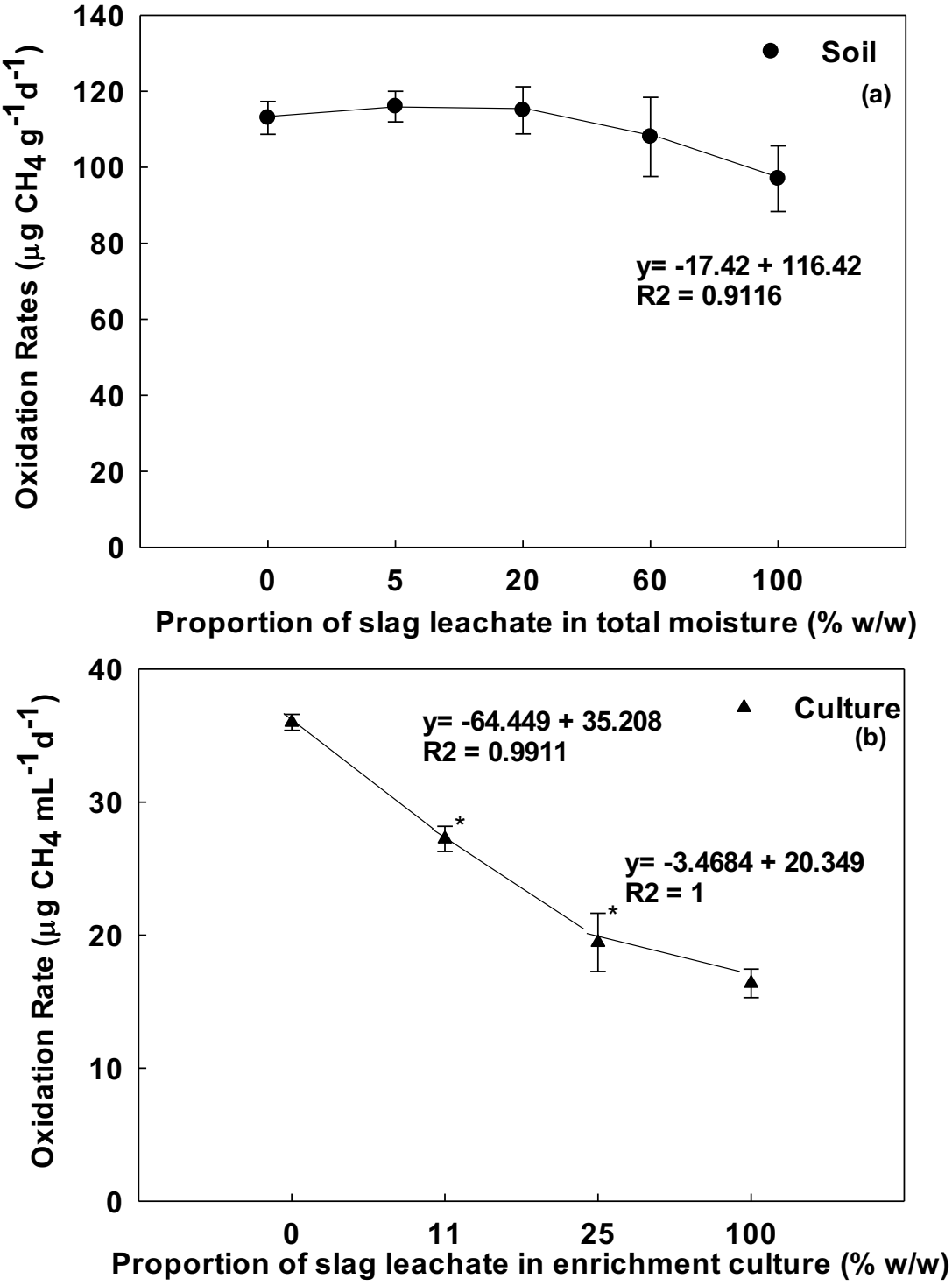


Fig. 7-2. Methane oxidation rates with different slag leachate proportions in: (a) Soil, and (b) Enrichment Culture (* indicates values that are statistically different between two slag leachate proportions at $p < 0.05$)

7.3.3 Methylotroph microbial community in soil and enrichment culture

Soil microcosms and enrichment cultures were inoculated with slag leachate in batch serum vials at different proportions from 0 to 100%, incubated for 5-6 days (enrichments) and 13-28 days (soil microcosms) at 23 ± 2°C. Microbial communities in enrichments were profiled using shotgun metagenome sequencing **(Fig. 7-3 and 7-4).** Majority of the microbial community that were found dominant in the soil microcosms belonged to the genus *Methylobacter* (~18%)*, Micromonospora* (~8.4%)*, Bradyrhizobium* (~3.3%), *Streptomyces* (~2.7%)**,** others (< 2%). However, the enrichment cultures were dominated by the bacteria from the genus *Methylosinus* (~10.1%), *Brevundimonas* (~5.3%), *Variovorax* (~5.4%), *Methylobacter* (~5.3%) and others (< 5%). The methylotrophic community in soil microcosms was dominated by the bacteria from the genus *Methylobacter* and species *M. luteus* (**Fig.7-3**). In addition to *Methylobacter* (82-84% of all methylotroph-annotated sequences), other genera of methylotrophs were detected, including *Methylocystis* (2.1-2.7%)*, Methylosarcina* (3-3.7%)*, Methylomicrobium* (2.7-3.3%) and others (<1-2%). Overall, sequences from MOB represented from 20 to 23% of all annotated sequences. Bacteria from the genus *Methylobacter* are type I methanotrophs, found most commonly in landfills (Chen et al. 2007; Chi et al. 2015; Yargicoglu and Reddy, 2017) and other ecosystems (Amaral and Knowles, 1995; Henckel et al. 2000), and are known to be mesophilic with an optimal pH of 6.5 -7 (Bowman et al. 1993). Prior studies have shown that type I methanotrophs can be favored under low CH_4 and high oxygen conditions (Chi et al. 2015; Chen et al. 2007; Li et al. 2013), and these conditions are consistent with our microcosms.

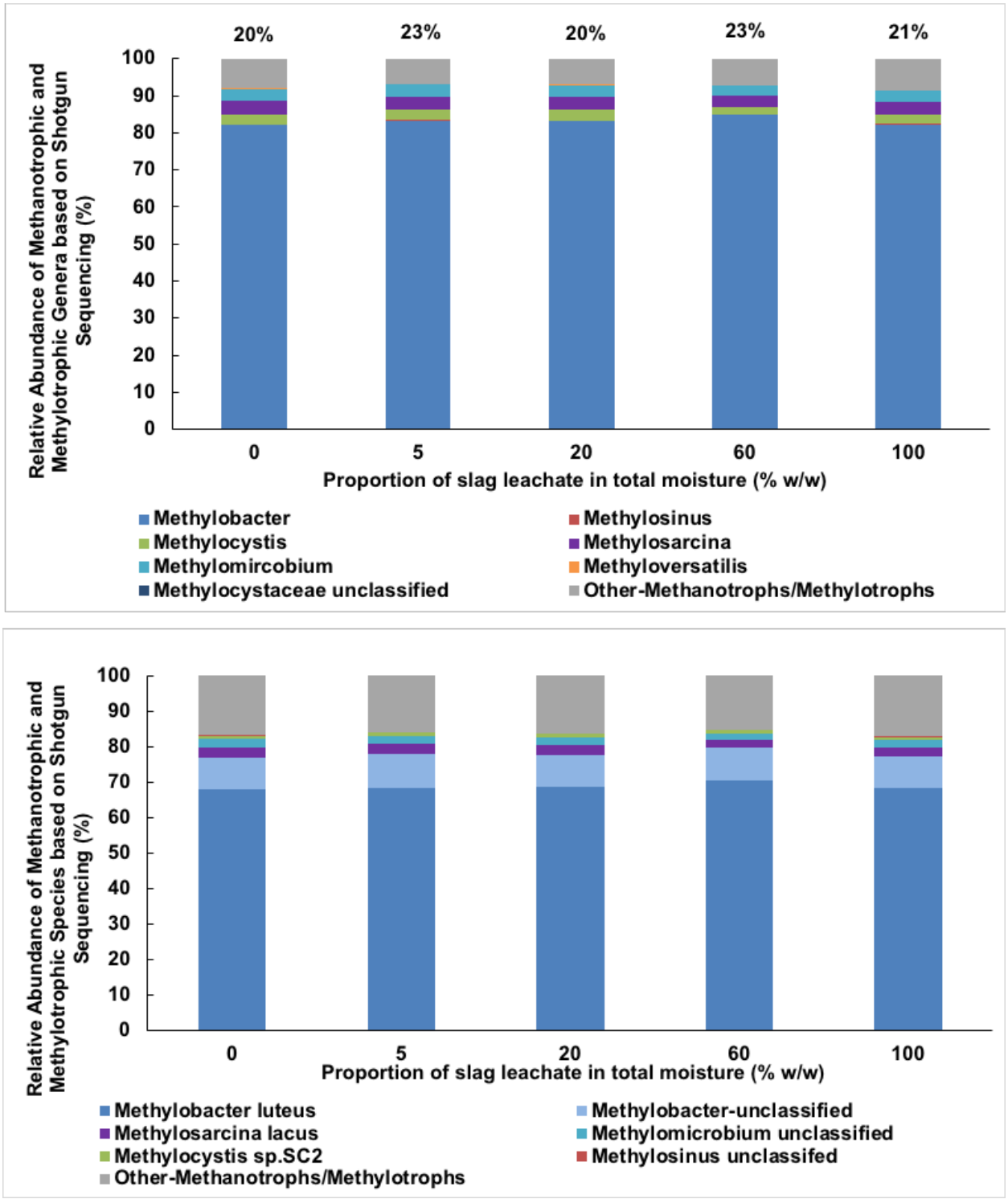


Fig. 7-3. Relative abundance (%) of sequences related to Methanotrophic and Methylotrophic community as determined by shotgun sequencing in soil microcosms, total of 20% moisture content (a) genera (b) species (percentage at top of each bars represent % of methanotrophic/methylotrophic community in total microbial community

Analysis of similarity (ANOSIM) was used to determine if microbial community structure was significantly different between groups in soil microcosms inoculated with slag leachate. The global ANOSIM R statistic was 0.119, with a p-value of 0.079 (999 permutations), indicating that microbial communities in soil microcosms were not significantly different between groups at different slag leachate proportions (**Fig. 7-5**). This is likely a result of the high buffering capacity of the soil, which shields the methanotrophic community from the leachate alkalinity. This finding suggests that in the field, any water infiltrating through the slag layer will not pose significant impact on the microbial activities in the biochar-amended soil layer. Pairwise comparisons also had R values ranging from -0.037 to 0.333, but due to the limited number of replicates, significance could not be properly assessed. Two-dimensional ordination resulted in substantial loss of information, as assessed by 2D stress value of 0.34.

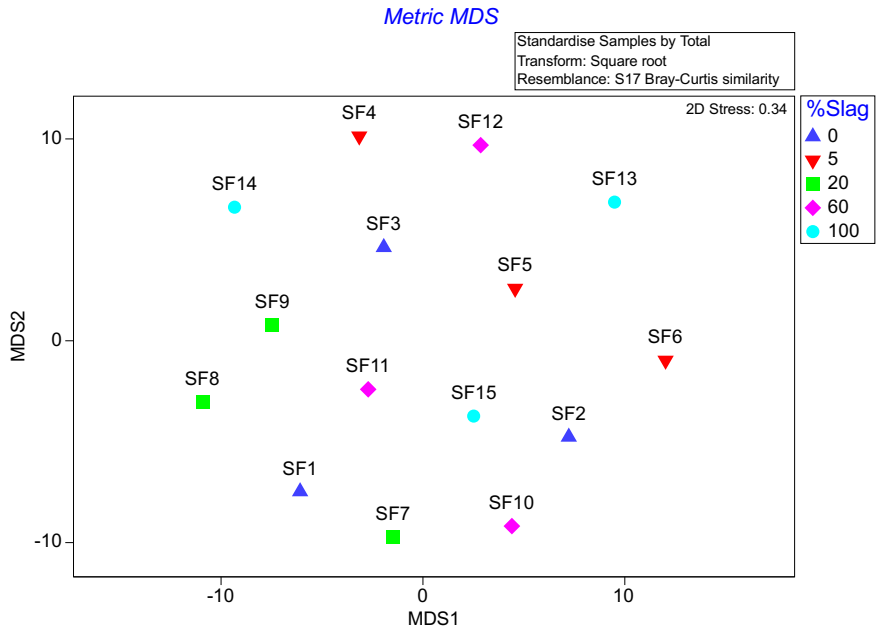
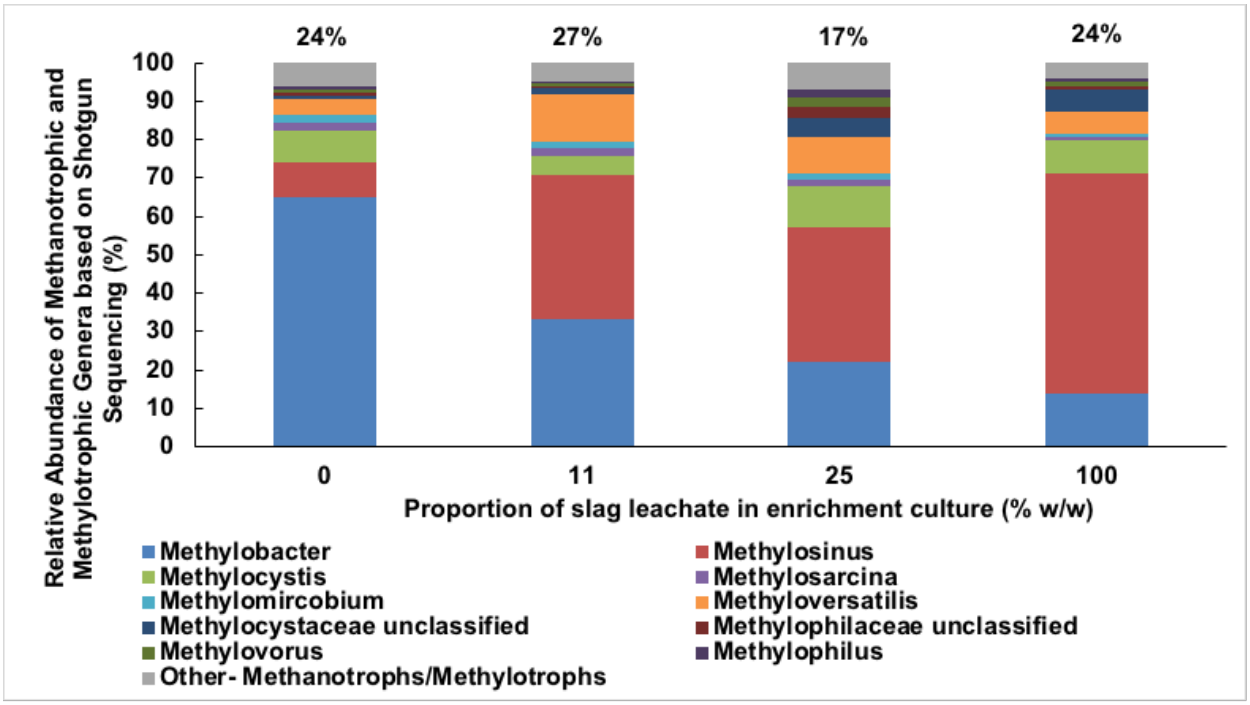


Fig. 7- 5. Metric multi-dimensional scaling (mMDS) plot of microbial community structure (taxonomic level of species) in soil microcosms inoculated with slag leachate proportions from 0 - 100% (n=3) using Bray-Curtis similarity metrics

Fig.7-4 shows the structure of methylotrophic communities in enrichment cultures inoculated with slag leachate at proportions varying from 0-100%. In contrast to soil microcosms, enrichment culture microbial communities were significantly affected by slag leachate proportions **(Fig. 7-4 and 7-6).** Methanotrophs of the genus *Methylobacter* and species *Methylobacter luteus* were most abundant in enrichments with no slag leachate, but the relative abundance of these organisms was significantly lower in enrichments with slag leachate (ANOVA, p=0.0075 and p=0.0017). The relative abundance of the genus *Methylobacter* decreased from 65% of all methylotrophic sequences to 33%, 22% and 14% in enrichments with 11%, 25% and 100% leachate respectively. However, the relative abundance of methylotroph sequences was consistent, ranging from 17-27% of all annotated sequences across all enrichment conditions (**Fig. 7-4**). The decrease in relative abundance of *Methylobacter* sequences was largely mirrored with an increase in sequences from bacteria of the genus *Methylosinus*. The relative abundance of the genus *Methylosinus* represented 9%, 37%, 35% and 57% of all methylotroph sequences at 0%, 11%, 25% and 100%, respectively. The shift in microbial community was associated with a decrease in CH_4 oxidation rates (**Fig.7- 2B)**, and this may be a result of different maximum oxidation rates of bacteria from the genera *Methylobacter* and *Methylosinus*, or the result of inhibition due to high initial pH levels in enrichments (**Table 7- 4**), or both. Slag leachate with elevated iron levels could have favored the growth of *Methylosinus* (a genus of type II methanotroph), as iron can serve as a cofactor for soluble methane monooxygenases (sMMO) (Park et al. 1991; Bowman and Sayler, 1994). Although methylotroph communities were dominated by bacteria from the genera *Methylobacter* and *Methylosinus*, other methanotrophic taxa were identified in the enrichment cultures, including *Methylosarcina* (Type I methanotrophs), *Methylomicrobium* (Type I methanotrophs) and *Methyloversatilis* (methylotrophs). Analysis of similarity (ANOSIM) was

used to determine if microbial community structure in enrichment cultures was significantly different between groups. The global ANOSIM R statistic was 1, with a p-value of 0.001 (999 permutations), indicating significant differences in microbial communities. Pairwise comparisons also had R values of 1, but due to the limited number of replicates, significance could not be properly assessed.

The results of both soil microcosm and enrichment culture tests demonstrate that the CH_4 oxidation rates were more strongly affected in the enrichment culture relative to soil, likely due to the elevated initial pH conditions induced by leaching of slag. In soil microcosms tests, a minimal pH change was observed due to the high buffering capacity of the soil, and CH_4 oxidation rates were largely stable across all leachate levels. Furthermore, the leachate had no significant effect on the methylotroph community composition in soil microcosms, though a strong effect was observed in enrichments. Thus, due to the high buffering capacity of the soil it is indicated that the slag leachate will have a minimal effect on the CH_4 oxidation potential of biochar-amended soil layers underlying the slag layer in the biogeochemical cover.



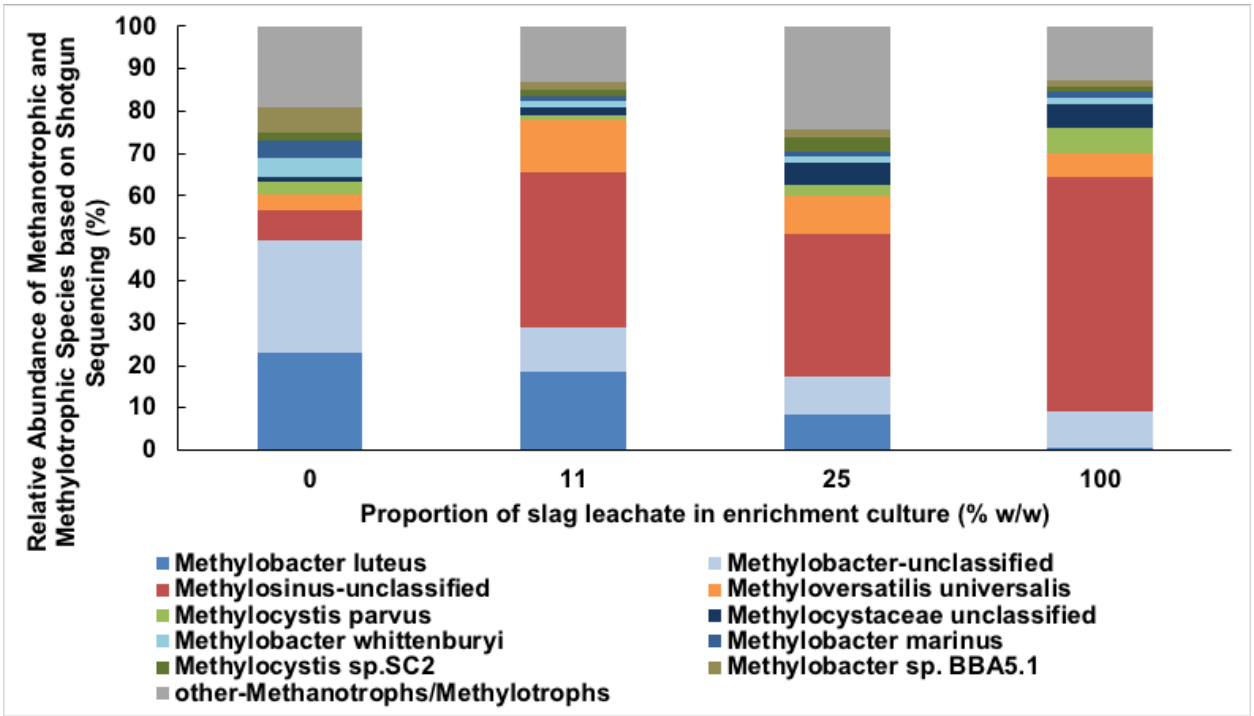


Fig. 7-4. Relative abundance (%) of sequences related to Methanotrophic and Methylotrophic community as determined by shotgun sequencing in enrichment culture (a) genera (b) species (percentage at top of each bars represent % of methanotrophic/methylotrophic community in total microbial community)

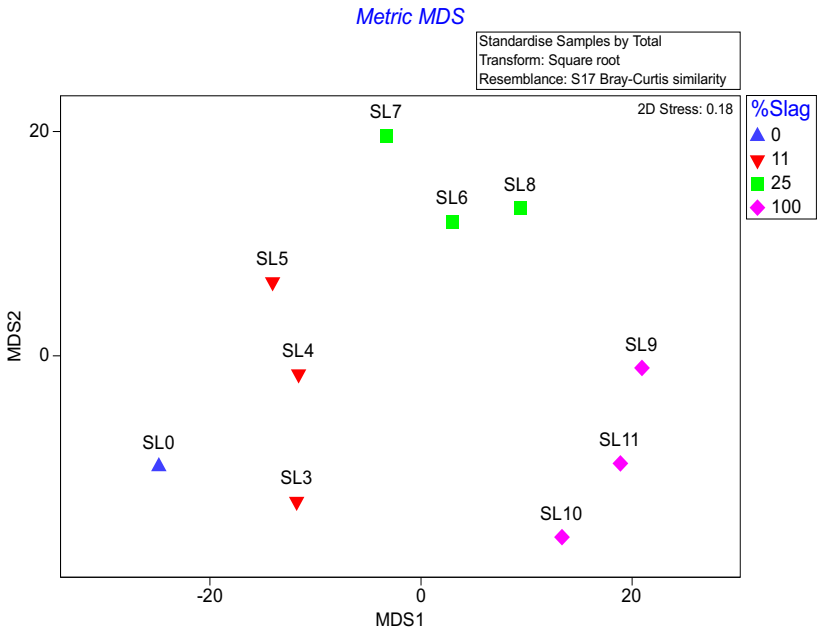


Fig.7- 6. Metric multi-dimensional scaling (mMDS) plot of microbial community structure (taxonomic level of species) in enrichment cultures inoculated with slag leachate proportions from 0 - 100% (n=1 or 3) using Bray-Curtis similarity metrics

7.4 CONCLUSIONS

An investigation of slag leachate on CH_4 oxidation and community composition was performed in soil and enrichment cultures. The soil microcosms and enrichment cultures responded differently to slag leachate, with soil microcosms largely unaffected. No significant change in oxidation rates and methylotrophic microbial community structure were observed in soil microcosms, and bacteria from the genus *Methylobacter* dominated. Conversely, enrichment culture showed a steep decline in oxidation rates with increased slag leachate proportions, and a concomitant shift in dominant methanotrophic taxon from the genus *Methylosinus* to the genus *Methylobacter*. The landfill soil is shown to possess significant buffering capacity which neutralizes the effect of elevated pH of the slag leachate, thereby facilitating effective CH_4 oxidation rates at varying slag leachate proportions. These results suggest that the use of BOF slag (isolated system) in the biogeochemical cover will be viable for maintaining CH_4 oxidation *in situ*. Long term column studies and field

scale studies are ongoing to evaluate the overall performance of CH_4 oxidation and CO_2 sequestration in the slag isolated biochar-amended soil cover systems.

7.5 CITED REFERENCES

Amaral, J. A., and Knowles, R. (1995). "Growth of methanotrophs in methane and oxygen counter gradients". *FEMS Microbiology Letters*, 126(3), 215-220.

Baesman, S., Miller, L., Wei, J., Cho, Y., Matys, E., Summons, R., and Oremland, R. (2015). "Methane oxidation and molecular characterization of methanotrophs from a former mercury mine impoundment". *Microorganisms*, 3(2), 290-309.

Bowden, L. I., Jarvis, A. P., Younger, P. L., and Johnson, K. L. (2009). "Phosphorus removal from waste waters using basic oxygen steel slag". *Environmental Science & Technology*, 43(7), 2476-2481.

Bowman, J. P., Sly, L. I., and Hayward, A. C. (1990). "Patterns of tolerance to heavy metals among methane-utilizing bacteria". *Letters in Applied Microbiology*, 10(2), 85-87.

Bowman, J. P., SLY, L. I., NICHOLS, P. D., and Hayward, 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 and Evolutionary Microbiology*, 43(4), 735-753.

Bowman, J. P., and Sayler, G. S. (1994). "Optimization and maintenance of soluble methane monooxygenase activity in Methylosinus trichosporium OB3b". *Biodegradation,* 5(1), 1-11.

Chand, S., Paul, B., and Kumar, M. (2017). "Short-term leaching study of heavy metals from LD slag of important steel industries in Eastern India". *Journal of Material Cycles and Waste*

Management, 19(2), 851-862.

Chaurand, P., Rose, J., Briois, V., Olivi, L., Hazemann, J. L., Proux, O., and Bottero, J. Y. (2007). "Environmental impacts of steel slag reused in road construction: A crystallographic and molecular (XANES) approach". *Journal of Hazardous Materials*, 139(3), 537-542

Chen, Y., Dumont, M. G., Cébron, A., and Murrell, J. C. (2007). "Identification of active methanotrophs in a landfill cover soil through detection of expression of 16S rRNA and functional genes". *Environmental Microbiology*, 9(11), 2855-2869.

Chi, Z. F., Lu, W. J., and Wang, H. T. (2015). "Spatial patterns of methane oxidation and methanotrophic diversity in landfill cover soils of Southern China". *Journal of Microbiology & Biotechnology*, 25(4), 423-430.

Clarke, K. R., and R. N. Gorley. "Getting started with PRIMER v7". PRIMER-E: Plymouth, Plymouth Marine Laboratory (2015)

Contin, M., Goi, D., and De Nobili, M. (2012). "Land application of aerobic sewage sludge does not impair methane oxidation rates of soils". *Science of the Total Environment*, 441, 10-18.

De Windt, L., Chaurand, P., and Rose, J. (2011). "Kinetics of steel slag leaching: batch tests and modeling". *Waste Management*, 31(2), 225-235.

Effler, S.W., Matthews, D.A. (2003). "Impacts of a soda ash facility on Onondaga Lake and the Seneca River, NY". *The Lake Reservoir & Management*, 19, 285e306.

Hanson, R. S., and Hanson, T. E. (1996). "Methanotrophic bacteria". *Microbiol. Molecular. Biology. Review*, 60(2), 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". *Environmental Microbiology*, 2(6), 666-679.

Huijgen, W. J., Witkamp, G. J., and Comans, R. N. (2005). "Mineral CO_2 sequestration by steel slag carbonation". *Environmental Science & Technology*, 39(24), 9676-9682.

Kalyuzhnaya, M. G., Khmelenina, V., Eshinimaev, B., Sorokin, D., Fuse, H., Lidstrom, M., and Trotsenko, Y. (2008). "Classification of halo (alkali) philic and halo (alkali) tolerant methanotrophs provisionally assigned to the genera Methylomicrobium and Methylobacter and emended description of the genus Methylomicrobium". *International Journal of Systematic and Evolutionary Microbiology*, 58(3), 591-596.

Khmelenina, V. N., Kalyuzhnaya, M. G., Starostina, N. G., Suzina, N. E., and Trotsenko, Y. A. (1997). "Isolation and characterization of halotolerant alkaliphilic methanotrophic bacteria from Tuva soda lakes". *Current Microbiology*, 35(5), 257-261.

Minot, S. S., Krumm, N., & Greenfield, N. B. (2015). "One codex: A sensitive and accurate data platform for genomic microbial identification". *BioRxiv*, 027607.

Mohanty, S. R., Bharati, K., Deepa, N., Rao, V. R., and Adhya, T. K. (2000). "Influence of heavy metals on methane oxidation in tropical rice soils". *Ecotoxicology and Environmental Safety*, 47(3), 277-284.

Motz, H., and Geiseler, J. (2001). "Products of steel slags an opportunity to save natural resources". *Waste Management*, 21(3), 285-293.

Park, J. R., Moon, S., Ahn, Y. M., Kim, J. Y., and Nam, K. (2005). "Determination of environmental factors influencing methane oxidation in a sandy landfill cover soil". *Environmental Technology*, 26(1), 93-102.

Park, S., Hanna, L., Taylor, R. T., and Droege, M. W. (1991). "Batch cultivation of Methylosinus trichosporium OB3b. I: Production of soluble methane monooxygenase". *Biotechnology and Bioengineering*, 38(4), 423-433.

Proctor, D. M., Fehling, K. A., Shay, E. C., Wittenborn, J. L., Green, J. J., Avent, C., and Zak, M. A. (2000). "Physical and chemical characteristics of blast furnace, basic oxygen furnace, and electric arc furnace steel industry slags". *Environmental Science & Technology*, 34(8), 1576-1582.

Rai, R. K., Chetri, J. K., Green, S. J., and Reddy, K. R. (2018). "Identifying active methanotrophs and mitigation of CH_4 emissions in landfill cover soil". *Proceedings International Congress on Environmental Geotechnics* (pp. 308-316). Springer, Singapore.

Reddy, K. R., Grubb, D. G., and Kumar, G. (2018). "Innovative biogeochemical soil cover to mitigate landfill gas emissions". *Proceedings International Conference on Protection and Restoration of the Environment XIV*, Thessaloniki, Greece.

Reddy, K. R., Gopakumar, A., and Chetri, J. K., (2019, a). "Critical review of applications of iron and steel slags for carbon sequestration and environmental remediation". *Reviews in Environmental Science and Bio/Technology*, DOI: 10.1007/s11157-018-09490-w.

Reddy, K. R., Chetri, J. K., Kumar, G., and Grubb, D. G. (2019, b). "Effect of basic oxygen furnace slag type on carbon dioxide sequestration from landfill gas emissions". *Waste Management*, 85, 425-436.

Reddy, K. R., Gopakumar, A., Rai, R. K., Kumar, G., Chetri, J. K., and Grubb, D. G. (2019, c). "Effect of basic oxygen furnace slag particle size on sequestration of carbon dioxide from landfill gas". *Waste Management & Research*, 0734242X18823948

Roadcap, G. S., Sanford, R. A., Jin, Q., Pardinas, J. R., and Bethke, C. M. (2006). "Extremely alkaline (pH> 12) ground water hosts diverse microbial community". *Groundwater*, 44(4), 511-517.

Saha, N., Kharbuli, Z.Y., Bhattacharjee, A., Goswami, C., Heaussinger, D. (2002). "Effect of

alkalinity (pH 10) on ureogenesis in the air-breathing walking catfish, Clarias batrachus". *Comparative Biochemistry & Physiology Part A*, 132, 353e364.

Semrau, J. D., DiSpirito, A. A., and Yoon, S. (2010). "Methanotrophs and copper". *FEMS Microbiology Reviews*, 34(4), 496-531.

Walkiewicz, A., Bulak, P., Brzezińska, M., Wnuk, E., and Bieganowski, A. (2016). "Methane oxidation in heavy metal contaminated mollic gleysol under oxic and hypoxic conditions". *Environmental Pollution*, 213, 403-411.

Whalen, S. C., Reeburgh, W. S., and Sandbeck, K. A. (1990). "Rapid methane oxidation in a landfill cover soil". *Applied Environmental Microbiology*, 56(11), 3405-3411.

Wnuk, E., Walkiewicz, A., and Bieganowski, A. (2017). "Methane oxidation in lead-contaminated mineral soils under different moisture levels". *Environmental Science and Pollution Research*, 24(32), 25346-25354.

Yargicoglu, E. N., and Reddy, K. R. (2017 a). "Microbial abundance and activity in biochar-amended landfill cover soils: evidence from large-scale column and field experiments". *Journal of Environmental Engineering*, 143(9), 04017058

Yargicoglu, E. N., and Reddy, K. R. (2017 b). "Biochar-amended soil cover for microbial methane oxidation: effect of biochar amendment ratio and cover profile". *Journal of Geotechnical and Geoenvironmental Engineering*, 144(3), 04017123.

CHAPTER 8 - EFFECT OF METHANOTROPHIC-ACTIVATED BIOCHAR-AMENDED SOIL IN MITIGATING METHANE EMISSIONS FROM LANDFILLS

8.1 INTRODUCTION

The content of this chapter has been previously published by Rai, Chetri and Reddy (2019) during the author's master's thesis work [Previously published as Rai. R. K, Chetri, J. K, and Reddy, K. R. (2019) Effect of methanotrophic-activated biochar amended soil in mitigating CH_4 emissions from landfills, Proc. 4th International Conference on Civil and Environmental Geology and Mining Engineering, Trabzon, Turkey, 20-22 April 2019.]

Municipal solid waste (MSW) landfills are regarded as the third largest anthropogenic source of methane (CH_4) emissions in the United States. The landfill gas (LFG), generated due to the anaerobic decomposition of the organic fraction in the waste, typically comprises of 50% CH_4 and 50% CO_2 (carbon dioxide) by volume, both of which are major greenhouse gases causing global climate change. The CH_4 emissions from the landfills are known to be partially converted to CO_2 by the naturally existing CH_4 oxidizing bacteria (methanotrophs) in the cover soil. For nearly two decades, many researchers investigated the CH_4 oxidation capacity of the landfill cover soils based on batch tests, small-scale to near full-scale column tests, and field-scale tests (Sadasivam and Reddy, 2014).

In recent years, a number of studies have investigated a variety of amendments to landfill cover soil to enhance CH_4 oxidation and promote microbial activity (Mor et al., 2006; Stern et al., 2007; Scheutz et al., 2011; Pedersen et al., 2011; Sadasivam and Reddy, 2014). Previous research indicates organic amendments such as compost or biosolids can increase CH_4 oxidation rates by enhancing the growth of methanotrophs (Wilshusen et al., 2004; Stern et al., 2007; Scheutz et al., 2009). However, the use of compost over long term is susceptible to degradation and has been

identified with performance issues such as pore clogging due to exopolymeric substance (EPS) formation and reduced activity due to heterotrophic bacteria (Sadasivam and Reddy, 2014). Hence, a more stable material "biochar", which is less prone to degradation and has good physico-chemical properties supporting microbial growth, was investigated in our laboratory as a soil amendment in biocovers (Reddy et al., 2014). Biochar is a carbon-rich solid product obtained from pyrolysis or gasification in the absence of oxygen. Studies from our laboratory have demonstrated biochar amendment to be effective in increasing methanotrophic population and promoting CH_4 oxidation in the long term due to its high porosity and surface area, which makes it a suitable habitat for methanotrophic bacterial growth and multiplication (Yaghoubi, 2012; Reddy et al., 2014; Yargicoglu et al., 2015; Yargicoglu and Reddy, 2017). However, microbial colonization and acclimatization in the biochar-amended soil was found to take relatively longer time in oxidizing CH_4. The present study investigates the use of methanotrophic-activated biochar-amended soil in comparison to non-activated biochar-amended soil in order to expedite the CH_4 oxidation process in the landfill biocovers and mitigate CH_4 emissions in MSW landfills.

8.2 MATERIALS AND METHODOLOGY

8.2.1 Soil

Soil was collected from Zion landfill site, located in Zion, Illinois, USA. Soil samples were collected from an interim cover at a depth of ~1 to 2 feet and were shipped to the Geotechnical and Geoenvironmental Engineering Laboratory at the University of Illinois at Chicago (UIC) where it was stored at room temperature (23 ± 2°C). Soil samples were air dried (moisture content <0.5%), pulverized and screened through a 2 mm sieve prior to conducting the experiments.

8.2.2 Biochar

Biochar was obtained from a commercial vendor in Illinois, USA. The biochar used in this study was produced from waste pinewood subjected to gasification at a high temperature of ~520°C. In this study, biochar in pellet form was used with fines sieved and discarded. The biochar was oven-dried at 105°C to remove any moisture content before conducting the experiments.

8.2.3 Methanotrophic Culture Consortium

The methanotrophic mixed culture consortium was prepared by enriching landfill cover soil in modified Nitrate Mineral Salts (NMS) (Whittenbury et al.,1997) and a mixture of 7% CH_4, 7% CO_2 balanced in air at a room temperature of 23°C. The culture consortium was then used for microbial colonization in the biochar.

8.2.4 Activating Biochar with Culture Consortium

The biochar was activated with methanotrophs by inoculating 10 g of biochar pellets in 10 mL of culture consortium, in the presence of 5% (v/v) CH_4 and 5% (v/v) CO_2 in 90% of air and was incubated at a room temperature of 23°C under static condition. The headspace gas concentrations were monitored regularly by collecting and analyzing the gas samples using gas chromatography (GC) until the headspace concentration dropped to less than 1%. The CH_4 oxidation rates were calculated from the linear regression analysis of CH_4 concentration versus elapsed time, based on the zero-order kinetics.

8.2.5 Long Term Batch Incubation Tests

For these tests, 10 g of the total material was placed in 125 mL-serum vials and the moisture content was adjusted to 40% (w/w) using deionized water (field capacity of the soil), except activated biochar that was soaked in the culture. The soil was amended with 2% and 10% (w/w) of non-activated biochar or methanotrophic-activated biochar. The vials were sealed airtight using butyl rubber septa and secured using crimp caps. Next, 20 mL of air from the headspace was replaced with equal volume of synthetic landfill gas comprising of 50% (v/v) CH_4 and 50% (v/v) CO_2 to achieve a headspace concentration of ~5-6% CH_4 (v/v), ~5-6% CO_2 (v/v) and a balance (~88-90%) of air. The change in the headspace gas concentrations were determined by collecting and analyzing the gas samples on a regular basis using gas chromatography (GC) until the headspace concentration dropped to less than 1%. Each time the vials were flushed with air to remove the CO_2 produced and replenished with ~5-6% (v/v) CH_4 and ~5-6% (v/v) CO_2 in ~88-90% of air to analyze the long-term microbial activity and evaluate the oxidation rates. All the experiments were conducted in triplicate along with the controls (with synthetic LFG without any material).

8.2.6 Gas Analysis

The gas samples were analyzed at regular time intervals using an SRI 9300 GC equipped with a thermal conductivity detector (TCD) and CTR-1 column capable of separating CH_4 and CO_2 as previously described (Yargicoglu and Reddy, 2017). Gas samples were withdrawn using 1 mL syringe where 0.5 mL of the sample was discarded and remaining 0.5 mL was injected into the GC to reduce any pressure effects due to sampling. A calibration curve for a minimum of three

points was established using high purity standard gas mixtures ranging from 1% to 50% (v/v) CH_4 and CO_2.

8.3 RESULTS AND DISCUSSION

Figure 8-1 shows typical CH_4 removal response by the methanotrophic-activated biochar. As seen, a gradual decrease in the headspace CH_4 concentration with time was observed in the first stage (before second replenishment). A second replenishment with the mix gas (CH_4/CO_2/Air) was performed on 7th day of the experiment to keep the microbes active and to analyze the CH_4 uptake trend in long term. The methanotrophic-activated biochar showed similar CH_4 uptake rate as in the first phase (before replenishment), indicating that the biochar was successfully colonized with methanotrophs and were not affected by substrate diffusion, thereby persisting CH_4 oxidation.

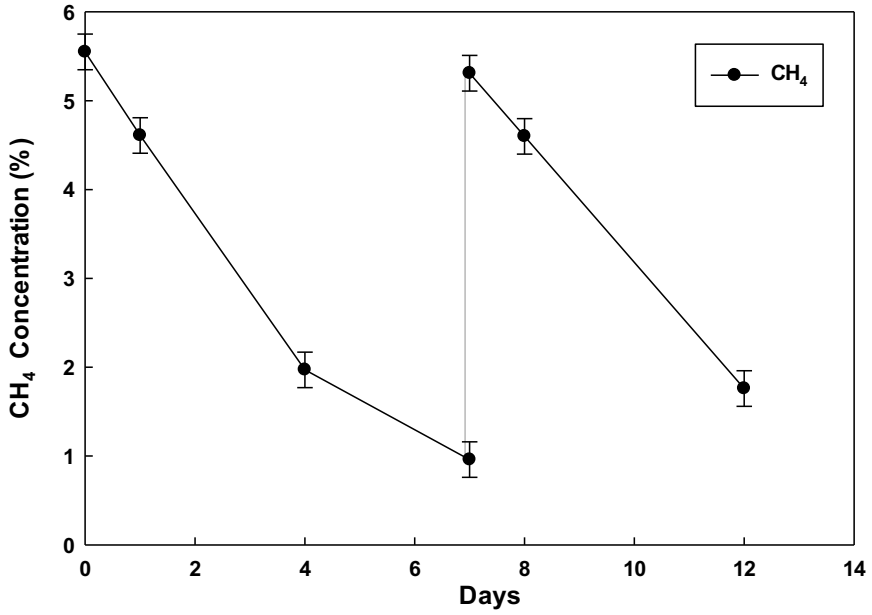


Figure 8-1 Typical methane removal response by methanotrophic-activated biochar

The long-term experiments were conducted in three phases: Phase I, Phase II, and Phase III. These phases and the corresponding results are described below:

8.3.1 Phase I Testing

In Phase I, the experiments were carried out for ~90-95 days. The CH_4 uptake/consumption and rates were calculated for each replenishment. The following experimental sets were investigated: soil, biochar-amended soil (2% w/w), biochar-amended soil (10% w/w), methanotrophic-activated biochar-amended soil (2% w/w), and methanotrophic-activated biochar-amended soil (10% w/w).

Figure 8-2(a) compares the cumulative uptake of CH_4 in the first 30 days for each experimental set. The methanotrophic-activated biochar-amended soil showed highest CH_4 uptake among all the experimental sets; the test with 10% methanotrophic-activated biochar-amended soil exhibiting highest CH_4 uptake (3371 µg CH_4 g^{-1}) followed by the 2% methanotrophic-activated biochar-amended soil (2341 µg CH_4 g^{-1}). The soil alone and 2% biochar-amended soil showed similar uptakes (1323 and 1311 µg CH_4 g^{-1}, respectively), and the 10% biochar-amended soil showed an uptake of 1278 µg CH_4 g^{-1}. The results from the methanotrophic-activated biochar suggests that the methanotrophs colonized in the biochar were in their growth phase and were able to oxidize CH_4 when amended with soil without substrate limitation to the microbes. On the other hand, in the biochar-amended soil, the CH_4 oxidizing bacteria present in the soil were not acclimated to the biochar, which could be the reason for the lower CH_4 uptake.

The CH_4 oxidation rates for all the experimental sets were calculated based on the zero-order kinetics. The average CH_4 oxidation rates for the initial 30 days in soil, 2% biochar-amended soil, 10% biochar-amended soil, 2% methanotrophic-activated biochar-amended soil, and 10% methanotrophic-activated biochar-amended soil were 46.4 µg CH_4 g^{-1} d^{-1}, 45.6 µg CH_4 g^{-1} d^{-1}, 36 µg CH_4 g^{-1} d^{-1}, 81.8 µg CH_4 g^{-1} d^{-1} and 111.8 µg CH_4 g^{-1} d^{-1}, respectively. The CH_4 oxidation rates in soil and biochar-amended soil were similar which implies that the biochar was not initially colonized with the methane oxidizing bacteria (MOB), thereby showing similar trend as the soil.

Whereas, in the methanotrophic-activated biochar-amended soil, the CH_4 oxidation rates were ~1.7 – 3.1 times of soil/biochar-amended soil due to the combined effect of colonization of the microbes in the biochar and the pre-existing MOB in the soil resulting in enhanced CH_4 uptake in the system.

Figure 8-2(b) shows the cumulative uptake of CH_4 in the experimental sets extended to time interval of 60 days. The 10% methanotrophic-activated biochar-amended soil showed continued increase in the CH_4 uptake (7287 μg CH_4 g^{-1}) followed by the 2% methanotrophic-activated biochar-amended soil (4466 μg CH_4 g^{-1}). Both of the biochar-amended soil sets (2% and 10%) showed similar cumulative CH_4 uptake (3243 μg CH_4 g^{-1}) and the soil alone system showed the lowest CH_4 uptake (2727 μg CH_4 g^{-1}) among all. The CH_4 oxidation rates also increased significantly at an interval of 60 days and resulted to be 139.2 μg CH_4 g^{-1} d^{-1} in 10% methanotrophic-activated biochar-amended soil and 92 μg CH_4 g^{-1} d^{-1} in 2% methanotrophic-activated biochar-amended soil. The 10% and 2% biochar-amended soil showed significant increase in the CH_4 oxidation rates from 36.01 μg CH_4 g^{-1} d^{-1} to 68.1 μg CH_4 g^{-1} d^{-1} and 45.6 μg CH_4 g^{-1} d^{-1} to 67.4 μg CH_4 g^{-1} d^{-1}, respectively. It suggests colonization of the MOB in the biochar in the long run thereby amplifying CH_4 oxidation rates. On the other hand, the soil alone system did not show significant increase in CH_4 oxidation rates (from 46.44 μg CH_4 g^{-1} d^{-1} to 49.8 μg CH_4 g^{-1} d^{-1}) which further confirms the role of biochar in the colonization of MOB in the long run.

Figure 8-2(c) shows the cumulative uptake of CH_4 in the experimental sets for further extended time interval of 90 days for soil and biochar-amended soils. The 10% biochar-amended soil showed significant increase in the CH_4 uptake (7221 μg CH_4 g^{-1}) when compared to 2% biochar-amended soil (6279 μg CH_4 g^{-1}) and soil alone system (5599 μg CH_4 g^{-1}). The corresponding CH_4 oxidation rates at this time interval were 140.7 μg CH_4 g^{-1} d^{-1}, 104.8 μg CH_4

g^{-1} d^{-1} and 98.5 µg CH_4 g^{-1} d^{-1}. The biochar-amended soil showed significant increase in the CH_4 uptake and oxidation rates in the long run which is in agreement with the previous study from our lab by Yargicoglu and Reddy (2017). It is to be noted that no data was available for methanotrophic-activated biochar-amended soil at this time interval, as these experimental sets were started 30 days later than the tests with soil and biochar-amended soil systems.

8.3.2 Phase II Testing

In Phase II, all the experimental sets were incubated by flushing the gas mixture of CH_4/CO_2/air on a weekly basis at a room temperature of 23°C without analyzing the samples for a period of 2 months. This phase allowed a long-term incubation of methanotrophs in the experimental sets.

8.3.3 Phase III Testing

In Phase III, the gas samples from all the experimental sets were analyzed on a regular basis to evaluate the performance of these systems after long-incubation period during Phase II. **Figure 8-3** shows the cumulative CH_4 uptake in the experimental sets after Phase II incubation period for an interval of 30 days. These results show that the 10% and 2% methanotrophic-activated biochar-amended soil systems continued to consume CH_4 at a faster rate when compared to the results from Phase I with a cumulative CH_4 uptake of 16039 µg CH_4 g^{-1} and 5969 µg CH_4 g^{-1}, respectively. However, soil and biochar-amended soil showed reduced and steady uptake of CH_4 throughout phase III, with a total CH_4 uptake of 3924 µg CH_4 g^{-1}, 2960 µg CH_4 $g^{-1}$and 2756 µg CH_4 g^{-1} in 10% biochar-amended soil, 2% biochar-amended soil and soil system, respectively. The plausible explanation for reduced CH_4 uptake during post-incubation could be that the microbes may have reached their capacity to further consume the substrates and reached stationary or death phase

following a typical bacterial growth curve. The CH_4 oxidation rates in 2% (169.2 μg CH_4 g^{-1} d^{-1}) and 10% (518.6 μg CH_4 g^{-1} d^{-1}) methanotrophic-activated biochar-amended soil were ~1.8–3.7 times the rates before incubation period (Phase I). Whereas, in soil and biochar-amended soil, the CH_4 oxidation rates declined after 2 months of incubation to a steady state condition with CH_4 oxidation rate of 88.3 μg CH_4 g^{-1} d^{-1}, 97.4 μg CH_4 g^{-1} d^{-1} and 116.1 μg CH_4 g^{-1} d^{-1} in soil alone, 2% biochar-amended soil and 10% biochar-amended soil, respectively. Similar trends of peak oxidation rates followed by a decline in the oxidation rates leading to a lower steady state values were reported in many column studies (Kightley et al., 1995; Hilger et al., 1999; Scheutz and Kjeldsen, 2003; Streese and Stegmann, 2003; Wilshusen et al., 2004; Yargicoglu and Reddy, 2017) and was attributed to the production of EPS, impeding substrate/nutrients transfer to the microbes (Hilger et al., 2000) or loss of moisture content. Our studies cannot confirm if production of EPS had limited CH_4 oxidation capacity in the soil or biochar-amended soil. Therefore, further tests determining the production of EPS are needed. However, loss of moisture due to air flushing from our samples that extended for > 150 days could be one of the factors causing decline in the CH_4 oxidation rates. As the final moisture content of all the samples at the end of the experiment showed significant loss of moisture by ~13-18%.

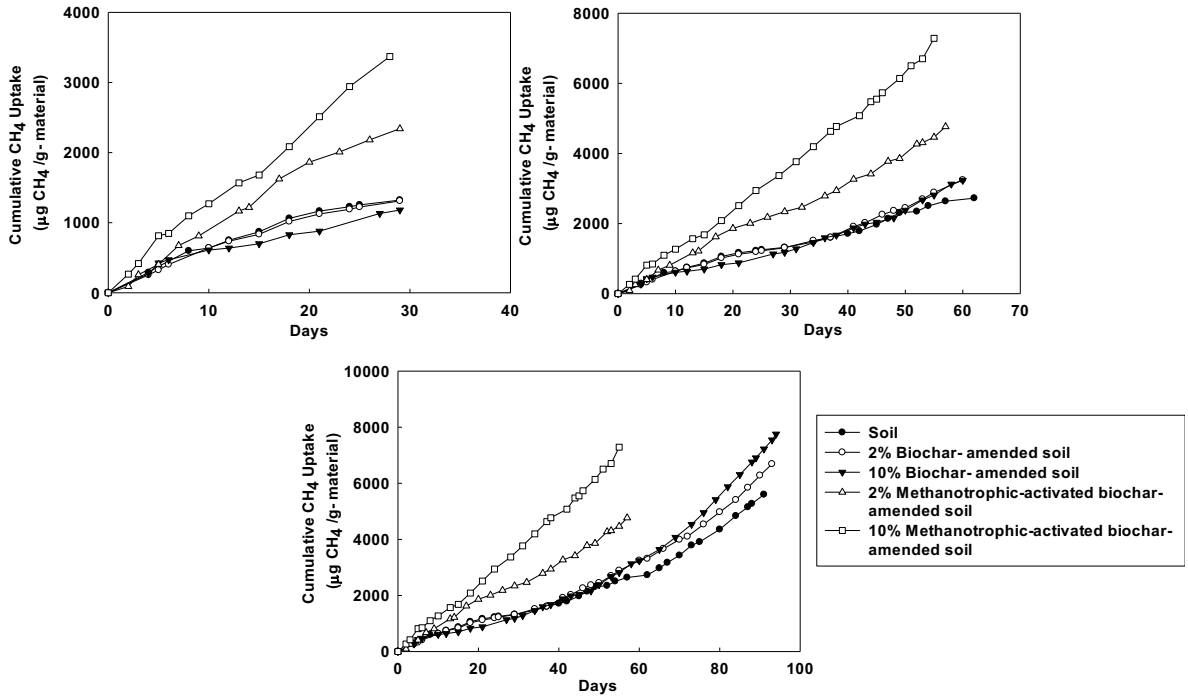


Figure 8-2. Phase I testing showing cumulative uptake of methane in soil, 2% biochar-amended soil, 10% biochar-amended soil, 2% methanotrophic-activated biochar-amended soil, and 10% methanotrophic-activated biochar-amended soil during test duration of: (a) 30 days, (b) 60 days, and (c) 90 days

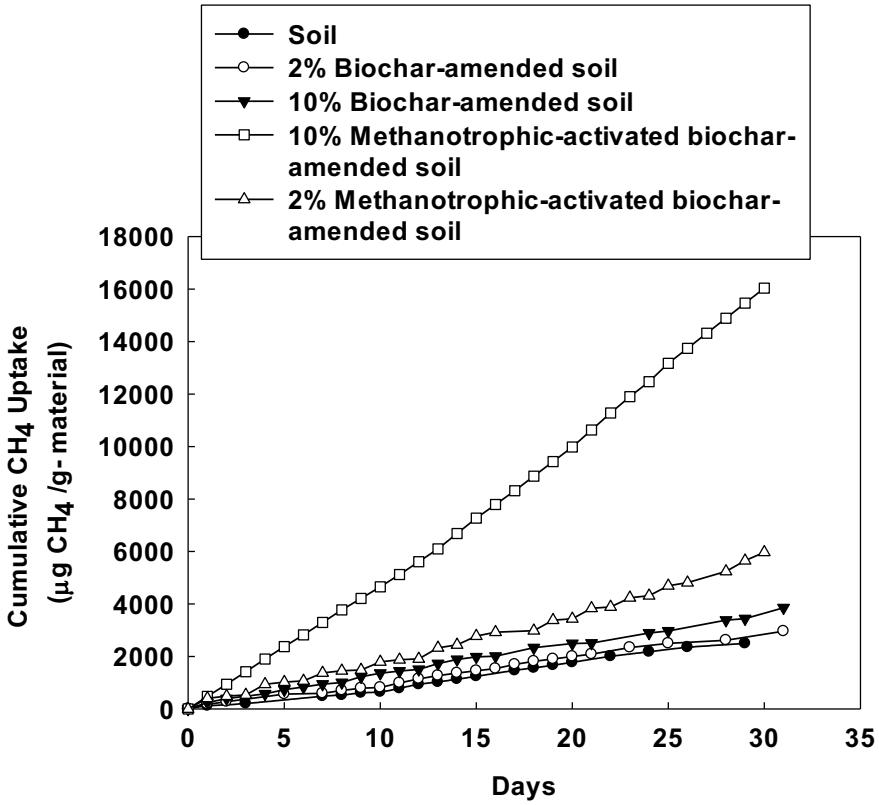


Figure 8-3. Phase III testing showing cumulative uptake of methane: soil, 2% biochar-amended soil, 10% biochar-amended soil (10% w/w), 2% methanotrophic-activated biochar-amended soil, and 10% methanotrophic-activated biochar-amended soil

Figure 8-4 shows the average CH_4 uptake rates for each gas replenishments with respect to time in soil, biochar-amended soil (2% & 10%), methanotrophic-activated biochar-amended soil (2% & 10%). All the systems showed decline or steady state condition in the beginning of the tests for a period of 20-30 days reflecting adaptation stages in the microbial growth (**Figure 8-4**). Thereafter, the samples showed increase in the oxidation rates that follows typical growth phase in the bacteria. After two months of incubation, all the experimental sets showed a decline or steady state in the oxidation rates which was followed by a stationary phase similar to the bacterial growth curve, except 10% methanotrophic-activated biochar-amended soil which showed increased uptake rates. The reason could be that the microbes were still in their growth phase and had not reached stationary phase.

Overall, these results demonstrate that the methanotrophic-activated biochar-amended soil showed significant potential in accelerating the CH_4 removal process when compared to soil or biochar-amended soil that takes time for colonization and acclimatization in the biochar. Similarly, 10% methanotrophic-activated biochar-amended soil performed better than the 2% methanotrophic-activated biochar-amended soil in removing CH_4 from the systems which suggests higher proportion of biochar amendment is beneficial in enhancing CH_4 oxidation.

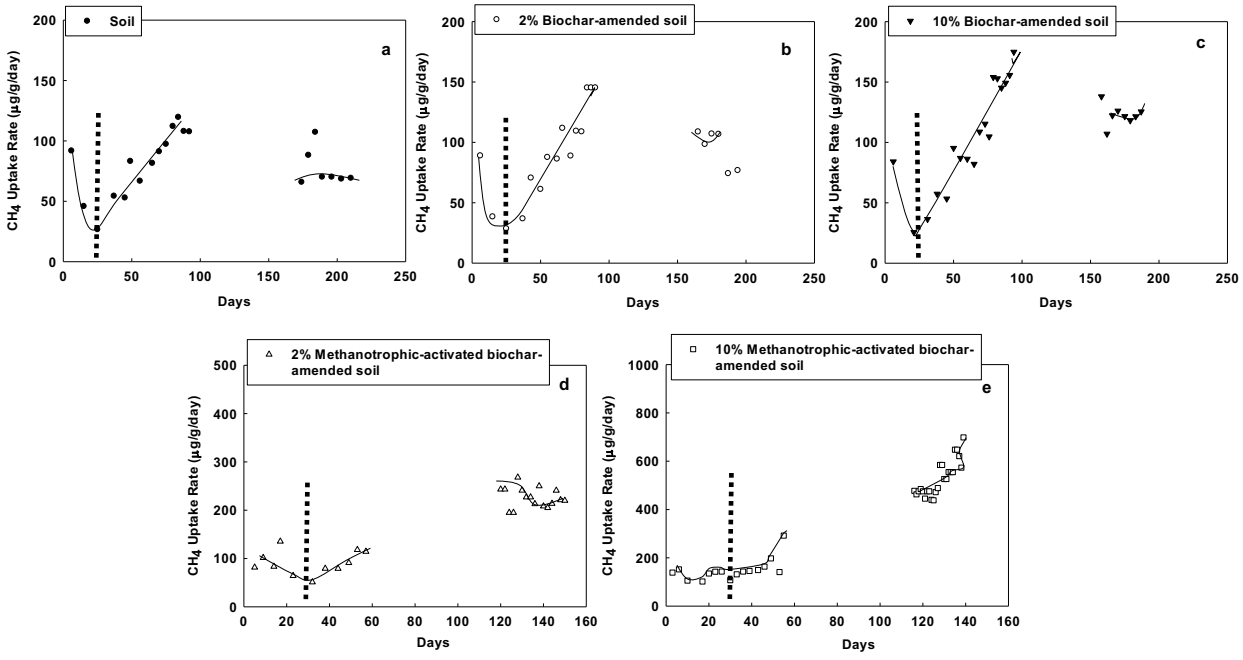


Figure 8-4. Average methane uptake rates for each gas replenishment with time in (a) soil, (b) biochar-amended soil (2% w/w), (c) biochar-amended soil (10% w/w), (d) methanotrophic-activated biochar-amended soil (2% w/w), and (e) methanotrophic-activated biochar-amended soil (10% w/w)

8.3.4 Methylotroph microbial community structure in soil and activated/non-activated biochar-amended soil

The microbial community in all the samples were initially tested for RNA sequencing. Due to very low biomass and difficulties in extracting nucleic acids, RNA sequencing failed in majority of the samples. The samples were then extracted for DNA followed by PCR amplification of 16S rRNA gene sequencing to identify the overall microbial community as previously described in Chapter 4. **Figure. 8-5** shows methylotrophic and methanotrophic community composition in soil, biochar-amended soil and methanotrophic activated biochar-amended soil. It was observed that the methylotroph sequences in these systems represented 10-12% of all annotated 16S rRNA gene sequences. Both biochar-amended soil and methanotrophic-activated biochar-amended soil showed similar community composition except the species *Methylobacillus-other* (2% and 9%) that were identified at 2% and 10% methanotrophic activated biochar-amended soil only. The most abundant species observed in these samples belong to the family Methylophilaceae and Methylomonaceae, taxa (genus and species) of which was not identified; followed by species from the genus *Methylocystis* and *Methylobacter*. Sequences derived from Methylophilaceae accounted for 31%, 35%, 31%, 21%, 15% of all methylotroph 16S rRNA sequences in soil (n= 2), 2% biochar-amended soil (n=1), 10% biochar-amended soil (n =2), 2% methanotrophic activated biochar-amended soil (n=1), and 10% methanotrophic activated biochar-amended soil (n=2), respectively. Methylomonaceae accounted for 23%,18%, 20%, 27% and 31% of all methylotroph 16S rRNA sequences in soil, 2% biochar-amended soil, 10% biochar-amended soil, 2% activated biochar-amended soil, and 10% activated biochar-amended soil, respectively. This study shows that activated biochar-amended soil (2% and 10%) showed similar community composition as soil

and biochar-amended soil. However, due to failure in extracting nucleic acids for RNA sequencing, this study fails in determining the active species behind CH_4 oxidation process.

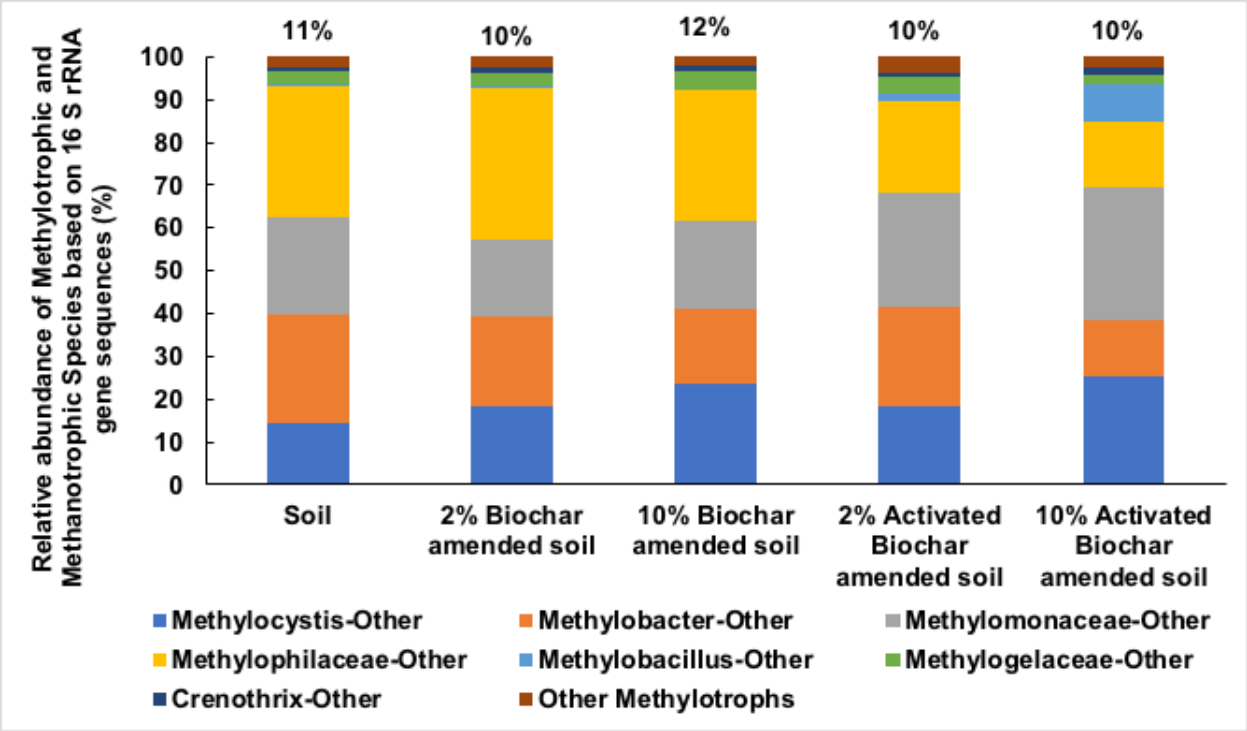


Figure 8.5. Relative abundance (%) of sequences related to Methanotrophic and Methylotrophic species as determined by 16S rRNA sequencing: soil, 2% biochar-amended soil, 10% biochar-amended soil (10% w/w), 2% methanotrophic-activated biochar-amended soil, and 10% methanotrophic-activated biochar-amended soil (percentage at top of each bars represent % of methanotrophic/methylotrophic community in total microbial community)

8.4 CONCLUSIONS

The biochar-amended soil and methanotrophic-activated biochar-amended soil at two different proportions (2% and 10%) were assessed to study the CH_4 uptake or removal capacity. The results demonstrate that the methanotrophic-activated biochar-amended soil had significant potential in the removal or uptake of CH_4 when compared to non-activated biochar-amended soil. Of which, the 10% methanotrophic-activated biochar-amended soil showed improved uptake of CH_4 over 2% methanotrophic-activated biochar-amended soil. The CH_4 oxidation rates at the end of the

study resulted to be in the following order 518.6 µg CH_4 g^{-1} d^{-1} >169.2 µg CH_4 g^{-1} d^{-1} >116.1 µg CH_4 g^{-1} d^{-1} > 97.4 µg CH_4 g^{-1} d^{-1} > 88.3 µg CH_4 g^{-1} d^{-1} for 10% methanotrophic-activated biochar-amended soil, 2% methanotrophic-activated biochar-amended soil, 10% biochar-amended soil, 2% biochar-amended soil and soil, respectively. Overall, this study demonstrated that when the biochar is activated with methanotrophs and amended with soil, the CH_4 removal is faster when compared to biochar-amended soil and therefore can be used to mitigate landfill CH_4 gas emissions. However, column and field-scale studies are recommended to evaluate the efficiency of methanotrophic-activated biochar-amended soil in the removal of CH_4 under dynamic field conditions.

8.5 CITED REFERENCES

Hilger, H.A., Liehr, S.K and Barlaz, M.A., 1999. Exopolysaccharide control of methane oxidation in landfill cover soil, Journal of Environmental Engineering., 125(12),1113-1123.

Hilger, H.A., Cranford, D.F and Barlaz, M.A., 2000. Methane oxidation and microbial exopolymer production in landfill cover soil, Soil Biology and Biochemistry., 32(4), 457-467.

Kightley, D., Nedwell, D.B and Cooper, M., 1995. Capacity for methane oxidation in landfill cover soils measured in laboratory-scale soil microcosms, Applied and Environmental Microbiology., 61(2), 592-601.

Mor, S., De Visscher, A., Ravindra, K., Dahiya, R.P., Chandra, A and Van Cleemput, O., 2006. Induction of enhanced methane oxidation in compost: temperature and moisture response, Waste Management., 26(4), 381-388.

Pedersen, G.B., Scheutz, C and Kjeldsen, P., 2011. Availability and properties of materials for the Fakse Landfill biocover, Waste Management., 31(5), 884-894.

Reddy, K.R., Yargicoglu, E.N., Yue, D and Yaghoubi, P., 2014. Enhanced microbial methane

oxidation in landfill cover soil amended with biochar, Journal of Geotechnical and Geoenvironmental Engineering., 140(9), 04014047.

Sadasivam, B.Y and Reddy, K.R., 2014. Landfill methane oxidation in soil and bio-based cover systems: a review, Reviews in Environmental Science and Bio/Technology., 13(1), 79-107.

Scheutz, C., Fredenslund, A.M., Chanton, J., Pedersen, G.B and Kjeldsen, P., 2011. Mitigation of methane emission from Fakse landfill using a biowindow system, Waste Management., 31(5), 1018-1028.

Scheutz, C and Kjeldsen, P., 2003. Capacity for biodegradation of CFCs and HCFCs in a methane oxidative counter-gradient laboratory system simulating landfill soil covers, Environmental Science & Technology., 37(22), 5143-5149.

Scheutz, C., Pedersen, G.B., Costa, G and Kjeldsen, P., 2009. Biodegradation of methane and halocarbons in simulated landfill biocover systems containing compost materials, Journal of Environmental Quality., 38(4),1363-1371.

Stern, J.C., Chanton, J., Abichou, T., Powelson, D., Yuan, L., Escoriza, S and Bogner, J., 2007. Use of a biologically active cover to reduce landfill methane emissions and enhance methane oxidation, Waste Management., 27(9),1248-1258.

Streese, J and Stegmann, R., 2003. Microbial oxidation of methane from old landfills in biofilters, Waste Management., 23(7), 573-580.

Whittenbury, R., Phillips, K.C and Wilkinson, J.F., 1970. Enrichment, isolation and some properties of methane-utilizing bacteria, Microbiology., 61(2), 205-218.

Wilshusen, J. H., Hettiaratchi, J. P. A and Stein, V. B., 2004. Long-term behavior of passively aerated compost methanotrophic biofilter columns, Waste Management., 24(7), 643-653.

Yaghoubi, P., 2012. Development of biochar-amended landfill cover for landfill gas mitigation,

University of Illinois at Chicago, PhD Thesis, USA.

Yargicoglu, E.N and Reddy, K.R., 2017. Effects of biochar and wood pellets amendments added to landfill cover soil on microbial methane oxidation: A laboratory column study, Journal of Environmental Management., 193, 19-31.

Yargicoglu, E.N., Sadasivam, B.Y., Reddy, K.R and Spokas, K., 2015. Physical and chemical characterization of waste wood derived biochars, Waste Management., 36, 256-268.

CHAPTER 9 - OVERALL SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS FOR FUTURE RESEARCH

This Master's thesis investigated the role of biogeochemical landfill cover materials individually and in combinations in CH_4 oxidation and CO_2 sequestration and mitigating fugitive landfill emissions. The initial results suggest that CH_4 oxidation is greatly inhibited when the steel slag is combined with soil (10%) or biochar-amended soil due to the combined effect of high pH (>11) and high metal constituents present in the slag. The effect of heavy metals on CH_4 oxidation was not a part of this study, therefore future studies to provide further insights into the effect of heavy metals on microbial CH_4 oxidation is entailed.

The initial batch scale experiments on pH using enrichment cultures and soil microcosms suggest that CH_4 oxidation is greatly inhibited at an extreme alkaline pH of 12. Therefore, for an effective CH_4 oxidation and simultaneous CO_2 sequestration, a slag layer isolated from the biochar-amended soil is highly recommended. However, in the layered cover profile it is possible that under rainfall or infiltration conditions, the heavy metals and alkaline metal ions may leach from slag exposing biochar-amended soil layer to high alkalinity and heavy metals.

For this reason, the effect of BOF slag leachate on microbial CH_4 oxidation and community was further studied as a part of this research. The results demonstrated significant CH_4 oxidation in both enrichments as well as soil microcosms containing slag leachate indicating no effect of metal constituents present in the slag leachate on the oxidation activity. Further, no significant difference in the community composition was noted when soil was inoculated with BOF slag leachate at different proportions thus confirming the same. However, column studies and field scale studies along with qPCR of methanotrophic community (targeting *pmoA* genes) is highly

recommended to study the performance of slag isolated biochar-amended soil under more dynamic environmental conditions.

A study on effect of temperature (6°C to 70°C) on methane oxidation and microbial community in the landfill cover soil was also studied. The maximum oxidation was observed at 30°C and decreased with increase in temperature at 40°C and 50°C with no oxidation at 70°C. No study was conducted between 30°C and 40°C and is therefore recommended to identify most optimal condition in the landfill cover as well as in the biochar-amended landfill cover system.

In this study, the methanotrophic community or structure was analyzed by targeting the 16S rRNA genes incubated at different conditions (pH, temperature, BOF slag leachate, cover materials) (shown in Table S8-1in Appendix A). Most of the results showed community taxa at the genus level and not at the species level, thus limiting our understanding in the role of specific species dominating in the experimental sets. Only few samples were selected and analyzed for shotgun metagenome sequencing and were successful in determining the bacteria at the species level. Due to the high cost involved in shotgun sequencing/Bioinformatics only limited samples were tested in this research. Shotgun sequencing is best recommended when compared to 16S rRNA gene amplicon sequencing in future as it determines the species level bacteria and prevents PCR bias.

A previous research at UIC on biochar amended soil (10%) had shown potential in enhancing CH_4 oxidation due to the favorable physicochemical properties of biochar. However, it was noticed that colonization and acclimatization of CH_4 oxidizing bacteria in the biochar-amended soil was time consuming. For this reason, this research focused on carrying out long term batch scale studies on methanotrophic-activated biochar-amended soil (2% and 10%) relative to non-activated biochar-amended soil (2% and 10%) with a goal to expedite mitigation of CH_4

emissions from the landfills. This study demonstrated promising results with 10% methanotrophic activated biochar-amended soil when compared to 2% and 10% non-activated biochar-amended soil, that took time in enhancing CH_4 oxidation. In soil and biochar amended soil, decrease in the oxidation rates were possibly due to loss of moisture content (~13-18%) and/or production of Exopolymeric substance (EPS) inhibiting substrate transport to the microbes. Further research in investigating the reasons behind reduced oxidation rates and production of EPS in biochar amended soil is strongly recommended. It is also recommended to target the methanotrophic specific genes (*pmoA*) in future studies to quantify methanotrophs colonized in the biochar. Further, column studies and field scale studies are suggested to study the performance of methanotrophic-activated biochar-amended soil under more dynamic environmental conditions.

APPENDIX A

Table S8-1: List of experimental samples used in this thesis and analyzed for microbial community by UICSQC & RIC

#	Sample Name to RRC	Sample Names
1	Reddy2_pH2_1	Culture - pH 2
2	Reddy3_pH2_3	Culture - pH 2
3	Reddy4_pH4_2	Culture - pH 4
4	Reddy5_pH4_3	Culture - pH 4
5	Reddy6_pH7	Culture - pH 7
6	Reddy7_pH9_2	Culture - pH 9
7	Reddy8_pH9_3	Culture - pH 9
8	Reddy9_pH10_2	Culture - pH 10
9	Reddy10_pH10_3	Culture - pH 10
10	Reddy11_pH12_2	Culture - pH 12
11	Reddy12_pH12_3	Culture - pH 12
12	Rai15_RpH7-1	Soil Suspension-pH 7
13	Rai16_RpH7-2	Soil Suspension-pH 7
14	Rai17_RpH7-3	Soil Suspension-pH 7
15	Rai18_RpH9-1	Soil Suspension-pH 9
16	Rai19_RpH9-2	Soil Suspension-pH 9
17	Rai19_RpH9-3	Soil Suspension-pH 9
18	Rai20_RpH10-1	Soil Suspension-pH 10
19	Rai21_RpH10-2	Soil Suspension-pH 10

20	Rai22_RpH10-3	Soil Suspension-pH 10
21	Rai23_RpH12-1	Soil Suspension-pH 12
22	Rai24_RpH12-2	Soil Suspension-pH 12
23	Rai25_RpH12-3	Soil Suspension-pH 12
24	Rai01_T50_1	Culture - Temperature 50°C
25	Rai02_T50_2	Culture - Temperature 50°C
26	Rai03_T50_3	Culture - Temperature 50°C
27	Rai04_T50_4	Soil - Temperature 50°C
28	Rai05_T50_5	Soil - Temperature 50°C
29	Rai06_T70_1	Culture - Temperature 70°C
30	Rai07_T70_2	Culture - Temperature 70°C
31	Rai08_T70_3	Culture - Temperature 70°C
32	Rai09_T70_4	Soil - Temperature 70°C
33	Rai10_T70_5	Soil - Temperature 70°C
34	Rai11_T70_6	Soil - Temperature 70°C
35	Rai12_T23_1	Soil - Temperature 23°C
36	Rai13_T23_2	Soil - Temperature 23°C
37	Rai14_T30_1	Soil - Temperature 30°C
38	Rai15_T30_2	Soil - Temperature 30°C
39	Rai16_T40_1	Soil - Temperature 40°C
40	Rai17_T40_2	Soil - Temperature 40°C
41	Rai18_T6_4	Soil - Temperature 6°C
42	Rai19_T6_5	Soil - Temperature 6°C

43	Rai20_T6_6	Soil - Temperature 6°C
44	Rai33_SL12	Culture - Temperature 6°C
45	Rai34_SL13	Culture - Temperature 6°C
46	Rai35_SL14	Culture - Temperature 6°C
47	Reddy6_pH7	Culture - Temperature 23°C
48	Reddy13_30C	Culture - Temperature 30°C
49	Reddy14_40C	Culture - Temperature 40°C
50	Reddy15_40C	Culture - Temperature 40°C
51	Rai21_SL0	0% Slag leachate- Enrichment Culture
52	Rai24_SL3	11% Slag leachate- Enrichment Culture
53	Rai25_SL4	11% Slag leachate- Enrichment Culture
54	Rai26_SL5	11% Slag leachate- Enrichment Culture
55	Rai27_SL6	25% Slag leachate- Enrichment Culture
56	Rai28_SL7	25% Slag leachate- Enrichment Culture
57	Rai29_SL8	25% Slag leachate- Enrichment Culture
58	Rai30_SL9	100% Slag leachate- Enrichment Culture
59	Rai31_SL10	100% Slag leachate- Enrichment Culture
60	Rai32_SL11	100% Slag leachate- Enrichment Culture
61	Rai36_SF1	0% Slag leachate- Soil Microcosm
62	Rai37_SF2	0% Slag leachate- Soil Microcosm
63	Rai38_SF3	0% Slag leachate- Soil Microcosm
64	Rai39_SF4	5% Slag leachate- Soil Microcosm
65	Rai40_SF5	5% Slag leachate- Soil Microcosm

66	Rai41_SF6	5% Slag leachate- Soil Microcosm
67	Rai42_SF7	20% Slag leachate- Soil Microcosm
68	Rai43_SF8	20% Slag leachate- Soil Microcosm
69	Rai44_SF9	20% Slag leachate- Soil Microcosm
70	Rai45_SF10	60% Slag leachate- Soil Microcosm
71	Rai46_SF11	60% Slag leachate- Soil Microcosm
72	Rai47_SF12	60% Slag leachate- Soil Microcosm
73	Rai48_SF13	100% Slag leachate- Soil Microcosm
74	Rai49_SF14	100% Slag leachate- Soil Microcosm
75	Rai50_SF15	100% Slag leachate- Soil Microcosm
76	Reddy1_B	Baseline (Field Soil -Unincubated)
77	Rai51_SS1	Soil + Slag (10%), Incubated at room temperature
78	Rai52_SS2	Soil + Slag (10%), Incubated at room temperature
79	Rai53_SS3	Soil + Slag (10%), Incubated at room temperature
80	Rai54_SS4	Soil + Slag (10%) + Biochar (10%), Incubated at room temperature
81	Rai55_SS5	Soil + Slag (10%) + Biochar (10%), Incubated at room temperature
82	Rai56_SS6	Soil + Slag (10%) + Biochar (10%), Incubated at room temperature
83	Rai01_RLT1	Long term experiment soil
84	Rai02_RLT2	Long term experiment soil
85	Rai03_RLT6	Long term experiment soil +2% Biochar

86	Rai04_RLT8	Long term experiment soil +10% Biochar
87	Rai05_RLT9	Long term experiment soil +10% Biochar
88	Rai06_RLT11	Long term experiment soil +2% Activated Biochar
89	Rai07_RLT13	Long term experiment soil +10% Activated Biochar
90	Rai08_RLT15	Long term experiment soil +10% Activated Biochar

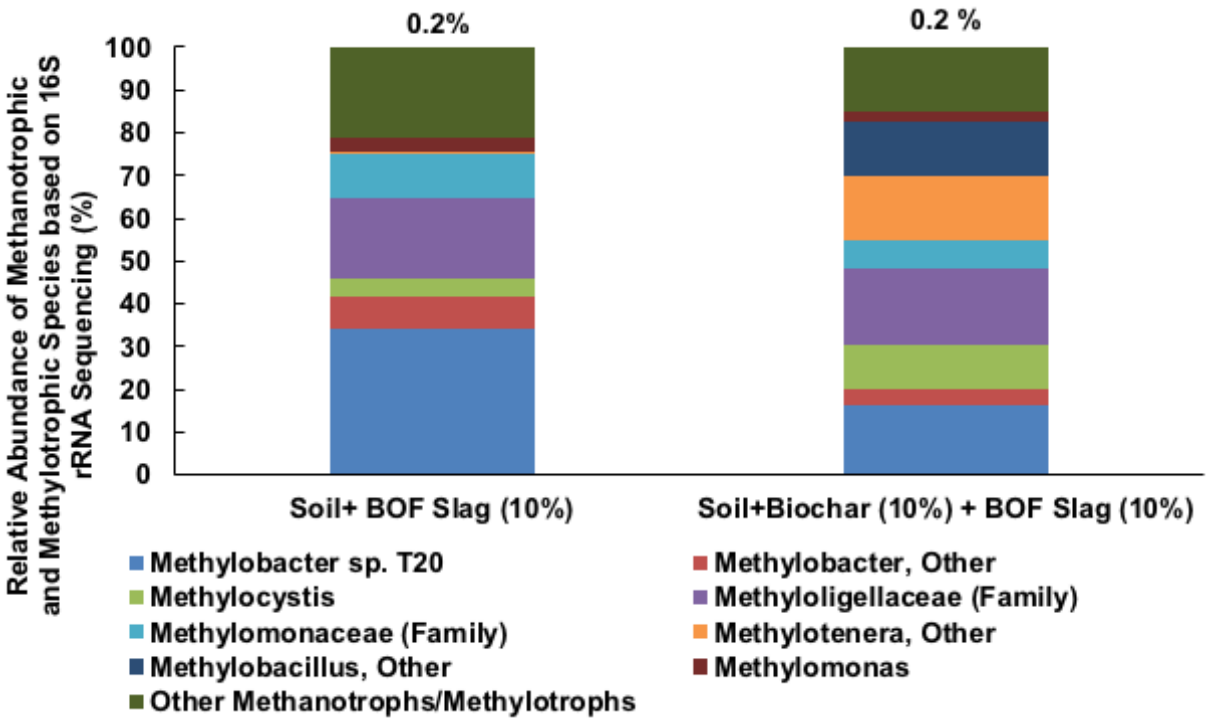


Fig S3-1. Relative abundance of methanotrophic and methylotrophic species in soil-amended slag and slag-biochar-amended soil

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Rai, R. K., and Reddy, K. R. (2019). "**Methanotrophic methane oxidation in new biogeochemical landfill cover system**." *Proc. 34th International Conference on Solid Waste Technology and Management*, Annapolis, MD, USA, March 31st – April 3rd, 2019.

Rai, R. K., and Reddy, K. R. (2019). "**Role of landfill cover materials in mitigating GHG emissions from landfill cover system**." *Proc. World Environmental and Water Resources Congress*, Pittsburgh, PN, USA, May19th – May 23rd, 2019.

Rai. R. K, Chetri, J. K, and Reddy, K. R. (2019). "**Effect of methanotrophic- activated biochar amended soil in mitigating CH_4 emissions from landfills**." *Proc. 4th International Conference on Civil and Environmental Geology and Mining Engineering*, Trabzon, Turkey, 20-22 April 2019.

Reddy, K.R., Kumar, G., Gopakumar, A., Rai, R.K., and Grubb, D.G. (2018). "**CO_2 Sequestration using BOF slag: Application in landfill cover**." *Proc. International Conference on Protection and Restoration of the Environment XIV, Thessaloniki, Greece*, July 2018.

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