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ENHANCING ANAEROBIC OXIDATION OF METHANE IN LANDFILL COVER SOIL

By

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Dedication

I dedicate this dissertation to my grandparents; late Zahra and Ali for their endless love and encouragement

You will always be missed

Abstract

ENHANCING ANAEROBIC OXIDATION OF METHANE IN LANDFILL COVER SOIL

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Methane (CH_4) is one of the major greenhouse gases (GHG) generated in landfills and has a global warming effect 28 times more than carbon dioxide (CO_2). Therefore, decreasing methane emissions into the atmosphere from landfills is critically important. In the upper portions of a landfill cover, methane is exposed to oxygen and oxidized aerobically to carbon dioxide while passing through the cover soil; this lowers the overall contribution of the landfill to climate change. However, because of the low permeability of the landfill cover, no aerobic oxidation occurs in the bottom of the cover because oxygen cannot penetrate to those depths. One possibility for increasing the overall oxidation of methane through landfill covers is to increase anaerobic oxidation of methane (AOM) in the lower depths. Although AOM has been studied by previous researchers in fresh water, sea water, and peat soil, no previous study has focused on AOM in landfill cover soil.

In this study, anaerobic oxidation of methane (AOM) in the landfill cover soil was studied. Specific objectives were:

1. To evaluate the ability of alternate electron acceptors (besides oxygen) to facilitate anaerobic methane oxidation in clay soil, using batch tests. Different concentrations of the electron acceptors such as sulfate, nitrate, and iron were evaluated.
2. To study the effect of environmental conditions such as different moisture contents, nutrients, and methane concentrations on anaerobic oxidation of methane through batch tests, as well as the effect of methane generation inhibitor.
3. Using the most promising electron acceptor concentrations determined from Objective 1, to measure rates of anaerobic oxidation of methane in clay landfill covers via column tests, which includes realistic conditions of gas flow, cover thickness, and cover compaction.

Compaction, permeability, sieve, hydrometer, liquid limit, plastic limit, and electron spectroscopy for chemical analysis tests were conducted to characterize the soil. Batch tests were conducted in 125 mL glass Wheaton bottles with 17 g soil. Electron acceptors (red mud-containing iron, iron chloride, iron oxide, hematite, sodium nitrate, potassium nitrite, sodium sulfate, manganese oxide, and ammonium chloride) were added to the soil, along with water (20% or 47% moisture content), nutrient solution, and/or methane generation inhibitor, as appropriate. After flushing the reactors with nitrogen gas, landfill gas (LFG) (50% methane, 50% carbon dioxide) was injected. Methane concentration in the headspace of the reactors was measured over time using a gas chromatograph. Maximum oxidation rate was also calculated using Michaelis-Menten kinetics.

Batch tests results showed that sulfate, nitrate, and a combination of sulfate+iron could remove more methane compared to the control test over the long-term and had higher maximum oxidation rates. Hence, they were chosen for testing in columns. Moreover, according to the

batch tests, methane removal decreased in the reactors with no added nutrients, lower moisture content, and low initial concentration of methane. The results also showed that adding inhibitor increased methane removal in some reactors while it lowered AOM in other reactors.

In columns, the soil was compacted to create a 2-foot layer of cover soil. Methane entered the column at a flux of 179.4 $gCH_4\ m^{-2}\ day^{-1}$ from the bottom and passed through the cover. Oxidation rate was obtained by measuring methane concentration at the port, where gas entered the column, and at the end of the anoxic zone.

The results of column tests showed that at a higher landfill gas flow rate, there was no significant difference in methane removal in the anoxic zone of the columns; however, at a lower flow rate, methane removal in the column amended with sulfate + iron had the highest (around 10%) removal of methane in the anoxic zone, followed by the column that contained sulfate. The results showed H_2S gas at the headspace of these two columns, which indicated that sulfate-reducing bacteria were likely responsible for methane removal in the anoxic zone of the columns.

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Chapter 1

General Introduction

1.1 Introduction

Methane (CH_4) is one of the major greenhouse gases (GHG) generated in landfills and has a global warming effect 28 times more than carbon dioxide (CO_2) (IPCC, 2014). Therefore, decreasing methane emissions into the atmosphere from landfill surfaces will reduce emissions of potent greenhouse gas. In a landfill cover, microbes convert methane to CO_2 through an aerobic process. However, since oxygen availability is limited in the deep layers of cover soil, utilizing anaerobic oxidation can play an important role in decreasing methane emissions into the atmosphere. Certain components (iron, nitrate, and sulfate) could facilitate anaerobic methane oxidation in landfill covers since they can potentially facilitate methane oxidation as alternative electron acceptors.

1.2 Landfill methane generation, use, and emissions

Landfill gas is produced when organic waste in a municipal solid waste (MSW) landfill is decomposed. Not only does capturing produced methane help prevent the emission of odors and hazardous air pollutants into the air, but it can also be converted to a source of energy. Many landfills collect and use LFG to take advantage of this renewable energy resource, while also reducing GHG emissions. Capturing methane also offsets the need for nonrenewable energy sources such as coal and oil, and thus helps reduce pollutants that contribute to local smog and acid rain (EPA, 2010). Recognizing the importance of CH_4 emissions from landfills in the US, in

Aug. 2015, EPA proposed updates to its 1996 Emission Guidelines for existing MSW landfills and its New Source Performance Standards for new and modified landfills. These updates require landfills to install landfill gas collection and control systems which will reduce CH_4 emissions by 436,000 metric tons per year by 2025 (EPA, 2016).

However, since landfills currently release 4.6 million metric tons of CH_4 per year, still more remains to be done. In landfills with low CH_4 generation, a gas collection system is not economical. Moreover, when landfills capture and burn methane to produce electricity, around 25% of methane still leaks through landfill covers (EPA, 2010). In the U.S., landfills are the second largest source of methane emissions (EPA, 2012) and according to the Intergovernmental Panel on Climate Change (IPCC), they are the third largest source of anthropogenic CH_4 worldwide, after releases from natural gas distribution systems and agricultural practices (IPCC, 2012). Therefore, it is necessary to reduce CH_4 emissions into the air by increasing methane oxidation in the landfill cover (Krishna, 2014).

1.3 Increasing methane oxidation in landfill covers

Generated methane passes through the cover soil before being emitted into the atmosphere. The process of methane oxidation reduces the emissions of methane and other volatile hydrocarbons from the surface of landfills (Chanton and Abichou, 2011). If the percent of methane oxidized could be increased, the landfill's contribution to climate change would be lowered. Conventional landfill covers, made from soil, contain aerobic microbes (methanotrophs) which oxidize 10-100% (average 36%) of the CH_4 passing through the cover to CO_2.

Various studies have assessed alternative landfill covers like bio-covers for facilitating aerobic CH_4 oxidation. Alternative covers are made of materials such as MSW compost, sewage sludge compost, yard waste compost, and mixtures of wood chips and compost, which has high nutrient content for microbes. Bio-covers, however, have several shortcomings, including high oxygen demand, competitive microbial activities, high maintenance, and low methane oxidation during the cold season.

Methane oxidation is challenging when lack of oxygen limits the ability of methanotrophs to oxidize methane. The lower parts of a landfill cover, in contact with waste, typically encounter an anaerobic environment of 50-60% methane and 40-50% carbon dioxide, since microbes have previously depleted available ambient oxygen. Final covers, by regulatory requirements, must be compacted to the permeability of at least 10^{-5} cm/sec, which may reduce diffusion and availability of ambient oxygen, particularly in the lower portion of landfill covers. Soil typically used to construct final landfill covers is clay, which has low permeability. Clay has been found inferior compared to coarser grained soil in terms of promoting oxygen diffusion and methane oxidation. Saturation of the soil lowers permeability even further and Exopolymeric Substances (EPS) produced by microbial activities can also lead to clogging of the pores of the soil.

Anaerobic oxidation of methane occurs in the absence of oxygen. In the lower parts of a landfill cover where there is almost no oxygen. If microbes were able to facilitate anaerobic oxidation of methane; this would likely increase the overall percent methane oxidized. However, no study to our knowledge has investigated the ability of alternate electron acceptors to promote anaerobic methane oxidation as an alternative landfill cover.

1.4 Research goals and objectives

The **overall goal** of this research is to increase methane oxidation through landfill covers via anaerobic oxidation. **Specific objectives** of the study are:

1. To evaluate the ability of alternate electron acceptors (besides oxygen) to facilitate anaerobic methane oxidation in clay soil, using batch tests. Different concentrations of the electron acceptors such as sulfate, nitrate, and iron will be evaluated.
2. To study the effect of environmental conditions such as different moisture contents, nutrients, and methane concentrations on anaerobic oxidation of methane through batch tests, as well as the effect of methane generation inhibitor.
3. Using the most promising electron acceptor concentrations determined from Objective 1, to measure rates of anaerobic oxidation of methane in clay landfill covers via column tests, which include realistic conditions of gas flow, cover thickness, and cover compaction.

Our hypothesis is that appropriate electron acceptors will increase the anaerobic oxidation in the lower portions of the cover. Therefore, by utilizing both anaerobic and aerobic oxidation of methane, the overall oxidation of the cover will increase.

Our batch and column tests will use chemicals as electron acceptors as the first step of testing because they are homogeneous and provide easy control in terms of concentration. If the chemicals produce promising results, it will be recommended to use waste materials containing the electron acceptors (such as iron-containing wastes like steel slag, iron and steel powder, mill scales, steel punching, and metal shavings) in the future research tests.

1.5 Dissertation organization

Subsequent chapters of this dissertation are organized as follows:

- Chapter 2 describes the previous studies that were conducted to evaluate and increase methane oxidation in the landfill cover. This chapter also summarizes some studies about the anaerobic oxidation of methane in peat soil and marine systems.
- Chapter 3 shows the methodology, and the process of collecting and preparing soil samples and conducting soil mechanical tests, batch and column tests.
- Chapter 4 summarizes the results of the soil, batch and column tests for different electron acceptors. The results will also include the optimum concentration of the promising chemicals along with their effect on total methane oxidation.
- Chapter 5 includes the conclusion of the study as well as the suggestions for future studies.

Chapter 2

Literature Review

2.1 Literature review organization

This chapter provides background concerning aerobic oxidation of methane in traditional and alternative landfill covers, as well as an introduction to anaerobic oxidation pathways for methane. The chapter concludes with the goals and objectives of the proposed research. Specific sections are as follows:

a) Landfill methane generation and models (Sections 1 and 2),
b) Factors affecting aerobic oxidation of methane in traditional landfill covers (clay soil), and models of landfill cover methane oxidation and emissions (Sections 3 and 4),
c) Alternative covers for increasing aerobic oxidation of methane (Section 5) and their limitations,
d) A proposed method of enhancing methane oxidation in landfill covers: anaerobic oxidation (Section 6),
e) Landfill cover laboratory testing methods (Section 7),
f) Goals and objectives of the proposed research (Section 8).

2.2 Background on methane generation and transport in landfills

Atmospheric methane has various sources such as rice production, ruminant animals, natural gas leakage, biomass burning and landfills (Bogner et al., 2010). In the landfills, methane is

generated due to the biodegradation of waste with the emission rates of 100–200 g m^{-2} d^{-1} (Adams et al., 2011). After waste disposal, microbes begin to use carbon and produce methane and as carbon sources decrease, methane generation decreases as well. Waste decomposition and gas generation occur through different stages and specific microorganisms are active during each stage. Methane generation is a result of waste degradation during an anaerobic stage (Larsson, 2014). Anaerobic biodegradation of organic materials such as cellulose, hemicelluloses, proteins, fats generates mostly CH_4 and CO_2 (Bogner et al., 2011). Landfill gases include roughly 50 to 55% methane and 45 to 50% carbon dioxide (CO_2) as major gases, a small amount (nearly 1%) of non-methane organic compounds (NMOCs) and trace amounts of inorganic compounds as minor components. Figure 2-1 shows different stages of waste decomposition and gas generation in a landfill.

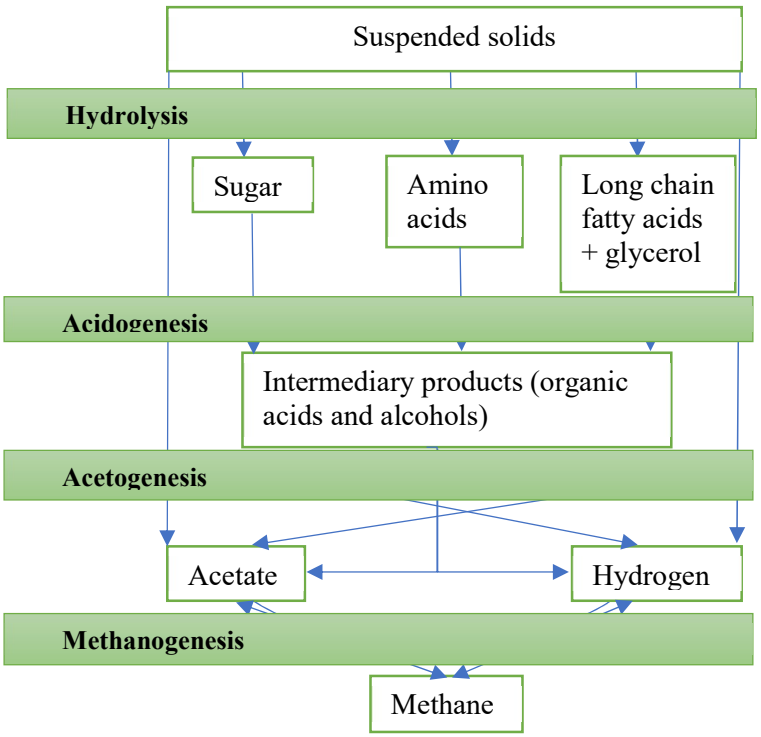


Figure 2-1 Phases of anaerobic decomposition of organic wastes (Larsson, 2014)

Landfill gas includes 40-60% methane, 30-40% carbon dioxide, 1-10% nitrogen, 0-2% hydrogen, 1-2% oxygen, and 10-1000 ppm hydrogen sulfide (Larsson, 2014).

As shown in Figure 2-1, microbes decompose landfilled waste in 4 phases, and different gases are produced in each phase (EPA, 2015). In the first phase, aerobic bacteria breakdown molecular chains of complex carbohydrates, proteins, and lipids that comprise organic waste. CO_2 is the most important component which is produced in this phase, and the phase continues until the depletion of available oxygen.

In the second phase, anaerobic microbes convert the products of the first phase to acetic, lactic and formic acids and alcohols, such as methanol and ethanol, and produced gases are carbon dioxide and hydrogen. In the third stage, anaerobic microbes convert the organic acids produced in the second phase to acetate and they consume CO_2 and produce methane.

Finally, in the last phase, the composition of landfill gases remains almost constant and landfill gas (LFG) is produced at a stable rate, typically for about 20 years for a conventional landfill (EPA, 2017). Figure 2-2 shows all 4 phases.

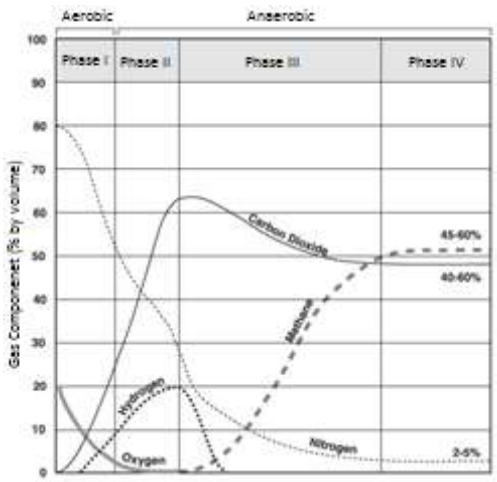


Figure 2-2, Changes in typical LFG Composition at different phases (EPA, 2005)

As mentioned before, methane (CH_4) has a global warming potential (GWP) 28 times more than CO_2 over a 100-year time horizon (IPCC, 2014). GWP is used to show relative pollutant potential of different gases and is defined as below (Larsson, 2014):

$$GWP_i = \frac{\int_0^{TH} RF_i(t)dt}{\int_0^{TH} RF_r(t)dt} \qquad \text{Equation 2-1}$$

Where:

TH: time horizon,

RF_i: radiative forcing for a gas,

RF_r: radiative forcing for the reference gas.

In the above equation, if CO_2 is considered as reference gas ($GWP_{CO_2} = 1$), for 100 years, GWP for methane would be equal to 28. Therefore, methane has global warming potential 28 times more than CO_2. High GWP of methane compared to that of CO_2 shows the importance of methane oxidation to CO_2 in a landfill cover.

As shown in Figure 2-3, generated methane follows different pathways, which include recovery, oxidation, and emission. If there is a gas collection system in the landfill, some of the methane can be collected/recovered into the gas wells due to negative pressure. The gas passes through the soil porous media of the landfill cover to escape from the places with high gas concentration to the places with low concentration and finally to the atmosphere. When the gas migrates through the soil pores, bacteria oxidize some of the methane to carbon dioxide in the presence of oxygen. So, emitted gas is a combination of methane and carbon dioxide. Minor amounts of methane may be stored in pores of the waste itself or migrate to the surrounding environment of the landfill via sub-surface pathways through the soil. The amount of methane emission can be estimated by the following equation:

$$CH_{4,\,Emitted} = CH_{4,\,Generated} - CH_{4,\,Recovered} - CH_{4,\,Oxidized} - CH_{4,\,Migrated} - \Delta CH_{4,\,Stored} \qquad \text{Equation 2-2}$$

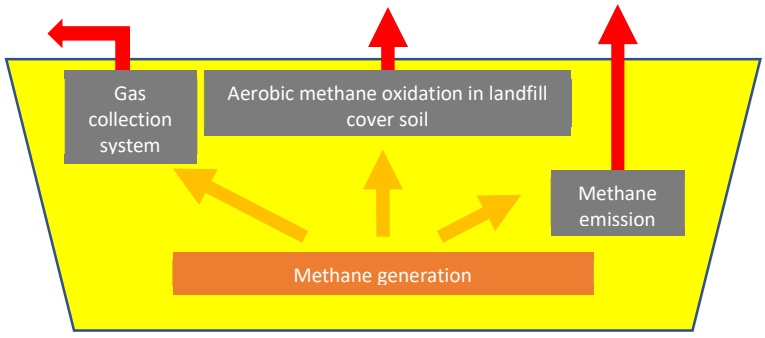


Figure 2-3 Different pathways for generated methane

2.3 Gas transfer through the landfill cover soil

Landfill gas is produced when organic waste decomposes and transfers through the pores of the soil. Diffusion is the dominant transport mechanism in vertical movements, where advection is not significant due to low permeability and pressure gradients. Diffusion is the movement of

pollutants from areas of high concentration to low concentration via the random motion of molecules, and can be described based on the Fick's law:

$$J_A = -D_A \frac{\partial c_A}{\partial x} \qquad \text{Equation 2-3}$$

This equation shows that the mass flux (J_A) is related to concentration gradient ($\frac{\partial c_A}{\partial x}$) with the proportionality constant of diffusion (D_A). The negative sign shows that the movement of molecules due to diffusion is from high concentration to low concentration (decreasing concentration gradient). The diffusion environment of interest in this study is that of soil. The permeability of the soil is lower than 10^{-5} cm/sec; therefore, it is reasonable to consider diffusion as the dominant transport mechanism and ignore advection (assuming that there is no crack in the cover soil).

2.4 Effective diffusion in porous media

Effective diffusion in porous media is a function of free diffusion of gas molecules in pores and physical properties of the media, and can be described as follows:

$$D_{A(eff)} = D_A \frac{\varepsilon}{\tau} \qquad \text{Equation 2-4}$$

Where, ε is the porosity of the media and τ is the tortuosity of the flow path. In a real landfill, the soil is not saturated, so we have both air-filled and water-filled pores. Then, effective diffusion would be:

$$D_{A(eff)} = D_A \frac{\varepsilon_{air}^{10/3}}{(\varepsilon_{air}+\varepsilon_{water})^2} \qquad \text{Equation 2-5}$$

Where, ε_{air} is air-filled porosity and ε_{water} is the water-filled porosity.

As a result, if we have completely dry (or completely saturated) soil, the effective diffusion is:

$$D_{A(eff)} = D_A\, \varepsilon^{4/3} \qquad \text{Equation 2-6}$$

2.5 Mass balance and transport equations

The mass balance equation for combined gas molecules' transport in the soil and reaction is as follows:

$$\begin{array}{c}\text{Rate of accumulation of contaminant per unit volume}\end{array} = \begin{array}{c}\text{Rate of contaminant flow in}\end{array} - \begin{array}{c}\text{Rate of contaminant flow out}\end{array} \pm \begin{array}{c}\text{Rate of Contaminant generation/ consumption}\end{array} \qquad \text{Equation 2-7}$$

Over a differential volume, the reaction will become

$$\frac{\partial c_A^{total}}{\partial t} = \Delta x \Delta y \Delta z = \left\{ j_{A,x}\big|_x - j_{A,x}\big|_{x+\Delta x} \right\} \Delta y \Delta z + \left\{ j_{A,y}\big|_y - j_{A,y}\big|_{y+\Delta y} \right\} \Delta x \Delta z \ldots\ldots\ldots +$$

$$\left\{ j_{A,z}\big|_z - j_{A,z}\big|_{z+\Delta z} \right\} \Delta x \Delta y \pm \left(\frac{reactions}{\Delta x \Delta y \Delta z}\right) \qquad \text{Equation 2-8}$$

By dividing the above reaction by $\Delta x \Delta y \Delta z$:

$$\frac{\partial c_A^{total}}{\partial t} = \left\{ j_{A,x}\big|_x - j_{A,x}\big|_{x+\Delta x} \right\} / \Delta x + \left\{ j_{A,y}\big|_y - j_{A,y}\big|_{y+\Delta y} \right\} / \Delta y \ldots\ldots\ldots + \left\{ j_{A,z}\big|_z - j_{A,z}\big|_{z+\Delta z} \right\} /$$

$$\Delta z \pm (reactions) \qquad \text{Equation 2-9}$$

If $\Delta v \to 0 \;\Rightarrow\; \Delta x \to 0, \Delta y \to 0, \Delta z \to 0$, then:

$$\frac{\partial c_A^{total}}{\partial t} = \nabla j_A \pm reactions \qquad \text{Equation 2-10}$$

Finally, based on a mass balance for one-dimensional diffusion, the equation will be:

$$\frac{\partial c_A^{total}}{\partial t} = D_{A(eff)} \frac{\partial^2 c_A}{\partial z^2} \pm reactions \qquad \text{Equation 2-11}$$

The last equation shows the change in concentration of contaminant due to diffusion transport and chemical reactions. Given appropriate boundary conditions, we can solve the equation and calculate the concentration.

2.6 Methane generation models

2.6.1 Intergovernmental Panel on Climate Change (IPCC) waste model

IPCC's Revised Guidelines (1996) and the IPCC Good Practice Guidance (2000) use a mass balance equation to calculate methane emission from landfill covers. The IPCC model was developed in 2006 and it is based on First Order Decay (FOD) and estimates all methane emitted from a landfill after waste disposal. In this model, if the waste disposal increases, the model will not give accurate methane emission values. Moreover, different composition of waste can be a potential source of error in this model. The FOD model is presented by:

$$E_{CH_4} = M.\sum_{i=1}^{4} C.f_i.D_i.D_F(e^{-(T-1).k_i} - e^{-T.k_i}).F.16/12.(1-R) \times (1-O) \qquad \text{Equation 2-12}$$

Where:

E_{CH_4} = CH_4 emitted in inventory year T,

M = mass of municipal solid waste disposed of in year 0,

C = correction factor,

f_i = fraction of the waste type I,

D_i = fraction of degradable organic carbon in the waste type i,

D_F = fraction of biodegradable organic carbon in the waste type i,

k_i = first-order reaction rate constant,

F = fraction of CH_4 in landfill gas,

R = methane recovery rate,

O = methane oxidation factor.

IPCC model assumes 75% gas recovery and default 10% methane oxidation, without doing any calculation for methane oxidation. Therefore, it is not an accurate model for methane emissions. Moreover, it considers just two dry and wet categories for moisture content, three categories for waste composition and two categories for temperature. Waste particle size and pH are not also considered in the model (Sattler, 2016).

Kumar et al. (2004) estimated methane emission from a landfill in India. They also proposed a triangular model for biogas from a landfill, if generated gas can be estimated based on First Order Decay (FOD) in two parts. In the first part, gas generation increases and reaches a peak; then it decreases in the second part. It was concluded that the proposed model can give realistic estimation of the gas emitted from a landfill. No oxidation was assumed to calculate emitted gas using the IPCC model, and higher gas emissions were thus obtained using the IPCC model.

2.6.2 US EPA's Landfill Gas Emissions Model (LandGEM)

This model does not consider methane oxidation; In fact, LandGEM is used to predict methane generation. Larsson (2014) evaluated gas mitigation form Kikås landfill in Mölndal. Three analytical models (LandGEM, IPCC model and the Dutch Afvalzorg's model) were used to estimate gas emission potential and the consequent environmental effects. The results showed

different gas releasing potential based on the models. Based on LandGEM, the amount of generated methane for 20 years was 7 times more than that of IPCC. Finally, due to the high uncertainty of the results, actual field measurements were recommended.

2.6.3 UTA's CLEEN (Capturing Landfill Emissions for Energy Needs)

This model predicts methane generation and is not as simplified as previous models. It includes rainfall, waste composition, and ambient temperature in methane generation calculations. However, the model considers the default value of 10% for methane oxidation. The 10% oxidation is based on the results for a New Hampshire landfill as obtained by Czepiel et al. (1996). Karanjkear et al. (2015) developed this model to estimate methane generation. This model can be used for the worldwide landfills since it considers different waste composition and climatic conditions such as annual rainfall and ambient temperature. The first-order decay constant estimated using this model is more reliable than the previous models since it considers the local climatic condition and waste composition.

2.7 Aerobic oxidation of methane

Aerobic bacteria that exist in the landfill cover facilitate methane oxidation with an oxidation rate in the range of 0.0024 μg CH_4 g^{-1} h^{-1} measured in a sandy clay loam to 173 μg CH_4 g^{-1} h^{-1} measured in silty loam (Fredenslund, 2010). In fact, methane oxidation controls gas emission into the atmosphere. Therefore, increasing methane oxidation in the cover soil can reduce methane emission from the cover soil in both landfills with and without gas collection system (Abushammala et al., 2014).

Aerobic methane oxidation occurs in the presence of oxygen. Generated methane passes through the cover soil before being emitted to the atmosphere. In the upper layers of the cover soil, where both methane and oxygen are available, methanotrophic microbes oxidize methane and produce CO_2 (Bogner et al., 2011).

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \qquad \text{Equation 2-13}$$

The quantification of methane oxidation is one of the major uncertainties in estimating national or global CH_4 emissions from landfills (Chanton and Abichou, 2011). Since methane oxidation occurs due to microbial activity near the surface of the cover soil of the landfill, its efficiency depends on climate parameters such as moisture and temperature as well as CH_4 concentration, soil type, and pH, which can affect the activity of the bacteria (Hilger et al., 1999). Many analytical and experimental research works have been conducted to estimate the amount of methane emission from landfills. In analytical works, numerical models have been developed to evaluate methane generation and in the experimental researches, lab column tests and field measurements have been done to quantify methane oxidation in cover soils (SWANA, 1997).

2.8 Factors affecting aerobic oxidation of methane

Methane oxidation rate in landfills varies based on seasonal changes (variations in factors like temperature and moisture), methane concentration under the landfill cover, and physical and chemical characteristics of the cover (e.g. cover particle size, compaction, permeability, cover thickness, microorganisms, and nutrient content). Methane oxidation in the landfill cover can reach a maximum rate in thick cover layers at optimum temperature and moisture content (Abushammala et al., 2014).

2.8.1 Soil texture

Soil structure, particle size, specific surface area (on which microorganisms can grow) and porosity of the soil affect landfill gas transfer and oxygen availability in the cover (Cao and Staszewska, 2011). Thus, these parameters directly affect methane oxidation efficiency. Based on previous studies, methane oxidation is higher in the soil with coarse particles because of higher gas transfer and sufficient available oxygen for microbial activity (Park et al. 2005; Abushammala et al., 2014).

Pawłowska and Stępniewski (2008) conducted lab column tests of four different mixtures of sand and gravel and studied the effect of particle size on methane oxidation capacity. Based on the results, methane, oxygen and carbon dioxide availability were affected by grain size. Maximum oxidation was achieved in the sample with coarse sand material since the material provided optimum gas transfer and available surface area for microorganisms to grow.

Röwer et al. (2011) measured gas concentrations in the soil, topsoil methane oxidation capacity and soil properties at depths of 10, 40, and 90 cm in an old landfill in north-western Germany at 40 locations. Although the difference in soil porosity, moisture content, compaction, cracks, and composition leads to the creation of preferential gas flow paths, these differences were too localized to be captured by the sampling method that was used. In the beginning, the oxidation capacity of all locations clearly exceeded the methane flux to the cover; therefore, the cover mitigated methane emissions very efficiently. Statistical model results showed that the methane oxidation capacity was enhanced by increasing the exposure to methane and that the oxidation capacity depends on methanotroph bacteria, optimum pH and nutrient availability.

2.8.2 Soil nutrient content

Studies have shown that soil with a high amount of organic matter has high methane oxidation efficiency. Microorganisms feed on the organic matter to decompose methane. Soil with high porosity and nutrition content like compost has 100% methane oxidation efficiency (Abushammala et al., 2014). Therefore, in some studies, researchers amended the landfill cover soil by adding compost or soil which is high in nutrition.

Stern et al. (2007) investigated the methane oxidation capacity of bio-covers in a landfill in Florida. Because of the high nutrition content of bio-cover, the high retention time for captured methane and ample thickness of the cover, methane oxidation increased by a factor of 2. At the beginning of the field measurements, the bio-cover showed higher methane oxidation. Compared to a pure soil cover, the bio-cover showed zero methane emissions and, in some cases, negative methane oxidation after three months of bio-cover replacement. In other words, bio-cover was so effective in methane oxidation that it could even oxidize atmospheric methane in addition to the methane generated in the landfill.

Albanna et al. (2007) conducted batch tests to investigate the combined effect of soil moisture, nutrient content, and cover thickness on methane oxidation efficiency. For the soil sample with the thickness of 200 mm and moisture content of 30%, in which fertilizer was added to the soil as a nutrition source, methane oxidation efficiency was increased from 38% to 81%. However, methane oxidation decreased when the moisture content was 15% for the soil with the same amount of nutrition and the same thickness.

Christophersen et al. (2000) conducted batch tests to study methane oxidation capability of an old landfill cover for low temperatures and different moisture contents. Results showed that

methane oxidation rate was 0.005 to 0.17 μmol g^{-1} h^{-1} at 2°C, depending on soil nutrient content and moisture, and the cover could oxidize all produced methane even during winter.

Lee et al. (2009) investigated methane oxidation in King Highway Landfill in Kalamazoo, Michigan. The soil had a water content of 5%, and 15 mg NH^{4+}/kg soil and 0.1 mg phenylacetylene/kg soil were added as nutrients. Though the soil was almost dry, it showed 28% greater oxidation capacity compared to the soil sample with the same water content and no added ammonium or phenylacetylene.

Lizik et al. (2013) conducted field studies for 2 ½ years in a closed landfill in Western Michigan. Nutrients (KNO_3, NH_4Cl, and phenylacetylene) were added to the soil, which resulted in an increase in methane oxidation. Measurements showed that after adding KNO_3 and NH_4Cl, methane flux dropped to half. Moreover, the addition of phenylacetylene lowered the production of N_2O and reduced the maximum methane oxidation rate. Finally, the addition of nutrients coupled with moisture content was suggested to decrease methane flux to the atmosphere.

2.8.3 Soil temperature

Most of the methanotrophs available in the soil for oxidation are mesophiles, which means that they grow best at the moderate temperature range (20 - 45°C). However, some of them can survive in the temperature up to 50°C (Hanson, R.S. and T.E, 1996). Soil temperature is an important factor that determines the microbial community and activity. Microbes have the highest activity at optimum temperature if the process is not limited by gas diffusion (Abushammala et al., 2014; Cao and Staszewska, 2013). The optimum temperature for methane oxidation ranges from 25-35°C; however, oxidation can occur at a low temperature as 1-2°C

(Scheutz et al., 2009), which shows that some microorganisms can survive and remain active at low temperature.

The Q_{10} coefficient represents a change in methane oxidation rate when the temperature increases 10°C. Studies show that when the temperature increases from 10°C to 30°C, the relationship between temperature and Q_{10} is almost exponential and Q_{10} increases from 1.7 to 4.1. However, Q_{10} is highly dependent on the temperature at high methane concentrations, which is likely because of gas transfer limitations at lower methane concentrations (Scheutz et al., 2009). Kettunen et al. (2006) examined the oxidation capacity of the cover soil of an engineered landfill at low temperature (4-12°C). The cover soil was sewage sludge compost and de-inking waste, which was amended with sand or bark chips. The thickness of the cover soil was 30 cm. According to the results, oxidation dropped just to half the initial amount when the temperature decreased from 21–23˚C to 4– 6˚C. High porosity of the soil and enough available oxygen level helped keep microbes active even at very low temperature. On the other hand, in the soil amended with bark chips, methane oxidation at the temperature of 4– 6˚C dropped to one-fourth of its initial value at the temperature of 21–23˚C. The reason for the significant drop of methane oxidation was low porosity of the second soil sample. Therefore, it was concluded that in the areas with low ambient temperature, soil characteristics such as porosity, moisture content and soil nutrition are important factors that determine methane oxidation efficiency of the cover.

Dunfield et al. (1993) studied anaerobic methane generation and aerobic methane oxidation in slurries of peat samples at a temperature range of 0-35°C. Based on the results, methane production showed much more temperature dependency than methane consumption. For example, in the temperature range of 1-10°C methane production was negligible, however, methane consumption was 13-38% of maximum value.

2.8.4 Soil water content

Soil moisture content is one of the most important factors that determines the methane oxidation efficiency of landfill covers. Rainfall, leachate recirculation, and microbial activity are the common sources of water in a cover. Enough water should be provided for microbes to oxidize methane, and microbial activity increases by increasing the water content of the soil. Water content affects methane oxidation by influencing microbial activity and gas transfer. However, the very high water content will decrease the permeability of the soil, which will limit landfill gas transfer within the cover layer. Moreover, low permeability will lower oxygen availability for microbes in the cover soil (Abushammala et al., 2014). High moisture content can also build up gas pressure in the cover and cause lateral emissions of the gas or gas transport due to advection. Another problem related to the high moisture content happens in clay soil. During the seasons of rainfall, clay becomes saturated and has a high moisture content. Then during the dry season, it loses the moisture and as a result, cracks and fissures occur in the clay. Therefore, gas can find preferential pathways to be emitted to the atmosphere, which causes a decrease in methane oxidation (Scheutz et al., 2009).

Researchers have obtained optimum moisture content for different cover soils. Dasselaar et al. (1998) evaluated methane oxidation capacity of sandy soil for different moisture contents. The results showed that methane oxidation is inhibited if the soil moisture content is either higher than 50% (w/w) or lower than 5% (w/w). The highest methane oxidation was obtained in the range of 20 – 35% (w/w).

Albanna and Fernandes (2009) evaluated the effect of environmental factors such as soil moisture and temperature on methane oxidation rate for two existing landfills. Methane oxidation was correlated to a decrease in oxygen and methane and an increase in CO_2

concentration. The highest oxidation was obtained at a moisture content of 20% and it decreased at the moisture content of 25 or 30%. In general, research works show the optimum moisture content of 11-25% for methane oxidation (Abushammala et al., 2014).

Einola et al. (2007) evaluated the effect of soil moisture content on methane oxidation and found that the microbial activity at different soil moisture contents varies with temperature. The results showed that for soil temperature between 1-6°C, the highest oxidation can be achieved at the soil moisture content of 33–67%, whereas for a temperature range of 12–19°C, maximum oxidation occurs at a water content of 50%. Finally, it was recommended to provide enough moisture to the cover soil for microbial activity, and a high share of coarse pores (like compost) that leads to oxygen penetration through the depth of cover. In this way, oxidation can occur in deep parts of the cover, where soil temperature and moisture have a more stable condition.

2.8.5 Permeability and oxygen availability

Studies have shown that a very low concentration of oxygen limits the activity of methanotrophs (Scheutz et al., 2009). Soil porosity is the most important factor that determines oxygen penetration into the depth of landfill cover. However, other parameters such as methane oxidation rate, soil moisture content, soil cover thickness, and texture have a combined effect with moisture (Abushammala et al., 2014). Soil porosity can be also increased by vegetation on the cover soil. The roots of vegetation penetrate through the soil and cause micropores and create pathways for oxygen transfer (Tanthachoon et al., 2012).

Bohn et al., (2011) conducted column tests to investigate the effect of vegetation on oxygen availability and subsequently methane oxidation in silty-clay soil. Two columns were set up: cover soil with vegetation and a cover with the bare surface. The results indicated that the cover

with bare surface did not have methane oxidation even when methane flux was low. On the other hand, the cover with vegetation had 91% oxidation for methane load of 5.6l CH_4 m^{-2} h^{-1}. High oxidation in the second column was due to an increase in soil porosity and oxygen availability. Moreover, water produced during methane oxidation could also be evaporated and hence more water-free pores were available for oxygen transfer.

Pinjing et al. (2011) studied the effect of water content and oxygen availability on methane oxidation and the behavior of two types of methanotrophs was investigated. The results showed that type I bacteria were mostly affected by water content; however, type II was more sensitive to oxygen availability and the highest methane oxidation occurred at an oxygen content of 3%.

Hilger (2000) conducted lab column tests to study the effect of Exopolymeric Substances (EPS) on methane oxidation. Based on the observations, methane oxidation increased over time in the samples and after reaching a peak, it declined to a steady-state condition. It was hypothesized that gradual accumulation of EPS clogged the pores of the sample and consequently limited gas transfer in the soil. Based on the results, oxygen availability and methane oxidation were limited due to clogging; however, it did not cause short circuiting inside the columns.

2.8.6 Soil pH

The optimum pH for methanotrophs available in the soil is the same as optimum pH for methanotrophs in pure cultures (Hanson, R.S. and Hanson, T.E., 1996). Researchers investigated optimum pH for methanotrophs during methane oxidation. Dunfield et al. (1993) studied the anaerobic methane generation and aerobic methane oxidation in slurries of peat samples at a pH range of 3.5-8. Optimum pH for both methane generation and consumption were obtained at pH

values higher than native pH in native acidic peats, and 0–1 pH unit higher in the more alkaline peat, which indicated a greater adaptation of methanotrophs to the acidic environment. In some cases, methanotrophic activity dropped dramatically below or above the optimum pH.

Scheutz and Kjeldsen (2004) studied the effect of pH changes on methane oxidation of the soil samples from Skellingsted Landfill in Denmark. The pH of the soil changed from 2.6 to 9.9 by adding HCl and NaOH solutions. The optimum pH for methanotrophic activity was identified to be in the range of 6.5-7.5, indicating that the microbes were adapted to the local soil pH equal to 6.9.

Cao and Staszewska (2011) and Stępniewski and Pawłowska (1996) also discussed different factors that can affect methane oxidation efficiency in landfills and found an optimum pH in the range of 6-8 for maximum methane oxidation.

2.8.7 Soil compaction

Rachor et al. (2011) conducted lab column tests to determine design criteria for effective methane oxidation in the landfill cover. Methane oxidation and corresponding soil gas composition of the different landfill cover soils (varying content of silt and clay) were measured and in all tests. Soils were compacted to 95% of their specific proctor density to lower the porosity and reflect unfavorable conditions for methane oxidation. They concluded that an increase in the methane flux rate decreases oxygen availability. It increases the absolute methane oxidation rate but decreases the relative proportion of oxidized methane. The specific maximum methane oxidation of each soil was obtained. The oxidation values were directly affected by air-filled porosity of the soil and diffusion-based movement of the air in the soil.

Based on the results, for the soils with a high quantity of fine particles and organic matter, methane oxidation is limited by an increase in methane flux rate into the cover soil. Because methanotrophs are not able to fully compensate for the increasing methane production by methanogens. High soil compaction was also determined as a limiting factor in methane oxidation because oxidation is restricted to the uppermost few centimeters of the cover soil depth and thus, CH_4 oxidation would strongly vary by near-surface conditions such as desiccation, oversaturation after rainfalls and harsh temperatures during winters. Therefore, in places where high compaction is not avoidable, more coarsely textured soil that retains higher porosity should be used for landfills. Finally, to design an efficient landfill cover, the expected methane flux rate, compaction of the soil and its texture are the key factors which determine soil methane oxidation capacity.

2.8.8 Methane concentration and kinetics

The concentration of methane in biogas is one of the important factors that affect methane oxidation by microorganisms (Pawłowska and Stępniewski, 2006). Kinetics of methane oxidation shows how fast oxidation occurs. The Michaelis-Menten equation is used to express the rate of oxidation as follows (Powelson et al., 2006; Cao and Staszewska, 2011; Walkiewicz et al., 2012):

$$V = \frac{V_{max}}{1+K_m/C} = \frac{V_{max} \times C}{C+K_m} \qquad \text{Equation 2-14}$$

Where;

V = methane oxidation rate ($m^3/ (m^3 s)$),

V_{max} = maximum methane oxidation rate ($m^3/ (m^3 s)$),

K_m = Michaelis constant,

C = methane concentration,

As shown in Figure 2.5, for low concentrations of methane, the oxidation equation will be first order. In this case, the maximum methane concentration is below the saturation level:

$$V = \frac{V_{max} \times C}{C + K_m} = \frac{V_{max} \times C}{K_m} \qquad \text{Equation 2-15}$$

However, for high concentrations of methane, the reaction will be zero-order, with maximum reaction rate as follows:

$$V = \frac{V_{max} \times C}{C + K_m} = V_{max} \qquad \text{Equation 2-16}$$

In the above equations, K_m is the half-saturation constant and as shown in Figure 2-4, it is equal to the concentration of methane when the oxidation rate is half of the maximum oxidation rate. A high K_m shows a low growth rate of microorganisms and consequently their low affinity for methane. In general, K_m depends on the type of soil and methane concentration. For forest soils, K_m is in the range of 2.2×10^{-3} to 9.9×10^{-3} %; however, K_m measured in lab column tests simulating landfill soil reached a peak value of 2.9% (Cao and Staszewska, 2011).

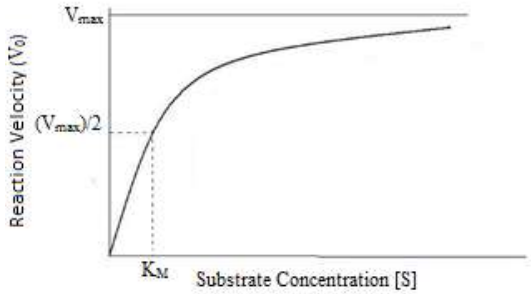


Figure 2-4, Variation of reaction rate (oxidation) with substrate (methane) concentration (Worthington Biochemical Corporation, 2016)

Studies show that there are two types of oxidizing methanotrophs in the soil. The first type of methanotrophs have low methane affinity and a high oxidation rate that leads to having a high Michaelis-Menten constant and high maximum oxidation rate; these conditions favor microorganisms living in an environment with low methane and high oxygen concentration. The second type has a high methane affinity and low oxidation rate that leads to a low Michaelis constant and low maximum oxidation rate; these conditions favor microorganisms living in an environment with high methane and low oxygen concentration (Yaghoubi, 2011). The maximum oxidation rate of the soils varies between 2 to 104 μg CH_4 g^{-1} soil h^{-1}, and Michaelis constant ranges from 1,000 to 25,000 ppmv. Based on previous studies, sandy soil with organic content of 2 to 5% (w/w) and methane concentration higher than 5% (v/v) has the highest methane oxidation capacity (Yaghoubi, 2011).

Scheutz and Kjeldsen (2004) also performed batch tests to determine the methane oxidation rate. They took the samples from the soil that was located 15 – 20 cm under landfill cover surface level and was incubated to 15%(v/v) methane concentration, with a moisture content of 5% w/w, and temperature of 22℃. To investigate the effect of temperature on the methane oxidation kinetics, soil samples were exposed to a specific temperature for two hours before the tests. The moisture content of the samples also changed from 6 to 50% w/w, to study the oxidation rate at different moisture contents. To obtain low moisture content, samples were air dried, and to increase moisture content, distilled water was added to the samples and samples were kept at the new moisture content for 12 hours before the test to let microbes equilibrate to the new climatic condition before the test. Control experiments were also carried out to verify that the methane consumption during batch tests was just due to methane oxidation and no other

mechanisms like adsorption. Therefore, in control experiments, 25 mg of sodium azide/kg soil was added to samples to prevent microbial growth. During the tests, methane concentration dropped while carbon dioxide concentration increased, which indicated methane oxidation in an active environment. No lag phase was observed, which showed that microbes could be adapted to oxidation. Reaching zero-order reaction verified that the oxidation was not methane limited and high oxygen concentration showed that the reaction was not oxygen limited.

Oxidation kinetics were used to calculate maximum oxidation rate (V_{max}) and half-saturation constant (K_m), and these values were obtained 104 μg CH_4 g^{-1} soil h^{-1} and 2.0% v/v respectively. Moreover, the effect of temperature, moisture content, and pH on methane oxidation rate was also studied.

Youn et al. (2007) conducted open-top lab column tests to simulate landfill covers. Soil samples were collected from a landfill in Korea and were passed through 4.76 mm sieve and incubated to facilitate oxidation. Water content and the pH of the samples were determined to be 8.28% and 7.84, respectively. 10 grams of soil sample was also put in 45 mL sterilized vials that were sealed with septa during batch tests. The moisture content of the samples was set at 8, 14, 17, and 23% w/w and methane concentrations were read at time intervals of 0, 30, 60, and 120 minutes. The slope of the graph that showed methane concentration versus elapsed time was then used to find the methane oxidation rate.

Whalen et al., (1990) evaluated kinetic parameters of natural and pure cultures of methanotrophs. K_s values were obtained in the common range that was previously reported in lab column tests and field measurements. A first-order reaction rate constant was obtained of 0.54 h^{-1} if the methane concentration was lower than 1 microliter per liter of gas, and k = -2.37 h^{-1} if the

K_s values were obtained between 2.5 to 9.3 μM, and methane oxidation rate was 6.1 (mg liter^{-1} day^{-1}) for the soil under waterlogged conditions (moisture content of 41%). This oxidation rate value was much lower than the 116 (mg liter^{-1} day^{-1}) oxidation rate for the soil with lower moisture content (11%). The difference between the two oxidation rates was because of lower gas transfer in the soil with higher moisture content.

Pawłowska and Stępniewski (2006) found that kinetic parameters for methanotrophs that have been exposed to high concentrations of methane (e.g. methanotrophs in landfill cover soil) are remarkably different from the ones for methanotrophs that were exposed only to atmospheric concentrations of methane. The first group have high V_{max} and low affinity ($1/K_M$); on the contrary, the second group has low V_{max} and high affinity. V_{max} and K_m were also investigated at different depths of a simulated sand cover.

Pawłowska and Stępniewski (2006) also investigated methane oxidation kinetics under various conditions. The results of lab column tests showed highest methanotrophic activity at the depth of 60 cm, with low K_m values in the range of 0.6 to 2.9% of CH_4 (v/v), and high range of V_{max} values was obtained 0.11×10^{-3} to 0.86×10^{-3} units.

2.9 Models for aerobic oxidation of methane

2.9.1 Background on analytical models to estimate aerobic methane oxidation in landfill covers

To quantify methane oxidation in landfill covers, several numerical models have been proposed (Ng et al., 2015; Liu, 2014) and fields measurements and laboratory column tests have been conducted (Hilger, 1999, Widory, et al., 2011). Bogner et al. (2004) proposed a model to

quantify methane emissions and oxidation through landfill covers. However, the model did not consider the seasonal micro-climatic changes in the soil media. They examined the rates and controlling variables of methanotrophic oxidation at a northeastern Illinois landfill. The landfill had a gas collection system and methane emissions were measured in the cover soil, which was clay soil without geomembrane. They used the static chamber method supplemented by soil gas concentration profiles and field incubations. The measurements were taken initially when the gas collection system was working and then when it was shut down, to evaluate the effect of methane flux to the cover soil on the oxidation. A three-dimensional analytical model was then developed, which was based on mass balance, and included both gaseous mass transfer (CH_4, CO_2, and O_2) and microbial CH_4 oxidation. The model simulated the movement of the methane through the landfill cover and net emissions of methane into the atmosphere. Diffusion was considered as the predominant mechanism of gaseous transport, and the landfill was modeled as porous media for diffusional transport of CH_4. Total porosity, volumetric soil moisture, geometric properties of landfill and gas concentration profiles through the cover soil were included in the model. Methane emissions and oxidation rate were obtained from the model and the analytical results were validated based on field measurements.

Ng et al. (2015) developed a numerical model to estimate methane oxidation in landfill cover soils. The model incorporated water-gas-heat coupled reactive transport in unsaturated soil, and it was validated using experimental data from lab column tests. Heat and water generated by microbial activity during methane oxidation were included in the model and parametric studies were conducted to evaluate the effect of microbial oxidation-generated heat and water. However, the effect of heterogeneity of the soil was not included in the model. Comparison between experimental and analytical results showed good agreement in predicting methane oxidation.

Based on the results, if the water content of the soil is above field capacity, oxidation-generated water decreases methane oxidation. Generated water reduces gas advection and diffusion and, consequently, oxygen availability in the cover soil. On the other hand, if the water content of the soil is below field capacity, generated water increases oxidation due to increased water availability for microbes. Moreover, results showed that heat generated from microbial oxidation can enhance the oxidation if the ambient temperature is below 25°C; it has the opposite effect if the ambient temperature is higher than 25°C.

Chanton et al. (2011) captured samples from the surface of 20 landfills and conducted isotope analysis on the samples to evaluate methane oxidation. They developed an integrated gas model that couples climate and soil model with a gas model to estimate methane oxidation variation with soil heterogeneity. The two-dimensional mass balance-based model represented the landfill cover soil hydraulic properties that affected soil gas transfer and permeability. The main feature of the model was the simulating of secondary porosity in the landfill cover that highly impacts methane oxidation in the soil. The modeled field, with randomly selected hydraulic properties, was 50m × 50m with the two cover thicknesses of 30cm and 60 cm. Porous parts of the field were simulating the cracks in the cover soil. They found that methane oxidation increases linearly by increasing the CH_4 flux to a point, after which the oxidation decreases exponentially with increasing CH_4 loading to the cover soil. Therefore, to enhance methane oxidation, CH_4 loading to the cover soil should be limited. They also concluded that methane oxidation is not a constant value. It is a varying quantity and is a function of cover type, climatic conditions and CH_4 loading to the cover soil.

Abichou et al. (2015) developed a numerical model that coupled water and heat flow in the soil with gas transport and oxidation to simulate methane emissions and oxidation in landfill

covers with and without vegetation in different climatic situations. Based on the analytical models, vegetated cover soil had the lowest methane emission from the surface. Plant roots increased the oxygen availability, soil porosity and consequently, the diffusion-based movement of the soil. Moreover, plant roots increased the organic material of the soil. So, it was concluded that vegetation can be applied to less permeable soils to increase methane oxidation. The maximum difference between the methane oxidation of vegetated and non-vegetated cover soils was obtained during the growing season. Soil moisture content was also lower in the top and middle of the vegetated topsoil compared to non-vegetated soil. However, there was no significant difference between the temperatures of two cover soils. Finally, a relation between methane flux rate to the soil layer and methane oxidation for both vegetated and non-vegetated cover soil was proposed.

Visscher et al. (2003) developed a model which incorporated Stefan-Maxwell diffusion, methane oxidation, and methanotrophic growth, and verified the model based on the data from previously published lab column test research. The results demonstrated that the model is most sensitive to the maximum possible methanotrophic activity. Soil moisture and temperature were also specified as the most important parameters that can affect methane oxidation. However, the results were obtained for steady-state conditions, which are not reachable in a real landfill.

Abichou et al., (2011) developed soil structure, temperature, and moisture correction and scaling factors for methane oxidation in different landfills. Lab column tests were conducted on the homogenized soil to define these factors and predict methane oxidation rate in a real landfill. K_m value was set at 5% to use the model for estimating methane oxidation rate in the landfill. After introducing the factors to the oxidation module of the model, good agreement between

field measurements and model predictions were obtained. So, the model was used to calculate methane oxidation and emission from the real landfill surface.

Bogner et al. (2010) developed CALMIM (CAlifornia Landfill Methane Inventory Model) from 2007 to 2010. CALMIM is an analytical model used to simulate methane oxidation and emissions through landfill covers. The model includes site-specific cover properties and seasonal factors. It also allows the application of both conservative defaults and custom data. In summary, the model includes:

- The effect of the gas collection system on methane emission,
- The effect of different materials for daily, intermediate and final cover,
- The effect of climatic conditions in different seasons on microbial methane oxidation and gas transport in the porous media.

The output of the model includes methane emissions and oxidation in the landfill cover based on various temperature and moisture of the soil throughout the year. Figure 2-5 shows an example of CALMIM output.

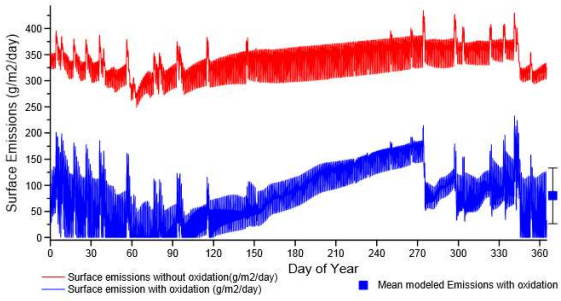


Figure 2-5 An example of CALMIM output

Extensive field validation has been done for verification of the model to apply it for different sites. The data from over 40 landfills, which was selected from 29 international sites, were used to validate the software. International sites were selected from North America, South America,

Asia, Europe, Africa, and Australia. The strong correlation between measured and calculated values using the software was observed (Sattler and Bhatt, 2017).

CALMIM is based on one-dimensional diffusion transport and does not consider other gaseous transport mechanisms. In fact, it considers the concentration gradient as the major driving force for gas transport through the soil, which is affected by the gas collection system and methane oxidation in the cover. Since clay with low hydraulic conductivity is used for cover soil (hydraulic conductivity lower than 10^{-5} cm/sec), considering diffusion as the dominant gaseous transport mechanism is reasonable; however, it does not consider the gas transfer through the cracks.

2.10 Alternative landfill covers

2.10.1 Bio-covers for promoting aerobic oxidation of methane

Alternative covers to increase the CH_4 oxidation rate include bio-covers and bio-filtration beds. The principle of these technologies is the use of methanotrophic bacteria which oxidize CH_4 to water, CO_2, and biomass (EPA, 2011).

2.10.1.1 Studies of compost bio-covers

Kristanto et al. (2015) added contaminated compost (contaminated by Cu) to the soil to increase methane oxidation capacity of the soil and find a way to reuse contaminated compost at the same time. The effect of compaction on methane oxidation was also studied and based on the results, increasing the compaction shifted the methane oxidation zone to the deeper part of the cover layer.

For the compaction of 800-900 kg/m^3, the oxidation zone was 62 cm from the media surface. The maximum methane oxidation was obtained in the sample with the thickness of 40 cm and compaction of 750 kg/m^3 (which occurred in the depth of 20-30 cm from the surface) and in the sample with the thickness of 80 cm and compaction of 800-900 kg/m^3.

Pedersen et al. (2010) determined the methane oxidation rate in a compost bio-cover system using batch incubation, column incubation and field measurements at Fakse Landfill in Denmark. The study was conducted to evaluate the oxidation rate in seven compost types, and batch and column incubations were used to determine the compost with the highest oxidation rate. Therefore, 20 g of compost material was placed inside 300 ml sealed bottles and 40 ml of air was withdrawn from the bottles and replaced with 40 ml of methane so that microbes were pre-equilibrated to methane for one night. Then, 140 ml of air was replaced with 40 ml (15% v/v) methane and 100 ml (35% v/v) oxygen in the bottles. Methane oxidation rates were also studied at different water contents of the soil (to show the effect of seasonal variation of moisture content) and different oxygen diffusive fluxes. Based on the lab results, methane oxidation was 32% when compost respiration was zero, and **23%** if the temperature dependent compost respiration was considered.

Researchers believed that higher oxygen demand and low phosphorous content of compost promoted oxidation rate; however, ammonium content did not affect the oxidation as much. Furthermore, the C/N ratio was another parameter that affected the oxidation rate. After determining the oxidation rate in the lab, field measurements were conducted to evaluate the real oxidation rate in the field. Based on the results, compost material could be utilized as landfill cover material in its mature and stable form, since immature and unstable compost can produce

methane under anaerobic conditions rather than oxidizing it. However, if the compost is too old, its oxidation capacity decreases.

The high nitrogen content of compost can also be another inhibiting factor because high nitrogen content of compost causes the consumption of oxygen by ammonia and lack of oxygen for methane oxidation (Bodelier and Laanbroek, 2004). Moreover, adding some inert materials like sand to the compost can increase the stability of compost and consequently enhance methane oxidation (Yaghoubi, 2011).

Scheutz et al. (2014) conducted batch incubations to determine methane oxidation rates in 10 compost samples. The samples consisted of two types of composts that were passed through 15 mm and 45 mm sieves. Samples were incubated to facilitate methane oxidation and then 70 grams of samples were placed in 100 ml bottles and were sealed using rubber septa. Batch tests were conducted at the temperature of 22℃ and moisture content of 42–44% (dry matter basis). It took 17 to 480 hours to run the tests and oxidation rates were calculated using the linear part of the curve. Based on the results, the highest methane attenuation was obtained **80%**. The results showed that the higher oxidation rate was obtained for compost samples that were passed through 15 mm sieve, since they had more available surface area for microbes to attach and grow.

Cassini et al. (2014) conducted batch tests on two types of garden waste compost to measure methane oxidation rate and respiration activity. 30 grams of samples were placed inside 200 ml containers and 200 ml of headspace was evacuated and filled with 120 ml of oxygen and 80 ml of methane. The change in methane concentration was monitored for 100 hours. Based on the results, biofilters reached the oxidation of almost **100%**.

Barlaz et al. (2004) used yard waste compost to increase the microbial activity of landfill cover soil and reduce methane and non-methane organic compounds from the landfill surface. Field measurements were carried out for 14 months and based on the observations, methane oxidation of the bio-cover varied from −1.73 to 1.33 g m^{-2} d^{-1}. In 52% of tests, which represented the tests when the gas collection system was turned on, the cover oxidized **100%** of methane generated in the landfill and oxidized some methane that existed in the atmosphere. Then the gas collection system was shut off to evaluate its effect on methane emissions. In both cases, methane emissions from the bio-cover did not change; however, the methane flux was high in the places covered with pure soil. Atmospheric methane uptake of the cover soil was also observed in 54% of tests when the gas collection system was active and 12% of tests when the system was shut off. Overall, it was concluded that the bio-cover could oxidize **55%** of methane that reached beneath the cover, whereas soil cover could just oxidize 21% of the methane.

Fredenslund (2010) implemented a bio-cover (composted yard waste) in Fakse Landfill, Denmark, to evaluate methane oxidation efficiency of the full-scale cover by measuring methane emissions through the cover before and after implementation. Flux chamber measurements were done for one year after implementation of the bio-cover. Results showed that bio-cover reduced methane emissions by **27%**.

2.10.1.2 Studies of biochar covers

Reddy et al. (2014) conducted batch and lab column tests to investigate the effect of biochar amendments on landfill cover soil, the growth of methanotrophic bacteria of the cover soil, and methane oxidation. Biochar is made of biomass and it is a stable solid, which is high in carbon content. The columns were filled with gravel, and either soil, or 20% biochar/80% soil and were

fed by humidified landfill gas for four months. Based on the results, the maximum oxidation rate obtained 0.38 and 1.35 nmol s^{-1}g dry $soil^{-1}$ at 22°C and 35°C in the amended sample and 0.18 nmol s^{-1}g dry $soil^{-1}$ in the control test. The results showed that the methane oxidation increased in biochar-amended soil in comparison to the soil alone. Therefore, the biochar-soil combination was considered as an effective cover layer for increasing the growth of methanotrophic microorganisms and consequently, achieving greater methane oxidation capacity.

Sadasivam and Reddy (2015) conducted several batch and column tests to investigate the adsorption and biodegradation of methane in soil that was amended with waste hardwood biochars. Samples were amended with different ratios of 2, 5 and 10% biochar by weight. Samples had different moisture contents from 0 to 75% water holding capacity of the soil, different temperatures (25, 35 and 45°C) and concentrations of methane within 0-1 kPa. The results showed 2 times more gas transport in the samples amended with biochar compared to conventional soil cover. Methane sorption capacity in the sample, which was amended with dry biochar, was 1.03×10^{-2} to 7.97×10^{-2} mol kg^{-1} which was 10 times higher than that of pure soil with 1.9×10^{-3} mol kg^{-1}.

In another study, Yaghoubi et al. (2014) conducted batch incubations to determine Michaelis-Menten kinetics for methane oxidation in a soil amended with biochar and evaluate the increase in methane oxidation rate due to the amendment. Batch tests were conducted in 250 ml rubber sealed bottles and the landfill gas that was injected to the bottles was composed of $5\%CH_4/5\%CO_2/90\%N_2$ and $25\%CH_4/25\%CO_2/50\%N_2$. 5 to 10 g of material (different combinations of soil and biochar with different moisture contents) were passed through #40 and #20 sieves and placed inside the bottles. Batch tests were conducted at room temperature and methane and carbon dioxide concentrations were measured using a gas chromatograph at specific

time intervals. The researchers also conducted batch tests to find the adsorption isotherm parameters of the combination of soil and biochar. 500 ml sealed bottles were used for adsorption batch tests and 5 to 10 g of different compositions of soil and biochar were passed through #10, #20 and #40 sieves and placed inside the bottles. Pseudo-second-order adsorption rate constants for different combinations were evaluated as presented for some dry samples in Table 2-1.

To completely inhibit methane oxidation during the adsorption tests, the soil was autoclaved at 121 °C to kill microorganisms. The results showed that the adsorption in dry biochar is 10 times higher than the adsorption of soil alone. Batch experiments were stopped after methane adsorption reached equilibrium; however, carbon dioxide had not reached equilibrium yet.

Table 2-1 Pseudo second-order adsorption rate constants k and maximum adsorption capacities q_e of methane and carbon dioxide for different biochar samples (Yaghoubi et al., 2014)

Adsorbent	**Pseudo-second-order**			
	CH_4		**CO_2**	
	k_2 ($kg.ml^{-1}min^{-1}$)	**q_e (ml/kg)**	**k_2 ($kg.ml^{-1}min^{-1}$)**	**q_e (ml/kg)**
Soil	0.038	32.9	0.07	67.0
10% Biochar as is	0.068	59.2	0.032	110.0
10% Biochar20	0.075	64.5	0.029	143.0
10% Biochar40	0.63	80	0.032	161.0
20% Biochar as is	0.056	82.6	0.028	137.0
20% Biochar20	0.058	111	0.023	167.0
20% Biochar40	0.068	156	0.024	204

Continuous column tests were also conducted for 4 months. After extracting the soil from the columns, the soil was divided into three sections: upper, middle, and lower layers. A specific amount of soil from each layer was then placed inside the sealed batch reactors to run batch tests.

The results of batch tests are presented in Table 2-2. According to the results, biochar-amended soil had a higher maximum oxidation rate than the pure soil, and the maximum oxidation rate was much higher in the top layer. Moreover, an increase in temperature enhanced the maximum oxidation rare in the amended soil (not in pure soil), which is probably because of the sensitivity of microbial community of biochar to temperature.

Table 2-2 Kinetic parameters for methane oxidation obtained from batch incubations of soil only and soil/biochar (Yaghoubi et al., 2014),

Column	Position	Temperature (°C)	V_{max} (nmol s^{-1} g^{-1} dry)	K_M (mol m^{-3})
Soil only	Top	22	0.18	0.83
	Top	35	0.16	0.27
	Middle	22	0.19	1.21
	Bottom	22	0.17	0.86
Biochar: 20%	Top	22	0.38	0.89
	Top	35	1.35	2.57
	Middle	22	0.28	1.27
	Bottom	22	0.24	0.52

Though this study showed promising oxidation potential of biochar, in some cases it can suppress methane oxidation. For example, in a study by Spokas et al. (2009), the oxidation rate of different combinations of soil and biochar was investigated, and the results showed that biochar suppressed oxidation of methane in all soil-biochar combinations.

2.10.1.3 Studies of other bio-cover materials

Pariatamby et al. (2015) studied methane oxidation rate of soil amended with organic wastes such as compost, sawdust, empty fruit bunch, black soil spent yeast, sewage sludge and spent tea leaves. 125 ml bottles with sealed rubber septa were used for batch tests and 20 g of samples

(different combinations of organic wastes) was placed inside the bottles. 15 ml of the air was removed from headspace and instead, 10 ml oxygen and 5 ml methane were injected into the headspace, which resulted in 4% methane (v/v) and 8% oxygen (v/v) in the headspace volume. Bio-cover Performance Index (BPI) was used to evaluate methane oxidation potential of the different combination of organic wastes, with a higher BPI value representing a higher methane oxidation rate. BPI was calculated as follows:

$$BPI = \frac{CH_4 - CH_{4_n}}{W \times N} \qquad \text{Equation 2-17}$$

In which:

CH_4: Initial methane concentration (µg/ml)

CH_{4n}: Methane concentration at time n (µg/ml)

W: Amount of bio-cover (g)

N: Time (hr)

Bio-cover Performance Indices for different combinations of organic waste were obtained as shown in Table 2-3.

Table 2-3 Bio-cover Performance Index for different combination of wastes (Pariatamby et al., 2015)

Organic waste	**BPI ($\times 10^3$µg g^{-1} h^{-1})**
20 % Sewage sludge + 80 % compost	8.33
20 % Spent yeast + 80 % compost	0.52
All ratios of EFB	1.754
20 % Sawdust + 80 % compost	4.16
40 % Sawdust + 60 % compost	4.16

Figure 2-6 also shows the methane percentage in the headspace for different waste combinations during batch incubations. Based on the results, sawdust and compost had the highest methane oxidation rate.

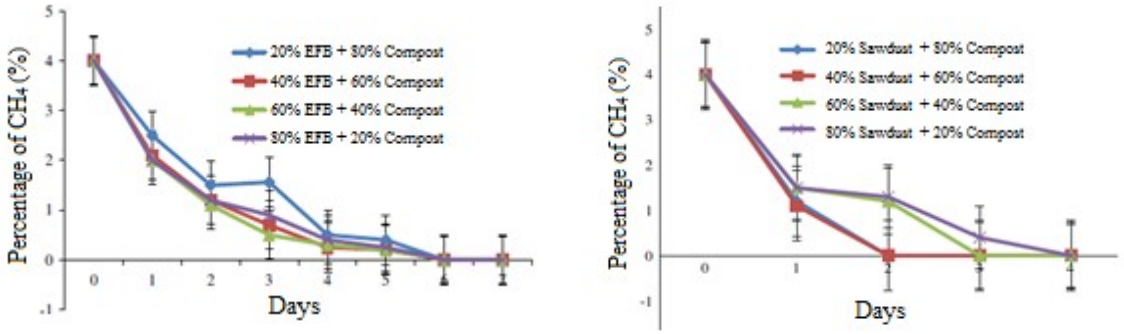


Figure 2-6 Reduction in methane concentration by different waste combinations (Pariatamby et al., 2015)

Einola (2010) measured methane consumption, methane oxidation, and carbon dioxide generation rates for soil and different bio-cover (made of compost from municipal sewage sludge and sludge from food-board factory effluent treatment) materials in batch tests. First, the soil was air dried to the moisture content of 7% at a temperature of 30℃. Deionized water was then added to the samples to give them the desired moisture content. After placing the samples inside the sealed bottles, samples were pre-incubated for one day and then were aerated for 30 min to run the batch tests. 10 ml of air was removed from the head space and 10 ml of methane was injected into the headspace. In another reactor, 5 ml of methane was added to the head space; however, no air was removed. To measure methane concentration, 0.1 ml gas samples were taken from the head space regularly and analyzed using gas chromatography. After plotting the moles of gas per sample weight versus time, zero-order rate constants were obtained for methane consumption and carbon dioxide production using linear regression,. Field tests were also conducted, and the

maximum methane oxidation was obtained higher than **96%**; however, the oxidation decreased during winter.

Wang et al. (2011) conducted experimental research on methane oxidation in bio-cover soil. The bio-cover material was taken from an organic waste landfill bioreactor. The effect of composition, ambient conditions and nitrogen stress on CH_4 oxidation was evaluated and the composition of the cover such as particle size, moisture and pH were optimized to achieve higher methane oxidation. Based on the results, bio-cover soil with original pH value, 45% soil moisture and particle size ≤ 4 mm oxidized methane and performed very well as a landfill cover. In fact, methane oxidation rate increased from 0.91 ± 0.08 to 3.60 ± 0.16 μmol (g d.w.)$^{-1}$ h^{-1}, when the particle size changed from ≤ 0.45 mm to ≤ 4 mm. Methane oxidation was enhanced by increasing CH_4 loading to the saturated concentration point of 10% (v/v). CH_4 oxidation rate varied based on the temperature, nitrogen content, and oxygen concentration. Moreover, ammonia volatilization from landfills and nitrification in the soil enhanced methane oxidation when the content of NH_4^+ and NO_3^- was lower than 600 mg kg^{-1}; however, it had a negative effect when the concentration of NH_4^+ and NO_3^- was higher than 1200 mg kg^{-1}. Based on the results, the addition of NH_4^+-N from 20 to 300 mg kg^{-1} increased the oxidation rate to 5.0–6.0 μmol (g d.w.)$^{-1}$ h^{-1}.

Adams et al. (2011) investigated methane oxidation capacity of bio-tarp covers through lab column tests with continuous 0.5 mL min^{-1} landfill gas flow. Bio-tarp is a bio-based system such as bio-cover, bio-window or biofilter, which can be removed or reactivated. It is a temporary cover that is placed on top of the waste after each workday to oxidize the methane that is produced by waste. Therefore, it reduces the methane emission from an active landfill cell (Alshareedah and Sallis, 2016 and Hilger et al., 2007). The flux through the bio-tarp was 23.2 g

m^{-2} d^{-1}, which simulated landfill gas flux lower than the maximum range that could fall in 100–200 g m^{-2} d^{-1}. The most suitable geotextiles to use with bio-tarp were specified. The results of the study showed that application of two geotextile layers along with bio-tarp decreased methane emission by 16% of fluxed methane, while trials of bio-tarps containing enriched landfill soil lowered methane emission by 26%. Moreover, adding landfill cover soil, shale or compost to the combination of bio-tarp and geotextile decreased methane emissions by **32%**. Field measurements were also carried out; however, a significant difference was not observed between methane emissions in the multilayered bio-tarp cover and controls without methanotrophs. The reason was probably variation in methane flux, so future field measurements were recommended to evaluate the applicability of proposed cover in a real landfill.

2.10.2 Limitations of bio-covers for promoting aerobic oxidation of methane

As mentioned in the previous section, in some cases, bio-cover lab studies have achieved 80-100% oxidation. Field studies have achieved removal ranging from 23% to 55%. In general, there are some disadvantages to these bio-covers as follows:

- Potential for methane generation: There is a risk of methane generation in bio-cover if either water saturation or high LFG generation causes anaerobic conditions in the absence of alternative electron acceptors in the bio-cover (Barlaz et al., 2004). In addition, the process of producing compost itself, although theoretically aerobic, in actuality is often partially anaerobic and produces methane.
- Reduced availability of oxygen: The oxygen demand for maintaining the plants in the bio-cover itself can compete with the oxygen demand for methane oxidation. Moreover, in a landfill with high LFG pressure under the cover, gas pressure may prevent oxygen

entrance into the bio-cover. This can especially be a critical issue in the landfills without a gas collection system (Pedersen et al., 2010). Finally, clogging due to EPS, discussed below, can limit oxygen availability.

- Maintenance issues/costs:
  - The material of bio-cover can get clogged due to microbially-produced Exopolymeric substances (EPS), (Scheutz et al., 2009). Thus, it is required to sweep the chamber's basal gravel layer, which increases operation/maintenance costs.
  - Degradation of bio-cover material can decrease the thickness of the cover and cause instability. Therefore, either the exchange of the cover or the addition of more bio-cover material would be needed to maintain the required thickness of the cover (Pedersen et al., 2010).
  - Maintaining constant moisture content also adds the maintenance cost (Pawlowska, 2014).
- Cold weather vulnerability: Bio-cover performance is highly affected by ambient temperature. Even if the oxidation rate is as high as 96%, it lowers during winter (Einola, 2010). Aerobic methane oxidation occurs in the top parts of the cover, which is the most vulnerable to changes in temperature.
- Competitive microbial activities might inhibit methanotrophic activities in the bio-cover because of high amounts of nutrients in the compost material (Sadasivam & Reddy, 2014, and Long et al., 2014). For example, the presence of ammonia can lower the methane oxidation rate, because of oxidation of ammonia by methane-oxidizing bacteria (Neill and Wilikinson, 1977).

2.10.3 Alternative covers for removing hydrogen sulfide

As discussed previously, the degradation of municipal solid waste in the landfills produces landfill gas, which can be emitted into the atmosphere after passing through the landfill cover. Other than methane and carbon dioxide, which constitute the major part of produced gas, there are also a trace amount of other gases like hydrogen sulfide. Sulfate-reducing bacteria utilize sulfate as an electron acceptor in the absence of oxygen and produce H_2S in the anaerobic zone of the cover layer as the result of sulfate reduction. Hydrogen sulfide contributes to a rotten egg odor and can cause health problems like neurotoxicity in humans and even death at high dosages (Du et al., 2014). Since adsorption and degradation of the produced gas can significantly decrease the amount of emitted gas, researchers try to find alternative cover materials which can improve adsorption and degradation of the landfill gas. Many of these research works focus on the reduction of H_2S in municipal landfills.

Bergersen and Haarstad (2008) studied the hydrogen sulfide emissions from landfill covers (simulated by 30-L reactors), including sulfur-containing plaster board. The effect of water content was also studied. Iron hydroxide was added to the landfill cover soil, and according to the following reaction, metal sulfide precipitated and reduced hydrogen sulfide emissions from landfill cover.

$$2Fe(OH)_{3(s)} + 3H_2S \longleftrightarrow 2FeS_{(s)} + 1/8S_{8(s)} + 6H_2O \qquad \text{Equation 2-18}$$

The influence of adding other wastes to the cover soil was also investigated. Among all wastes, adding Iron oxides, bottom ash/iron oxides (3:1) and sludge compost gave the lease hydrogen sulfide emissions from the cover.

Metal-rich filters have been shown to reduce H_2S more effectively than organic filters. H_2S removal by filtering through mineral wool waste products was studied (Bergersen and Haarstad, 2014) and wool waste products (that are high in metal content especially Fe), could effectively remove H_2S with the flow 100 times less than the actual gas flow. This low flow rate, which was 0.3 L min^{-1}, did not cause visible gas cracks in the filter material. 98% of H_2S gas was removed over 80 days of using filter made of the mixture of mineral wool waste and rod mill waste. It was concluded that if enough retention time and moisture content is provided, the filter can effectively remove sulfide gas. Considering H_2S gas concentration as 45 ppm and wool and rod mill waste of 14 g/Kg and 261 g/Kg, removal capacity would last 11-308 days.

Ducom et al. (2009) used municipal solid waste incineration (MSWI) bottom ash to reduce the amount of sulfur-containing compounds such as hydrogen sulfide, methyl mercaptan, and dimethyl sulfide in the landfill gas. Field studies were conducted in Biovale Company landfill to evaluate the application of the proposed cover soil in a real landfill and the effect of bottom ash on the concentration of landfill major gases such as methane and carbon dioxide. The landfill had a gas collection system and the produced gas was used to generate electricity via six gas engines. The results showed that the bottom ash filter can significantly reduce that amount of sulfur compounds as well as CO_2. However, it had no effect on CH_4. Gas retention happened due to diffusion and chemical reaction. A transfer coefficient for diffusion was calculated using Equation 2-19, and the results showed that diffusion was not a limiting parameter in the experiment.

$$k_D = \frac{ShD_{H_2S-gas}}{d_P} \qquad \text{Equation 2-19}$$

Where,

Sh is Sherwood number,

D_{H_2S-ga} is the H_2S gas diffusivity, and

d_P is the average diameter of media particles.

In the experiments, k_D (rate of gas removal) was higher than the gas speed, showing that diffusion was not controlling gas retention speed. In the same way, high kinetics of chemical reaction in the aqueous phase did not limit the retention speed. Therefore, due to the high speed of retention, the filter trapped the gases fast in the beginning; however, retention speed decreased over time due to the saturation of water.

He et al., (2012) studied H_2S concentrations in two types of soil: Landfill Cover Soil (LCS) and Waste Bio-cover Soil (WBC). In the beginning, H_2S was removed through adsorption to the solid phase and absorption into the liquid phase and biodegradation in LCS, while WBS showed more H_2S removal by biotransformation in addition to sorption. Over time, WBC showed higher H_2S attenuation compared to LCS because aerobic conditions can stimulate bacteria to have faster biotransformation activity. Finally, WBS was proposed as an effective alternative landfill cover to trap H_2S and solve the odor problem in landfills.

Xu et al. (2010) studied H_2S attenuation in the cover soil with different materials by doing both laboratory bottle experiments and field measurements. The study showed that H_2S removal is higher in compost, yard trash, or the soil amended with quicklime or calcium carbonate compared to sandy soil. In addition to physical/chemical sorption and biological degradation in alternate cover soils, some materials create unfavorable conditions for sulfate-reducing bacteria and subsequently decrease H_2S generation.

Hurst et al. (2005) studied the removal of odorous emission from the surface of landfills using municipal solid waste compost by conducting column tests. According to the results, compost with the bulk density of 590kg/m^3 and 740 kg/m3 could remove 69% and 97% of odor correspondingly. In summary, compost showed the ability to remove 63-100% of the inlet concentration of the selected sulfurous compounds in the initial 10 cm of compost depth.

Plaza et al. (2007) investigated the reduction of H_2S emissions from construction and demolition landfills with different cover layers: sandy soil, sandy soil amended with lime, clay soil, fine concrete with particle size less than 2.5 cm, and coarse concrete with particle size greater than 2.5 cm. Lab column tests were conducted to study the H_2S attenuation capacity of five cover layers under anaerobic conditions. Maximum hydrogen sulfide removal (more than 99% efficiency) was obtained in lime-amended soil and fine concrete, probably because of two mechanisms: physical sorption and high pH of the cover which inhibited the activity of sulfate-reducing bacteria. Clay and sandy soil had lower H_2S attenuation capacities of 65% and 30%, respectively, and coarse concrete had the lowest removal efficiency. It was hypothesized that the removal in clayey soil, sandy soil and coarse concrete was just due to physical sorption and physical removal was the least for coarse concrete because of the low internal surface area of the cover.

Adding iron can increase methane generation. Since sulfate-reducing bacteria inhibit methanogenic activity, methane generation increases when H_2S generation decreases. Adding iron to landfilled waste was suggested by the researchers to decrease odor problems in landfills. H_2S can be removed by adsorption and oxidation, however, these methods can remove H_2S for a short time.

In an study by Du Y. et al. (2014), 48 reactors with the volume of 50 ml were used to simulate landfill cover soil for three groups of; R_1: without $Fe(OH)_3$, R_2: $Fe(OH)_3$ added to the soil, and R: $Hg(Cl)_2$ added to the soil to inhibit microbial activity. The results showed that the presence of $Fe(OH)_3$ in the biodegradable sulfur-containing substrate can lower H_2S emission about 95%. Researchers concluded that the iron present in landfilled refuse could be used to lower H_2S emission from the surface of landfills. An advantage of using iron-rich landfilled refuse in the alternative cover is the high iron content of the refuse in the landfill, and alternative cover that contains iron-rich landfilled refuse, can both reduce the space occupied by cover material and also reduce the emission of H_2S gas from the surface of landfills into the atmosphere.

2.11 Anaerobic oxidation of methane

As indicated, oxygen acts as an electron acceptor for methane oxidation in the aerobic part of the landfill cover, while other electron acceptors can contribute to methane oxidation reactions in the anaerobic parts of the soil. Anaerobic oxidation of methane (AOM) occurs in the absence of oxygen and it has been shown to consume around 90% of the methane produced in marine sediments, where oxygen diffusion is limited. In this case, sulfate, ferrous iron, and other compounds act as electron acceptors and an alternative of oxygen. AOM is not thermodynamically feasible unless an electron acceptor is provided.

Studies show that the Anaerobic Methanotrophic Archaea (ANME) which oxidize methane in an anaerobic environment, are closely related to the ones that generate methane (Blazewicz et al., 2012). Mueller, T.J. et al., (2015) suggested that AOM has higher carbon efficiency compared to

aerobic oxidation of methane. Reverse methanogenesis produces Acetyl-CoA without losing carbon, while aerobic oxidation of methane lose some carbon in the form of CO_2.

2.11.1 Anaerobic oxidation of methane by sulfate

Sulfate reduction, as shown in$CH_4 + SO_2^{-4} \rightarrow HS^- + HCO_3^- + H_2O$ Equation 2-20, has been shown to be the dominant mechanism of anaerobic oxidation of methane in marine environments due to the abundance of sulfate, with ΔG_r^0 of -16.6 kJ mol^{-1} (Caldwell et al., 2008).

$CH_4 + SO_2^{-4} \rightarrow HS^- + HCO_3^- + H_2O$ Equation 2-20

Bicarbonate can reduce the available energy for this reaction. However, since seawater is high in calcium carbonate, the concentration of bicarbonate will be reduced when it is accumulated due to AOM. This reaction would have higher energy as the pressure (depth in marine systems) increases, however, this effect is negligible (Caldwell et al., 2008). Another parameter that is affecting in sulfate-dependent AOM is the concentration of methane. According to Nauhaus, K., et al. (2002), by increasing the partial pressure of methane from 0.1 to 1.1 MPa, sulfide generation will increase by 4-5-fold. Moreover, if methane enters this reaction in the gas phase, the reaction will yield more energy. Finally, if the concentration of sulfide falls between 10-15mM, it will have a toxic effect on the reaction. Figure 2-7 Shows the required mM of each component. If the combination of the concentrations falls on the left side of the curve, methane oxidation reaction will not happen. The concentration of sulfate and methane should also be enough to trigger the reaction.

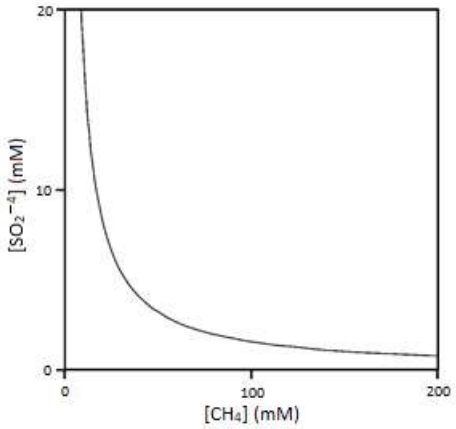


Figure 2-7 Combination of methane and sulfate that yield 30kJ $mole^{-1}$. Concentration combination that falls on the left side of the curve, yield less energy and cannot trigger the reaction (Caldwell et al., 2008)

Segarra et al. (2015) studied anaerobic oxidation of methane in the wetlands in Florida, Georgia, and Maine. According to the results, the maximum methane oxidation rate was observed in Georgia. Among electron acceptors, nitrate, nitrite, sulfate, iron, and manganese, sulfate was recognized as the electron acceptor with the highest methane oxidation rate. The results showed that freshwater wetlands can consume 200 Tg of methane per year, which decreases methane emissions by 50%. It was also concluded that microorganisms can consume carbon dioxide as a carbon source in addition to methane.

According to the previous studies, three mechanisms are proposed as the possible sulfate AOM mechanisms (Caldwell et al., 2008):

Reverse methanogenesis: This is the most studied mechanism for AOM, in which methanogens reverse the methanogenesis pathway and methane generation and oxidation occur simultaneously. Studies show that methanotrophs involved in AOM require the same environmental condition as methanogens (Glass and Orphan, 2012). Based on the differences between the gross methane generation and oxidation rates, the concentration of methane varies in

the soil. In an enclosed anaerobic system, where generation and oxidation of methane occur simultaneously, the isotopic composition of methane depends on the mass of generated and consumed methane.

Acetogenesis: In this case, there are two mechanisms for sulfate-dependent methane oxidation. Based on the first mechanism, acetic acid and H_2 are produced from methane and are finally consumed by sulfate-reducing bacteria. The hypothesis for the second mechanism is that acetate is produced from CO_2 and CH_4 and is finally consumed by sulfate-reducing bacteria.

Methylogenesis: In this mechanism, methane-oxidizing and CO_2-reducing archaea produce methyl sulfides, which are finally consumed by sulfate-reducing bacteria.

65% of methane generated in freshwater Canadian Shield Lake (Lake 227) is consumed anaerobically (Cicerone and Oremland, 1988). Oremland et al. (1987) identified four sources of methane generation in Monto Lake: two sources in the lake and two other sources from microbiological activities. The overall amount of gas seep in the lake was estimated as 2.1×10^6 moles of CH_4. Sulfate concentration decreased from 133 mM at the surface to 35 mM at the bottom, which was attributed to sulfate-reduction and methanogenic activities. Both aerobic and anaerobic methane oxidation resulted in a 98% reduction in the methane concentration. However, methane flux from the surface of the lake was still supersaturated with respect to atmospheric concentration.

2.11.2 Anaerobic oxidation of methane by non-sulfate species

AOM has been observed in the absence of both oxygen and sulfate, which is related to the other electron acceptors such as Fe^{3+}, NO_3^-, or Mn^{4+} (Caldwell et al., 2008, and Segarra et al.,

2015). The standard free energy for the reactions between methane and different electron acceptors are given in Table 2-4.

Sulfate as electron acceptor: Methanotrophic archaea ANME and sulfate-reducing bacteria use sulfate to oxidizes methane according to the following reaction;

$$CH_4 + SO_4^{2-} \longrightarrow HCO_3^- + HS^- + H_2O \qquad \text{Equation 2-21}$$

As shown in Table 2-4, the standard free energy for the equation in which sulfate acts as an electron acceptor is relatively low; however, sulfate is the most prevalent electron acceptor during AOM. Therefore, the reason for prevalent methane oxidation by sulfate is probably the availability of sulfate in marine systems, not the feasibility of the equation. Moreover, in general, AOM in marine systems is slow due to low temperatures and low availability of organic carbon (Smemo and Yavitt, 2011).

Iron as electron acceptor: Oxidized solid phases such as iron (Fe) oxides are more thermodynamically favorable electron acceptors for the biological oxidation of CH_4 and have been shown to stimulate anaerobic methane oxidation. Table 2-4 shows Standard free energy (kJ mol^{-1} CH_4) of reaction for anaerobic microbe-mediated iron oxidation of CH_4 (Caldwell et al., 2008):

$$CH_4 + 8\ Fe(OH)_3 + 15\ H^+ \longrightarrow HCO^-_3 + 8\ Fe^{2+} + 21\ H_2O \qquad \text{Equation 2-22}$$

Table 2-4 Standard free energy of reactions between methane and electron acceptors (Caldwell et al., 2008)

Reaction	$CH_{4\,(g)}$	$CH_{4\,(aq)}$
$CH_4+SO_4^{2-} \longrightarrow HCO_3^-+HS^-+H_2O$	-16.6	-33.0
$CH_4+SO_4^{2-}+2H^+ \longrightarrow CO_2+H_2S+2H_2O$	-92.8	-109.2
$CH_4+2O_2 \longrightarrow HCO_3^-+H_2O+H^+$	-806.0	-822.4
$CH_4+2O_2 \longrightarrow CO_2+2H_2O$	-842.3	-858.7
$CH_4+4NO_3^- \longrightarrow HCO_3^-+4NO_2^-+H^++H_2O$	-467.0	-483.4
$CH_4+4NO_3^- \longrightarrow CO_2+4NO_2^-+2H_2O$	-503.4	-519.8
$5CH_4+8MNO_4^-+19H^+ \longrightarrow 5HCO_3^- +8Mn^{2+}+17H_2O$	-991.7	-1008.1
$5CH_4+8MNO_4^-+24H^+ \longrightarrow 5CO_2+8Mn^{2+}+22H_2O$	-1028.1	-1044.5
$CH_4+8Fe^{3+}+3H_2O \longrightarrow HCO_3^-+8Fe^{2+}+9H^+$	-418.3	-434.7
$CH_4+8Fe^{3+}+2H_2O \longrightarrow CO_2+8Fe^{2+}+8H^+$	-454.6	-471.0

Nitrate as electron acceptor: Nitrate has been used by researchers as a terminal electron acceptor to anaerobically oxidize methane (Haroon et al., 2013). Further studies have shown that denitrifying bacteria can oxidize methane anaerobically. Anaerobic oxidation of the methane using NO_3^- produces almost the same free Gibbs energy as aerobic oxidation and its mechanism is very different from anaerobic oxidation using sulfate as an electron acceptor.$5CH_4+8NO^-_3 +8H^+ \rightarrow 5CO_2+4N_2+14H_2O$ Equation 2-23 shows AOM coupled to denitrification:

$$5CH_4+8NO^-_3 +8H^+ \rightarrow 5CO_2+4N_2+14H_2O \qquad \text{Equation 2-23}$$

However, NO_2^-, which is an intermediate during the denitrification process, can suppress methane generation and consequently AOM. When NO_2^- is reduced, N_2 and O_2 will be produced along with the oxidation of CH_4 (Smemo and Yavitt, 2011).

In a research by Ettwig et al. (2008), it was concluded that denitrifying bacteria can anaerobically oxidize methane, which may be related to the reduction of nitrite and production of oxygen that changes the anaerobic environment to aerobic and triggers aerobic oxidation of methane. Ettwig et al. (2010), also used both nitrite and nitrate as electron acceptors to promote AOM. Based on the results, nitrate could not facilitate AOM, however, nitrite promoted AOM using the oxygen produced by denitrification.

2.12 Studies of AOM in the soil

AOM has mostly been studied in marine systems and it was not discussed in the soil until recently. Anaerobic oxidation in cover soil is much faster than oxidation in wetlands, because of higher diffusion in gaseous space compared to liquid space, which enhances methane transport to methanotrophs (Whalen et al., 1990).

Blazewicz et al. (2012) studied anaerobic oxidation of methane in soils from Alaska and Puerto Rico. Experiments were carried out in an anoxic environment and isotope analyses were used to evaluate the amount of oxidized methane. Batch tests were conducted by putting 10 g of samples inside 100 ml bottles. Bottles were flushed using N_2 gas and were filled with N_2/CO_2 (98/2%) to create an anaerobic environment inside the bottles. To evaluate the effect of different electron acceptors such as NO_3^-, Fe(III), and SO_4^{2-} on anaerobic oxidation of methane, $NaNO_3$

(5 mM), soluble Fe(III) nitrilotriacetic acid (NTA) (10mM), and Na_2SO_4 (5 mM) were added, respectively. Then, the rates of aerobic and anaerobic oxidation of methane were evaluated.

The aerobic oxidation rate was obtained using the concentration of methane at the beginning and the end of the tests, while anaerobic oxidation rate was calculated by subtracting the methane oxidized at first time point (when the quantity of the oxidation was significant) and the immediately preceding time point. The results showed that the anaerobic oxidation had the highest rate when the soil was not amended. In fact, the addition of electron acceptors inhibited both methane generation and oxidation. Based on the results, methane oxidation happened at the same time as methane generation. There was a strong correlation between generated and oxidized methane; the anaerobic oxidation rate was lower than the generation rate. This agreed with the previous studies by Hoehler et al. (1994) that showed anaerobic oxidation of methane and generation occur simultaneously. Hoehler et al. (1994), also concluded that the addition of methyl-coenzyme M reductase (MCR) will catalyze the reverse equation (methane generation).

Several studies have demonstrated that the addition of electron acceptors can enhance anaerobic oxidation of methane. Pozdnyakov et al. (2011) evaluated the anaerobic oxidation of methane capacity of drained peat and automorphic sod-podzol soils, which were picked from central parts of the floodplain. KNO_3 and Na_2SO_4 were also added to the soil to study the effects of different electron acceptors on methane oxidation in the soil. 15 ml vials were used for batch incubations and 3 g of soil along with 0.4 mg KNO_3/g soil, 0.3mg Na_2SO_4/g soil, 0.21mg ammonium chloride /g soil, and 2.5mg ammonium chloride /g soil were placed inside the vials. Then, the samples were saturated to field capacity and the saturated samples were purged with argon gas for a minute. After purging, 1 ml of acetylene was added to prevent N_2O reduction.

The concentration of methane was measured using 0.5 ml samples and after four days, both anaerobic oxidation of methane and denitrification of N_2O were observed.

Based on the results, the addition of nitrate and sulfate increased the anaerobic oxidation of methane. It was concluded that the increase in the oxidation could be because of increase in the mineral nutrient content of the sample, growing of microorganisms on the other substrates, availability of different electron acceptors, and activity of aerobic microorganisms on the residual oxygen in the vials. Anaerobic oxidation in the control sample was 6.8 to 35.7 nmol g^{-1} day^{-1} (an average of 18.7), while it increased from 2.3 to 65.7% (an average of 26%) in the samples with additional electron acceptors.

Smemo and Yavitt (2007) studied AOM in several northern peatlands. Three independent methods (addition of methanogenic inhibitors, stable isotope enrichment, and natural abundance stable isotope analysis) were used to verify the results. The results showed that AOM can happen only if enough concentration of methane is available. So, it will be methane-limited in the laboratory studies where methane does not flow continuously.

Murase and Kimura (1994) investigated anaerobic methane oxidation in paddy fields by conducting lab column tests. Leachate was collected from the bottom of the samples and incubated using methane and electron acceptors such as NO_3^-, MnO_2, $Fe(OH)_3$, SO_4^{2-}. Oxidants were added with a concentration of 0.01 gr/gr soil. The samples were then placed inside the glass containers with a height of 10 cm and a diameter of 3 cm. Results showed a decrease in methane concentration in the presence of MnO_2 and SO_4^{2-}. Adding sulfurous compounds such as $CaSO_4$, $FeSO_4$ resulted in methane oxidation in both plow layer soil and subsoil.

2.13 Batch reactor tests and column tests for evaluating landfill cover materials

Batch reactor experiments are a relatively quick and straightforward method of evaluating the methane oxidation rate in landfill cover soils. Adsorption of gas particles to the surface of the soil can also be studied through these tests. Before the isotopic method was developed, batch incubations were the most common way to evaluate methane oxidation rate in landfill cover soils. They have some advantages, such as technical simplicity, reduced lab work, and lower cost, over column tests with the continuous gas flow. Therefore, batch tests are preferred when we need to evaluate the effect of some parameters on the methane oxidation rate for a high number of samples. On the other hand, these experiments cannot represent the dynamic gas transfer mechanism, which is observed in column tests. Therefore, comparing the methane oxidation rate obtained from batch tests with that of column tests is not appropriate (Scheutz et al., 2009).

Oxidation rate depends on the pre-exposure of the soil to methane, temperature, moisture content, and methane concentration (Pedersen et al., 2010). During batch incubation, gas samples should be withdrawn at specific time intervals to read the methane concentration of the headspace using gas chromatograph. After evaluating the methane concentration of the headspace, the oxidation rate can be obtained using Equation 2-14 presented earlier (Scheutz et al., 2009).

Compared to the batch tests, column tests are more complex and time-consuming but provide more realistic real-world test conditions, including gas flow, soil thickness and compaction (Rachor et al., 2011). The weak point of column tests is the formation of exopolymeric substances (EPS), which can cause deterioration of the sample over long periods. Therefore, the

long-term performance of the field cannot be simulated in columns. In general, columns are operated with a gas flow in which methane concentration is 50 or 100% v/v and CH_4 ranges from 200 to 300 g CH_4 m^{-2} day^{-1}, which represents the middle to high range of landfill CH_4 fluxes. Steady-state methane oxidation rates of cover soils range from 100 to 150 g CH_4 m^{-2} day^{-1}, which represents 30 to 60% removal, and the maximum rates are 200 to 250 g CH_4 m^{-2} day^{-1}, representing 80 to 100% removal (Scheutz et al., 2009).

2.14 Goal and objectives of this research

As mentioned above, landfills are the third largest anthropogenic source of potent greenhouse gas, methane, worldwide. Landfill gas collection systems are only about 75% efficient; 25% of methane still escapes through landfill covers. The ability of typical landfill cover soils to oxidize this escaping methane ranges from 0%-100%, depending on the type of soil and conditions. Bio-covers have been investigated extensively to increase aerobic oxidation of methane compared to traditional soil landfill covers. However, despite their high removal efficiencies in lab studies, bio-covers have several disadvantages, including the potential for methane generation, limited oxygen availability, maintenance issues (including clogging), and reduced performance in cold weather.

In this study, we propose a new method for reducing methane emissions from landfills: anaerobic oxidation of methane in landfill covers. Anaerobic oxidation of methane facilitated by electron receptors such as sulfate, nitrate, and iron has been observed in natural systems (wetlands). In the lower parts of a landfill cover, there is almost no oxygen. Therefore, if microbes in the lower portions of the landfill cover could facilitate anaerobic oxidation of methane, this would increase the overall percent methane oxidized.

Several previous lab studies have evaluated the potential of anaerobic electron acceptors to promote anaerobic oxidation of methane in typical wetland soil, which is peat. *However, no previous study has evaluated the potential of electron acceptors to promote anaerobic oxidation of methane in typical landfill cover soil, which is clay*. The **overall goal** of our study is thus to increase methane oxidation through landfill covers, by evaluating the potential for anaerobic oxidation. **Specific objectives** of the study are:

1. To evaluate the ability of alternate electron acceptors (besides oxygen) to facilitate anaerobic oxidation of methane in clay soil, using batch tests. Different concentrations of the electron acceptors sulfate, nitrate, and iron will be evaluated under different conditions of moisture, nutrients and methane concentration.
2. Using the most promising electron acceptor concentrations determined from Objective 1, to measure rates of anaerobic oxidation of methane in clay landfill covers via column tests, which include realistic conditions of gas flow, cover thickness, and cover compaction.
3. To evaluate the potential for rates of anaerobic oxidation of methane in clay landfill covers, via column testing.

Our hypothesis is that appropriate electron acceptors will increase the anaerobic oxidation in the lower portions of the cover. Therefore, by utilizing both anaerobic and aerobic oxidation of methane, the overall oxidation of the cover will increase.

Our batch and column tests will use chemicals as electron acceptors as the first step of testing because they are homogeneous and provide easy control in terms of concentration. Assuming that the chemicals produce promising results, it will be recommended that future research test

waste materials containing the electron acceptors (such as iron-containing wastes like steel slag, iron and steel powder, mill scales, steel punching, metal shavings).

Anaerobic oxidation of methane has the potential to address several shortcomings associated with bio-covers, including:

- Reduced availability of oxygen: Oxygen availability is not a challenge here since the process of oxidation is anaerobic.
- Maintenance issues: The instability and degradation associated with compost should not be a problem concerning inorganic waste materials containing electron acceptors
- Cold weather vulnerability: Anaerobic oxidation occurs in the lower parts of the cover and would thus presumably be less impacted by low temperature.

Chapter 3

Methodology

3.1 Overview

In this chapter, first the details of sample collection will be explained, then the methodology of all the experiments including soil mechanical tests, Electron Spectroscopy for Chemical Analysis (ESCA) on some waste samples, batch tests, column experiments, and microbe test on some soil samples will be discussed in detail.

Soil tests were performed to determine soil mechanical properties such as liquid limit, plastic limit, plasticity index, size distribution, maximum soil dry density, and permeability. Using the obtained results, we were able to classify the soil.

Batch tests were conducted to evaluate anaerobic oxidation of methane using different electron acceptors. Moreover, the effect of some parameters such as adding inhibitor, moisture content, the initial concentration of methane, and nutrient content was evaluated through batch tests. Short-term and long-term gas concentrations in the headspace of the reactors were evaluated and finally, maximum oxidation rates for all batch reactors were obtained.

After finding the promising electron acceptors through the batch tests, column experiments were set up to evaluate the effect of electron acceptors on anaerobic oxidation of methane considering the effect of gas transfer, and oxidation rate for the anoxic zone of the column reactors was obtained.

To evaluate the microbial community of the soil samples, microbe tests were conducted on the soil samples taken from the bottom of column reactors.

3.2 Soil sample collection

To perform the experiments on the cover soil in this study, first, the samples were collected from Arlington landfill. The samples were collected from the final cover that has been exposed to methane for almost 30 years. Figure 3-1 to Figure 3-3 shows the procedure of cover soil sample collection at the landfill.

Figure 3-1 Arlington landfill cover soil where samples were collected

Figure 3-2 Sample collection at the landfill

Figure 3-3 All collected samples from the landfill

The vegetation was first removed from the surface of the cover. Then the cover was dug to collect soil. After sample collection, the removed soil was replaced with fresh clay soil in the landfill to prevent gas emissions from the places of the cover that were dug. After collection, the soil was sealed in buckets and incubated with methane and carbon dioxide gas to keep microorganisms active. The soil was spread on the lab floor for a couple of days to be air dried. The soil sample was then placed inside the Isotemp 500 series oven by Fisher at 100°F for 48 hours. This temperature was chosen to be high enough to dry the samples but not too high that it could kill microorganisms.

3.3 Soil mechanical tests

3.3.1 Compaction test

It is necessary to compact the soil for building the cover soil of the landfills. According to the EPA regulations (EPA, 1992), the permeability of the landfill cover soil should be equal or less than 10^{-5} cm sec^{-1}. Permeability higher than 10^{-5} cm sec^{-1} causes gas emissions from the surface of the landfill into the atmosphere. To reach permeability less than the recommended value, the soil needs to be compacted. Landfill cover soil is typically clay due to its low permeability, and to find the permeability of the cover soil, we need to conduct the compaction test followed by falling head permeability test, which will be discussed in this section.

The compaction test was used to find the maximum dry unit weight of the soil and based on the results of the test, a compaction curve was determined in which soil dry unit weight was related to the moisture content. Therefore, by having the curve, we could find how much soil moisture was needed to obtain specific soil dry density. To perform this test, the soil was air

dried; then the soil was crushed using the crusher and plastic hammer. Figure 3-4 shows the procedure of crushing the soil.

(a) (b)

Figure 3-4 (a) Crushing the soil before the compaction test, and (b) Breaking big particles using a hammer

Water was added to the soil to give almost 5% water content and the soil was well mixed with water to make a homogeneous mixture. The compaction mold and base were weighed, and the extension of the compaction mold was attached to the mold. Moist soil was poured into the compaction mold in three equal layers and each layer was uniformly compacted by 25 blows using a standard proctor hammer, as shown in Fig. 3-5. After compacting each layer, the surface of each compacted layer was scratched to make sure that the next layer would attach to the compacted layer and after pouring all three layers of moist soil into the mold and compacting the soil, the top of the compacted soil should stand slightly higher than the rim of the mold. Then the mold extension was removed, and the top of the compacted sample was trimmed carefully to make the top of the sample the same height as the top of the mold. The mold along with the

sample inside was weighed and the sample was extruded from the mold using a mechanical extruder. The sample was placed in the Fisher Isotemp 500 series oven at 100°C for 72 hours to be completely dry. Finally, the moisture content of the sample was calculated.

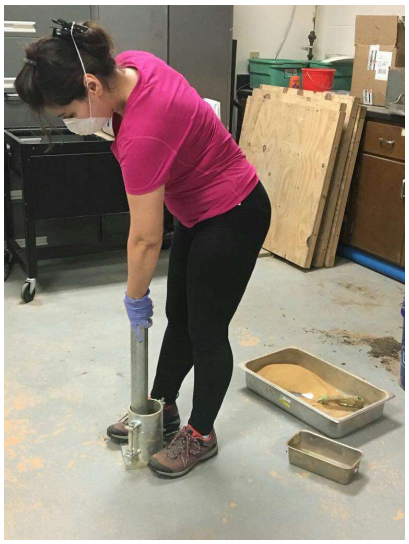


Figure 3-5 Conducting the compaction test

Six samples of the soil were compacted using the same proctor compaction method (ASTM D-698). The samples had different moisture contents and were weighed after compaction. After calculating the moisture content, moist soil unit weight, and dry unit weight of the soil for each sample, and the compaction curve was obtained based on the results.

The samples from the compaction test were oven dried for 24 hours and moisture content of the samples was calculated according to Equation 3-1, then moist and dry unit weight were calculated according to Equation 3-2 and Equation 3-3 (Das, 2013);

$$w(\%) = \frac{M_2 - M_3}{M_3 - M_1} \times 100 \qquad \text{Equation 3-1}$$

In which;

M_1 is the mass of moisture content can (g)

M_2 is the mass of moisture content can + moist soil (g)

M_3 is the mass of moisture content can + dry soil (g)

$$\gamma = \frac{Weight\ of\ compacted\ moist\ soil}{Volume\ of\ mold} \quad \text{Equation 3-2}$$

$$\gamma_d = \frac{\gamma}{1+(\frac{w(\%)}{100})} \quad \text{Equation 3-3}$$

Zero-air-void unit weight was also obtained by substituting γ_w and $1/G_s$ with γ and 1 in Equation 3-3.

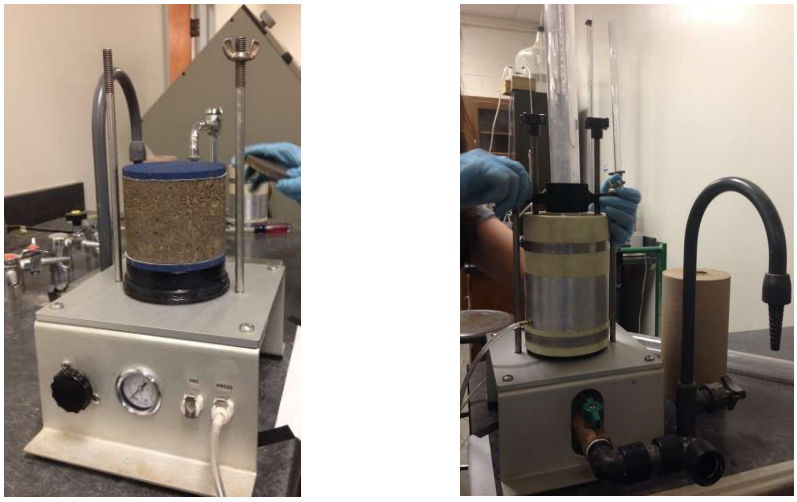
3.2.2. Permeability test

Soil consists of solid particles and the pores, which are filled with either water or air. Permeability of porous media of the soil is the most significant parameter that shows the potential of gas transfer in soil media. The permeability test was carried out according to standard method ASTM D5084 on the soil with a specific compaction ratio. Therefore, the permeability of the soil for gas transport was found for a given compaction ratio.

Based on EPA regulations, the permeability of landfill cover soil should be lower than 10^{-5} cm s^{-1} to prevent the emission of contaminants from the landfill into the atmosphere. Therefore, we conducted permeability tests for soil samples with different compaction ratios to evaluate the hydraulic conductivity of the samples. In this test, a sample of soil was first compacted with different ratios. Since the compaction ratio was less than 100%, static compaction was conducted. Figure 3-6 shows the compaction of the sample.

Figure 3-6 Compaction of the permeability test sample

After compaction, the sample was trimmed carefully, and the dimensions of the trimmed sample were measured. A membrane was attached to the permeability mold and a vacuum was applied to the permeability device. The filter papers and porous stones were placed at the top and the bottom of the sample and then the sample was placed inside the permeability mold. Since the diameter of the compaction mold was slightly smaller than the permeameter cylinder, we applied small pressure to make sure that the membrane was attached to the sample and water could not penetrate between the membrane and the sample. The permeability test had a deviation from standard methods. There is a pressure valve on the permeability device, and it is used to apply pressure before running the water through the sample. So, the pressure would not let the sample expand and it prevented the water from flowing between the sample and membrane. However, after applying the pressure, it was dropping over time to zero, so we connected the permeability device to a pressure valve and a pressure gauge was connected to the valve. In this way, the small pressure between the membrane and the sample was kept at a constant value during the test. The setup for the permeability test is shown in Figure 3-7.



(a) (b)

Figure 3-7 Permeability test setup

Water was filled in the burette and the sample was saturated for at least 10 days; then permeability was calculated according to the value of water level drop inside the burette. Since the permeability obtained was less than 10^{-5} cm/sec for our sample, we used the same compaction ratio for the column test setups. After the test, permeability was calculated according to the following equation:

$$k = 2.303 \frac{aL}{At} log \frac{h_1}{h_2} \qquad \text{Equation 3-4}$$

In which:

k: Permeability coefficient of the soil sample

a: Cross-sectional area of cylindrical pipe

L: Height of the sample

t: Time interval between initial and final water level

A: Cross-sectional area of sample

h_1: Initial water level in the burette

h_2: Final water level in the burette

3.3.2 Size distribution

3.3.2.1 Sieve analysis

The distribution of the grain sizes of a soil is determined to classify the soil. Sieve analysis is used to find the grain sizes of the soil. In this method sieves with different opening sizes were used. As the number of sieve increases, the size of the openings decreases. Sieve #200 with an opening size of 0.075 mm was the smallest size of sieve that was used in this method.

The soil that was used in this study had a high amount of clay content. Therefore, wet sieve analysis along with a hydrometer test was used to classify the soil. For sieve analysis, sieve number 4, 10, 20, 30, 40, 60, 140, and 200 were used according to standard method ASTM D-422.

Since the sample had aggregates of the smaller particles stuck together, the soil was soaked in water for more than 24 hours before the test, as it is presented in Figure 3-8. After washing the soil through sieve #200, the soil that passed through the sieve was placed in the oven for 24 hours, as well as the soil that was retained on the sieve. Figure 3-8 shows the process of washing the soil through sieve #200 until clear water is passing through the sieve.

Figure 3-8 Soil sample soaked in water for 24 hours

The soil that was retained on the sieve #200 was weighed and broken using a mortar and pestle. Since the clay part of the soil was removed, the soil particles segregated into individual particles easily. The sieves were then weighed and stacked in order to run the sieve analysis. The sieve #4 was placed at the top and the other sieves were placed in order of large opening size to the smallest, sieve #200, from top to bottom**.** Figure 3-10 shows the sieve test procedure.

(a) (b)

Figure 3-9 Washing and passing the soil through sieves for wet sieve analysis

Figure 3-10 Sieve shaker and sieve stack for the sieve analysis

The soil was poured into the stack of sieves from the top, and the sieve stack was placed inside the sieve shaker to shake for 10 minutes. Then the sieves that contained the soil samples were weighed to calculate the soil size distribution. The soil that was retained on sieve #200 was again washed; however, almost nothing passed through the sieve.

The percentage of soil that was retained on each sieve was calculated according to the following equation (Das, 2013);

$$\frac{Mass\ retained\ M_n}{Total\ mass\ M} \times 100 = R_n \qquad \text{Equation 3-5}$$

And cumulative percent retained on each sieve was;

$$\sum_{i=1}^{i=n} R_n \qquad \text{Equation 3-6}$$

Therefore, cumulative percent passing through each sieve was;

$$Percent\ finer = 100 - \sum_{i=1}^{i=n} R_n \qquad \text{Equation 3-7}$$

3.3.2.2 Hydrometer analysis

The soil that passed through sieve #200 was used for the hydrometer test according to standard method ASTM D7928-17. This test was used for the part of the soil that has particle smaller size than 0.075 mm. The test can represent the size distribution of soil particles with the size larger than 0.001 mm and smaller than 0.0745 mm. In the hydrometer test, soil particles that are soaked in the water, settle individually with different velocities, and the sizes of particles are determined based on the settlement velocity, according to Stokes' law (Das, 2013):

$$v = \left(\frac{2}{9}\right)\frac{\rho_p - \rho_f}{\mu} g R^2 \qquad \text{Equation 3-8}$$

In which:

g : Gravitational acceleration (m s^{-2})

R : Radius of the spherical particle (m^2)

ρ_p : Mass density of the particles (kg m^{-3})

ρ_f : Mass density of the fluid (kg m^{-3})

μ : Dynamic viscosity (kg m^{-1} s^{-1}).

For this test, ASTM 152-H type hydrometer was used. Before the experiment, distilled water was mixed with sodium hexametaphosphate to obtain zero correction and meniscus correction, as shown in Figure 3-11.

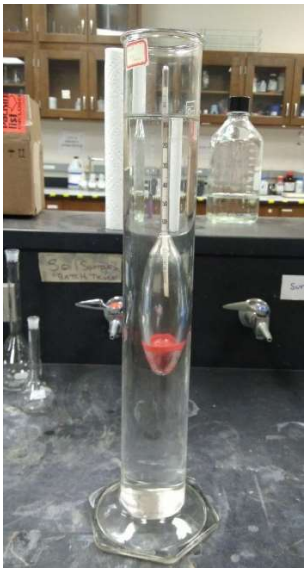


Figure 3-11 Obtaining zero correction and meniscus correction

The values of zero correction and meniscus correction were obtained as 5.5 and 1, respectively. To run the test, 50 g of oven-dried and well-pulverized soil that had passed through sieve #200 was soaked in 4% sodium hexametaphosphate solution for 24 hours.

10 grams of sodium hexametaphosphate was added to 125 ml of water and was mixed well before adding to 50 g of the soil sample. After 24 hours, the sample was transferred to the mixer

bowl from the container. Distilled water was used to wash all the remaining soil particles from the container. Then more distilled water was added to the sample to fill two-thirds of the mixing bowl and the sample was mixed for 2 minutes using the mixer. Figure 3-12 shows the mixing of the sample before the hydrometer test.

Figure 3-12 Process of mixing the sample before the hydrometer test

After mixing the sample, it was poured into a 1000 ml cylinder and distilled water was added to the sample to fill the cylinder to 1000 ml. A rubber stopper was put on the top of the cylinder and the cylinder was turned upside down for 30 times in a minute to mix the solution. The hydrometer was placed inside the sample slowly to take measurements in time intervals of 0.25, 0.5, 1, 2, 4, 8, 15, 30, 60, 120, 240, 480, 1440, and 2880 minutes.

Another cylinder with the same size was also filled with distilled water and the hydrometer was placed inside it after each measurement, so clay particles were detached from the hydrometer. The temperature of the solution was also taken several times using a thermometer. Figure 3-13 shows the hydrometer test setup.

Figure 3-13 Hydrometer test setup

After obtaining hydrometer readings *(R)*, the values of R_{cp} were calculated according to the following equation (Das 2013);

$$R_{cp} = R + F_T - F_Z \qquad \text{Equation 3-9}$$

In which,

F_T is the temperature correction and is equal to +0.18 for the temperature of 21°C.

F_Z is zero correction.

Percent finer is calculated according to the following equation;

$$Percent\ finer = \frac{\alpha R_{cp}}{M_s} \qquad \text{Equation 3-10}$$

In which;

Ms is the mass of dry soil sample that is used for the test (50 g).

α is the correction for specific gravity and was considered equal to 1.

R_{CL} value is calculated according to;

$$R_{CL} = R + F_m \qquad \text{Equation 3-11}$$

In which;

F_m is meniscus correction and was observed as 1.0 in this test.

The size of the particles is calculated according to the following equation;

$$D(mm) = A\sqrt{\frac{L(cm)}{t(min)}} \qquad \text{Equation 3-12}$$

In which;

A is average specific gravity shift and is 0.0135 for the specific gravity of 2.65 and temperature of 21°C.

L is effective depth and was obtained from ASTM 422-63 for each hydrometer reading (R).

t is the time of the reading.

3.3.3 Liquid limit test

When a cohesive soil is mixed with water, it will almost have a liquid state, and by losing moisture content, it will change from liquid state to plastic state, and finally to a solid state. The water content of the soil when it transfers from a liquid to plastic state is the liquid limit, and the water content in which soil changes from a plastic state to semisolid state is the plastic limit (Das, 2013).

To run the liquid limit test, first, the weight of 4 empty cans that were used to place moist soil inside the oven was measured. Oven-dried soil that was passed through sieve #40 was placed in a container and water was added to it. Soil and water were mixed to make a homogenous mixture. After calibration of the liquid limit device, some of the prepared sample was placed inside the brass cup of the liquid limit device. The surface of the soil was smoothed using a spatula and

maximum depth of the soil was 10 mm (ASTM D4318-17). Then, using a grooving tool, a groove was formed at the surface of the soil while holding the grooving device perpendicular to the soil surface. Then the device was turned on and the cup was lifted and dropped at a rate of 2 drops per second until the soil on two sides of the groove came into contact for the length of 13 mm. The number of blows that was applied to close 13 mm of the groove was recorded. Some of the soil sample was placed inside the moisture can to put inside the oven for 24 hours and obtain an exact moisture content. The remaining sample was completely removed from the liquid limit device. Figure 3-14 shows the liquid limit test procedure.

Figure 3-14 Liquid limit test

Water was added to the dry soil sample in different amounts to give different levels of moisture content, and the test process was repeated to obtain the number of blows for samples with different moisture contents. The number of blows in the liquid limit device should fall between 16 and 20, between 21 and 25, between 26 and 30, and between 31 and 35. So moisture

content of the soil should be set in an appropriate range to obtain the desired number of blows. We conducted enough numbers of liquid limit tests to obtain the number of blows in the desired ranges. After running the test, the moisture content of the samples was obtained according to Eq. 3-1.

Finally, the number of blows versus moisture content of the soil was drawn and the liquid limit was obtained. The moisture content of the soil for which the number of blows was equal to 25 according to the graph, was considered as the liquid limit of the soil.

3.3.4 Plastic limit test

As mentioned before, the plastic limit is the water content of the cohesive soil when it changes from the plastic state to the semisolid state. The soil sample was passed through the sieve # 40 and was dried in the oven for 24 hours before the test. The weight of two moisture cans was obtained and recorded. The soil was mixed well with water to form a homogeneous mixture and was rolled by hand on a glass plate to make a thread. Rolling the sample continued untill the thread broke to smaller threads. The test was repeated until the thread with the diameter of 3 mm broke to smaller threads.

If the broken thread had a diameter larger than 3 mm, more water was added to the soil and the experiment was redone. On the other hand, if the broken thread diameter was smaller than 3 mm, making of the soil thread was repeated until the soil lost enough moisture content to be broken at the diameter of 3 mm. After the tests, the samples were placed inside the moisture cans and placed in the oven for 24 hours. By measuring the weight of samples, the plastic limit was obtained. Figure 3-15 shows the samples of the plastic limit test.

Figure 3-15 Plastic limit test samples

Plasticity index was calculated using the following equation:

$$PI = LL - PL \qquad \text{Equation 3-13}$$

In which:

PL is the plastic limit

LL is the liquid limit

Figure 3-16 Unified Soil Classification System (USCS) shows the classification of the soils based on the plastic index and liquid limit values. According to the results, the soil used for this study is classified as clay with low plasticity (CL).

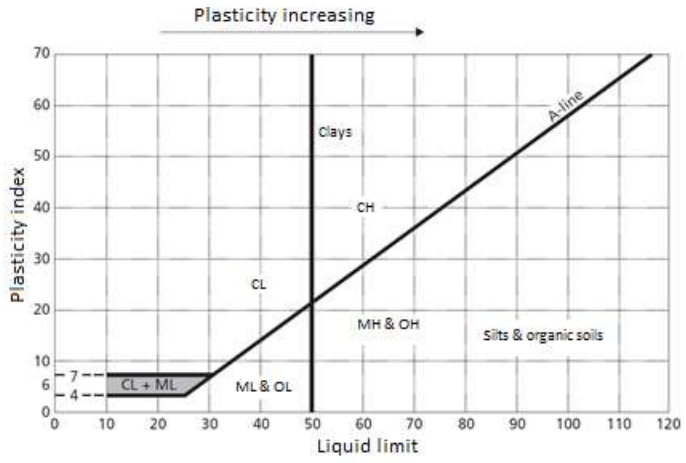


Figure 3-16 Plasticity chart: British system (USCS)

3.4 Batch reactor tests

3.4.1 Selection of electron acceptors

Anaerobic oxidation of methane (AOM) occurs in the absence of oxygen and the presence of another electron acceptor as an alternative for oxygen. Based on previous studies, different electron acceptors such as sulfate, nitrate, and iron III can promote AOM in saturated peat soil.

In this study, we first tried to find wastes with high iron content. The Electron Spectroscopy for Chemical Analysis (ESCA) test was conducted on the selected wastes to measure the iron and other elements in these wastes.

The ESCA analysis was conducted by Innovatech Labs, and the analysis equipment is shown in Figure 3-17. The test was started by sprinkling the powders onto double-sticky tape. Then the excess amount of samples were removed and the ESCA data were obtained from an analyzed area having a diameter of 1 mm using a monochromatic Al Kα x-ray source.

Low energy resolution survey scans were acquired from each sample to evaluate which elements were present in the samples. The atomic concentrations of these elements and their local chemistries were specified using higher energy resolution multiplex scans. This test selected a spot on the surface of the material, so the results may or may not be necessarily a very good representative of the material composition.

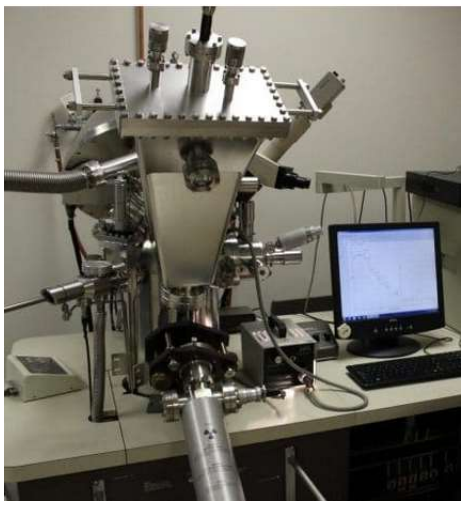


Figure 3-17 ESCA analysis equipment (Innovatechlabs.com)

According to the results of the ESCA test, among all the tested wastes, red mud had the highest iron content. So, it was selected for batch tests. Since it was time-consuming and expensive to select other wastes and run the ESCA test to find the electron acceptor content, we directly added chemicals as the source of electron acceptor to the soil for the other batch tests.

Based on previous studies of AOM in natural wetlands and peat soil, we tested six electron acceptors in the form of chemicals, as shown in Table 3-1 (Steven et al., 2012; Pozdnyakov et al., 2011; Beal, et al., 2009; Timmers et al., 2016; Orit et al., 2014).

When $FeCl_3$ was used, high amounts of CO_2 were measured inside the reactors. Even if an electron acceptor can remove CH_4, it will not be helpful if it produces a high amount of carbon dioxide and moreover, accumulation of high pressure inside a glass container which is not designed for high pressure was not safe. Moreover, sample amended with $FeCl_3$ had a very low pH which was toxic for methanogens. Hence, in subsequent experiments, iron in the form of Fe_2O_3 and $Fe(OH)_3$ was used.

Table 3-1 Added chemicals and the electron acceptors in this study

Added chemical	**Electron acceptor**
Red mud, $FeCl_3$, Fe_2O_3, $Fe(OH)_3$	Iron(III)
KNO_2	Nitrite
$NaNO_3$	Nitrate
Na_2SO_4	Sulfate
MnO_2	Manganese
NH_4Cl	Ammonium

Concentrations tested were the same as in previous studies, and in initial tests higher. We initially tested higher concentrations because of the potential low accessibility of chemicals for microorganisms in the soil samples in this study (previous researches had used water or saturated soil). However, Sets 1 and 2 with high chemical concentrations did not exhibit substantial oxidation. Since the high concentrations may have been toxic to microorganisms, in later sets we tested lower concentrations.

3.4.2 Organization of batch reactor experiments

Batch experiments were conducted to evaluate the anaerobic oxidation of methane in soil samples that were amended with different electron acceptors. Other parameters such as moisture content of the soil samples, the initial concentration of methane in the headspace of the reactors, and the effect of inhibitor were also evaluated through the batch experiments. As mentioned before, batch reactors have a straightforward procedure without the complexity of column tests. So, oxidation rates were initially studied through batch tests and the most promising electron acceptor was selected for column tests studies. Table 3-2 shows the organization of batch reactor experiments.

Table 3-2 Sets of batch reactor tests to evaluate the effect of initial methane concentration, added nutrient, added inhibitor, and soil moisture content

Set No.	Reactor No.	Added chemical	Concentration of Chemical (mg/g sample)	Solution added to sample	Moisture Content (%)	Inhibitor	Volume of injected gas (mL)
1	1	-	-	Water	20%	-	30
	2	Red mud	60.00	Water	20%	-	30
	3	Na_2SO_4	27.20	Water	20%	-	30
	4	$NaNO_3$	16.40	Water	20%	-	30
	5	$FeCl_3$	31.20	Water	20%	-	30
2	6	-	-	Water	20%	Yes	30
	7	Red mud	60.00	Water	20%	Yes	30
	8	Na_2SO_4	27.20	Water	20%	Yes	30
	9	$NaNO_3$	16.40	Water	20%	Yes	30
	10	$FeCl_3$	31.20	Water	20%	Yes	30
3	11	-	-	RAMM	47%	-	90
	12	$NaNO_3$	0.84	RAMM	47%	-	90
	13	$NaNO_3$	1.64	RAMM	47%	-	90
	14	Na_2SO_4	3.28	RAMM	47%	-	90
		Fe_2O_3	1.84				
	15	Na_2SO_4	2.72	RAMM	47%	-	90
	16	Na_2SO_4	1.36	RAMM	47%	-	90
	17	Fe_2O_3	3.08	RAMM	47%	-	90
	18	KnO_2	1.64	RAMM	47%	-	90
	19	$Fe(OH)_3$	1.72	RAMM	47%	-	90
	20	MnO_2	1.68	RAMM	47%	-	90
	21	NH_4Cl	0.40	RAMM	47%	-	90
	22	$FeCl_3$	3.12	RAMM	47%	-	90
4	23	-	-	RAMM	47%	Yes	90
	24	Na_2SO_4	2.72	RAMM	47%	Yes	90
	25	KNO_2	1.64	RAMM	47%	Yes	90
	26	$Fe(OH)_3$	1.72	RAMM	47%	Yes	90
5	27	-	-	Water	47%	-	90
	28	Na_2SO_4	2.72	Water	47%	-	90
	29	KNO_2	1.64	Water	47%	-	90
	30	$Fe(OH)_3$	1.72	Water	47%	-	90
	31	MNO_2	1.68	Water	47%	-	90
	32	$NaNO_3$	1.64	Water	47%	-	90
	33	$FeCl_3$	3.12	Water	47%	-	90
6	34	-	-	RAMM	47%	-	30
	35	$NaNO_3$	1.64	RAMM	47%	-	30
	36	NH_4Cl	0.40	RAMM	47%	-	30

Each set is described in more detail below. A duplicate was run of each reactor to verify the measurements. As shown in the table, some electron acceptors such as nitrate, sulfate, and iron were tested in more reactors compared to the other electron acceptors. Preferential testing with nitrate, sulfate, and iron over other electron acceptors was based on the success that previous researchers had achieved in AOM using theses electron acceptors. Therefore, though theoretically, all used electron acceptors could promote AOM, we preferred to use the ones that were promising according to the literature review in the design of more reactors.

Set 1: In Set 1, plain soil was tested as a control. Soil with red mud was also tested, along with chemical sulfate, nitrate, and iron. Based on previous studies (Dasselaar et al. 1998), optimum moisture content for methane oxidation is in the range of 20-50%; therefore, 20% moisture content was selected for Sets 1 and 2.

In Sets 1 and 2, 30 mL of CH_4/CO_2 (representing landfill gas) was injected into the headspace, equivalent to a 12% concentration. According to the previous studies, AOM can occur with initial methane concentrations as low as 6% (Steven et al., 2012). This concentration was doubled to 12% so that anaerobic oxidation of methane would not be methane limited.

Set 2: In Set 2, the same electron acceptor concentrations, moisture content, and methane concentration were evaluated as in Set 1, but sodium 2-bromoethanesulfunate (BES) was also added to the samples to inhibit methane generation. Since there was an anaerobic environment inside the batch reactors, methanogenesis could generate methane and as a result, methane generation and oxidation happen simultaneously. Therefore, if the addition of inhibitor prevented methane generation, we would get only oxidation in the batch experiments.

Set 3: In Set 3, nutrients and additional moisture were added, the concentration of methane in the headspace was increased. Nitrite, manganese, and ammonium were tested as electron

acceptors, along with iron, sulfate, and nitrate in different concentrations from Sets 1 and 2. In one of the reactors, a combination of sulfate and iron oxide (hematite) was tested (Orit et al., 2014).

Nutrient addition. Nutrients were added to ensure that AOM was not limited by nutrient availability. As mentioned before, reverse methanogenesis is the most studied mechanism of AOM. Based on previous studies, the nutrients that methanogens need to generate methane are the same required nutrients for microbes to consume methane anaerobically.

It was hypothesized that if enough nutrients such as Fe, Ni, Co, and Zn were provided for anaerobic methanotrophic archaea (ANME), the oxidation rate could increase (Glass and Orphan, 2012). Hence, the Revised Anaerobic Mineral Medium (RAMM) developed by Shelton and Tiedje (1984) was added to the samples in Sets 3, 4, and 6 to increase the availability of nutrients for anaerobic microbes.

Table 3-3 shows the amounts of minerals and metals used to make 1 liter of RAMM solution.

Table 3-3 RAMM solution components (Shelton & Tiedje, 1984)

Material	Mass (mg)
Phosphate buffer (adjusted to 7.0 pH)	
KH_2PO_4	270
K_2HPO_4	350
Mineral Salts	
Ammonium Chloride (NH_4Cl)	530
Calcium Chloride ($CaCl_2.2H_2O$)	75
Magnesium Chloride ($MgCl.6H_2O$)	100
Ferrous Chloride ($FeCl_2.4\ H_2O$)	20
Trace Metals	
Manganous Chloride ($MnCl_2.4H_2O$)	0.5
Boric Acid (H_3BO_3)	0.05
Zinc Chloride ($ZnCl_2$)	0.05
$CuCl_2$	0.03
$NaMO_4.2H_2O$	0.01
Cobalt Chloride ($CoCl_2.6\ H_2O$)	0.5
Nickel Chloride ($NiCl_2.6\ H_2O$)	0.05
Sodium Selenide Anydrous (Na_2SeO_3)	0.05

Moisture addition. As mentioned previously, Dasselaar et al. (1998) found optimum moisture content for methane oxidation to be 20-35%, so 20% was used for Sets 1 and 2. However, in another study by Einola et al. (2007), in the temperature range of 12-19°C, maximum methane oxidation was obtained at 50% moisture content. So, in Sets 3, 4, 5, and 6, moisture content was increased to 47%. The reason for considering 47% moisture content rather than 50% was that the syringes that were used for injecting the solution could only show the 8 mL accurately (47% moisture content), not 8.5 mL (50%).

One of the reasons that high moisture content may inhibit methane oxidation is the fact that water blocks pathways available for oxygen transfer. However, in our study, since we are focusing on anaerobic oxidation of methane, oxygen limit is not a problem. Increased moisture content can provide more accessibility of nutrients for microbes.

Increased methane concentration. In Sets 1, 2, and 6, 12% methane concentration in the headspace was used. However, since the solubility of methane in water is very low, increasing the methane pressure in the headspace would increase dissolution into the liquid phase and thus increase the accessibility of methane for microorganisms. Therefore, 90 mL of CH_4/CO_2 was injected into the reactors in Sets 3-5 to increase the concentration of CH_4/CO_2 from 12% to 36%.

Set 4. Three of the electron acceptor concentrations tested in Set 3 were re-tested in Set 4 with methane generation inhibitor, to evaluate the effect of methane generation inhibitor.

Set 5. Set 5 was designed to evaluate the effect of adding nutrients. The reactors tested in Set 5 were duplicates of 6 of the reactors tested in Set 3, except that no RAMM solution was added in Set 5.

Set 6. Set 6 was designed to evaluate the effect of the lower initial concentration of methane in the headspace. Corresponding reactors in set 3 and 6 had the same moisture content and nutrient content (RAMM solution). So, by comparing the results of these two sets, we can evaluate the effect of methane concentration.

3.4.3 Setting up batch reactor tests

125 ml Wheaton glass bottles were used for batch tests and 17 g of dry soil was placed inside each batch reactor. Then chemicals were also weighed and added to the soil. Lids with rubber septa were used to seal the bottles using a crimper. The reactors were shaken to mix the soil and chemicals. Sealed bottles were washed with nitrogen gas to remove oxygen and create anaerobic condition inside the reactors.

As shown in Figure 3-18, two 22-gauge and 2-inch needles were used to flush the bottles. One needle was inserted all the way into the bottle and into the soil to flush the soil particles; this needle was connected to the nitrogen gas cylinder (99.9% purity) through a hose with valve and a pressure gauge. The other needle was connected to the lab vacuum valve in the lab through a hose with valve and vacuum gauge. This needle was inserted slightly through the septa and was held at the top of the bottle to prevent the clogging of the needle due to the suction of the particles.

Figure 3-18 Pressure and vacuum gauge to control the flushing process

First, the bottles were evacuated to the vacuum pressure of -67 kPa for 30 seconds; then the vacuum valve was closed, and the nitrogen valve was opened to apply nitrogen into the bottle at a pressure of 3-5 psi for 30 seconds. This process was repeated 10 times. Then both vacuum and pressure valves were open for 5 minutes to pressurize and vacuum the bottles at the same time. Since vacuuming and flushing the nitrogen gas were occurring at the same time, the pressure was lower than 5 psi during the last 5 minutes.

Flushing the bottles for 15 minutes ensured us that oxygen was completely removed from the bottles even if some short-circuiting occurred during the process. The pressure and vacuum

gauge were constantly monitored during the flushing process to keep the process safe and accurate. 10 µl sample of headspace was taken after flushing the bottles, to measure the oxygen content of the headspace. The samples were injected into the gas chromatograph (GC, SRI 8610) and based on the results, there was no oxygen left in the bottles.

The distilled water was then flushed with nitrogen gas to remove the dissolved oxygen and was added to the samples give the samples 20% or 47% moisture content, depending on the set of experiments. After flushing the water, it was added to the reactors to give samples 20% or 47% moisture content.

According to previous research (Steven et al., 2012), the bottles were left in the lab for 48 hours. This time allowed the microorganisms to consume any remaining oxygen in the bottles. 30 ml or 90 ml of headspace gas, depending on the experiment, was then removed using a 26-gauge needle and 30 ml or 90 ml of landfill gas (methane- carbon dioxide, 50%-50%, Matheson) was injected into the bottles. To do so, a Tedlar bag was filled with 50% CO_2/50% CH_4 (representing landfill gas), then the syringe was purged using 50% CO_2/50% CH_4 from the Tedlar bag before each injection. The reactors were covered with aluminum foil to prevent oxygen generation by microorganisms (photosynthesis due to light exposure). Duplicates of all reactors were also made to compare the results. Figure 3-19 shows all the batch reactors used in this study.

Figure 3-19 Six sets of batch reactors

3.4.4 Methane concentration measurement

Gas chromatograph (GC, SRI 8610) was used to analyze gas samples in this study. Flame Ionization Detector (FID) was the detector that measured the concentration of methane and carbon dioxide in the samples. Before each day's reading, the GC was calibrated to check the accuracy of the machine. Figure 3-20 shows the gas chromatograph.

Figure 3-20 Gas chromatograph (GC, SRI 8610)

At time t = 0 (right after the 30 or 90 mL of CO_2/methane gas was added), 10 µl of gas was withdrawn from headspace using a 26-gauge needle and was injected into the GC to measure the methane and carbon dioxide content of the reactor headspace. Samples were withdrawn at subsequent time intervals and were injected into the GC to read the amount of methane and carbon dioxide in the sample over time for at least 40 days. First, the gas samples were withdrawn every day to measure the even small changes of concentration of the gases. However, since the change in gas concentration did not occur very quickly, the measurements were done every 4 or 5 days.

The needle was purged using a Tedlar bag containing nitrogen gas before taking samples to prevent cross-contamination and oxygen from entering the reactors. The gas samples were taken for 40 days and the concentration of methane and carbon dioxide was evaluated over time.

3.4.5 Adsorption tests

Methane and carbon dioxide adsorption onto the soil (rather than biological oxidation) can potentially affect the amount of headspace gas of the reactors. Adsorption tests were also set up to study the amount of methane gas that the samples can adsorb. To make sure that the only mechanism that decreases the amount of initial methane is just the adsorption and anaerobic oxidation does not occur in these bottles, the soil was oven dried for 24 hours to remove moisture content of the soil. Therefore, because of the 0% moisture content of the soil, microorganisms could not oxidize methane. After removing the sample from the oven, it was immediately weighed and placed inside a batch reactor. The reactor was sealed, and 30 ml of headspace gas was removed from the headspace. 30 ml of 50% CO_2/50% CH_4 was then injected into the reactor, and variation in methane and carbon dioxide concentrations was monitored over time. Since the

amount of chemicals that was added to the samples was very small compared to the amount of soil, the adsorption test samples contained just the soil. By measuring the methane removal in the adsorption bottle, we could find the amount of adsorbed methane in the reactors. Then by subtracting methane removal in the adsorption bottle from methane removal in the other reactors, we could find the amount of methane oxidized in the reactors.

3.5 Column experiments

Lab column tests were conducted on soil samples with electron acceptors that showed promising results during the batch tests, as well as pure soil as a control. Sulfate, nitrate, and sulfate + hematite were tested in concentrations of 2.72 mg sulfate/g soil, 1.64 mg nitrate/g soil, and 3.28 mg sulfate/ g soil +1.84 mg hematite/g soil, respectively. The moisture content of 20% was used because 47% would decrease the permeability of the soil (according to the compaction curve) and as a result, the soil would be clogged, causing the inhibition of gas transfer inside the column. RAMM solution was used to give the soil moisture content, since according to the results of the batch tests, adding nutrient increased the anaerobic oxidation of methane. The inhibitor was not added to the soil since it decreased anaerobic oxidation of methane when AOM was linked to methanogenesis. Moreover, adding inhibitor would be costly in a real landfill and can cause leakage of heavy metals to the leachate.

3.5.1 Installation of columns

Column tests are an experimental setup used to evaluate methane oxidation in landfill cover soil. The landfill cover is simulated inside the column, and the LFG, that is generated inside the landfill due to the biodegradation of solid waste, transfers through the cover soil and column.

Column tests are more complex than batch tests; however, since they simulate gas transfer, the results of column tests are more accurate and the oxidation rate which is calculated based on the results on the column tests is closer to the real oxidation rate in the landfill than the oxidation rate obtained from batch tests.

The schematic setup of column tests in this study is shown in Figure 3-21.

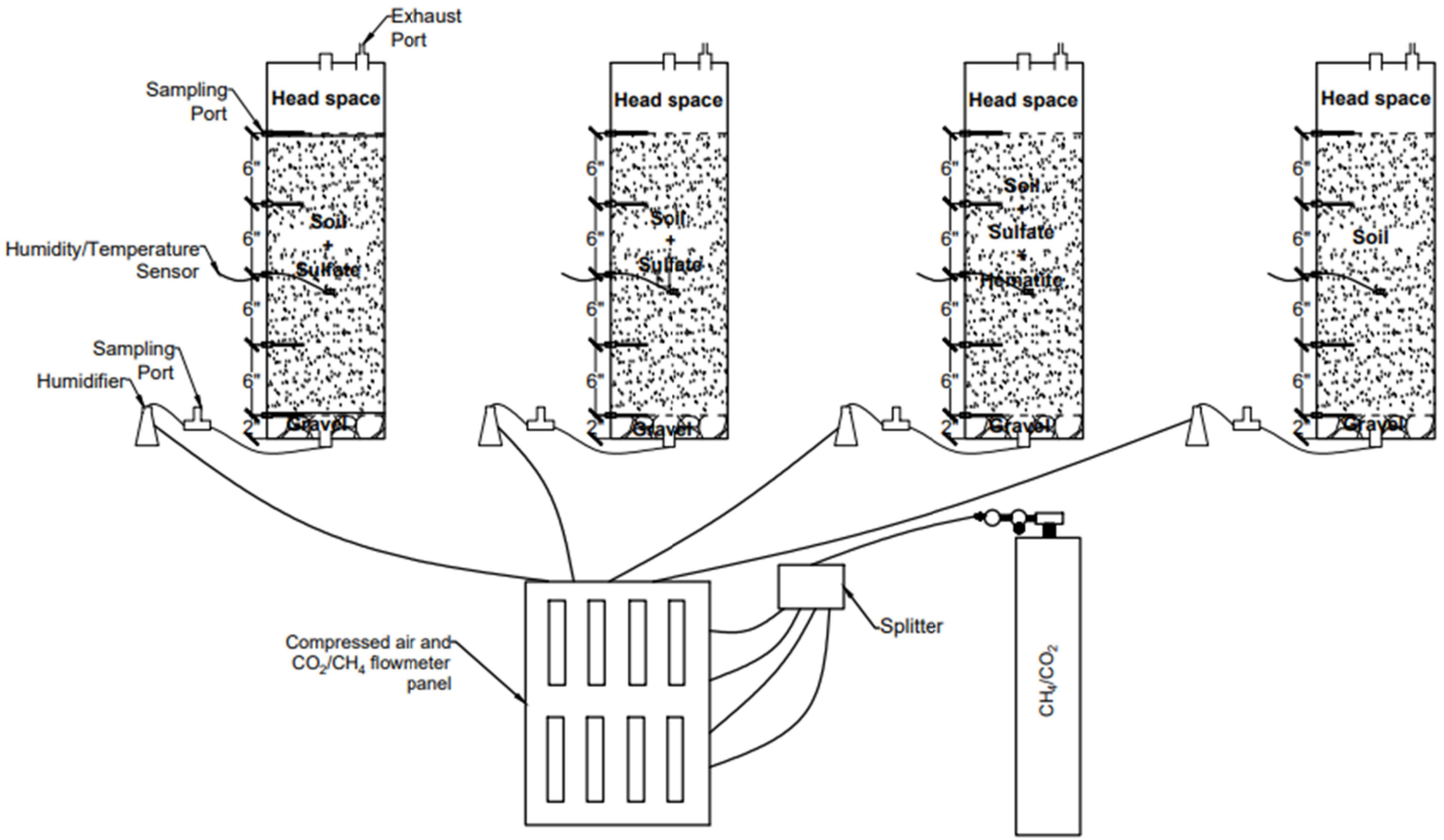


Figure 3-21
The design of column tests

As shown in the schematic figure, 4 polycarbonate columns were used for columns tests. The gravel at the bottom of the column was used to distribute gas evenly before entering the soil. Cover soil with a height of 2 feet was compacted in 4 layers (6 inches in each layer). Therefore, the design satisfies the minimum required 18″ thickness for landfill cover. Gas (50% methane and 50% CO_2) passed through the soil from the bottom to top through gravel, soil cover and topsoil, respectively. The top of the columns was closed all the time (during the time of running test and taking measurements), and the exhaust port was open to let exhaust gas leave out.

The soil had 85% compaction ratio. First, the mass of soil needed to give 85% compaction for each layer was calculated according to the compaction curve obtained previously. Next, the big particles and vegetation were removed, and the soil was crushed and air dried. Electron acceptors were also weighed and added to the soil. Then RAMM solution was added to achieve 20% moisture content (w/w). The soil, electron acceptor and RAMM solution were well mixed to make a homogeneous mixture. Finally, the prepared sample was compacted in each layer to obtain 85% compaction. Figure 3-22 shows the process of mixing and compacting soil inside the columns.

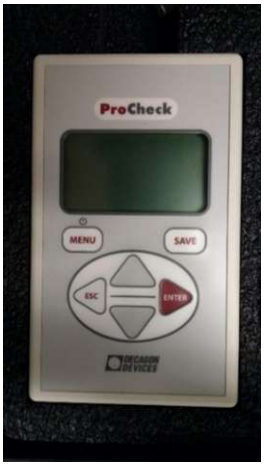
Figure 3-22 The process of mixing and compacting soil inside the column

After filling each column, top and bottom plates were placed and after passing the steel rebars, the plates were screwed, and columns were placed upright next to the flow meters and cylinders. Figure 3-23 shows the process of setting up the first column reactor.

Figure 3-23 The process of setting up the first column reactor

As shown in the column design, there were five ports on the sides of the column. Ports were sealed to prevent gas leakage. One of these ports in the middle was used to measure moisture and temperature using Decagon sensors (5TE Moisture, Temp & EC), which was placed during the compaction of the soil. The sensors were connected to ProCheck, which is a handheld readout device to use with all environmental monitoring sensors by Decagon. Figure 3-24 shows the ports and instruments used to measure the moisture and temperature during the tests.

x



(a) (b) (c)

Figure 3-24 (a) Column port to measure the moisture and temperature of soil during the test, b) Moisture/humidity sensor, and (c) ProCheck

Four remaining ports were used to measure the concentration of methane and carbon dioxide at different depths of the soil during the experiment, as shown in Figure 3-25. To measure the concentration of the methane accurately, we inserted a 2.0″ needle into a plastic cover and connected that to the port and sealed the port. Then a thread was created in the glass cover of the port to let the gas pass into the port and reach the needle. This design was fragile, and the compacted soil could damage and break the needle and the glass cover. Therefore, we inserted rebars into the soil, which had the same diameter as the plastic cover, to create a hole in the

location of the port. Then, the rebars were pulled out and the ports were inserted without being damaged or clogged. The gas samples were taken through these ports during the test.

(a)

(b)

Figure 3-25 (a) The design of the port to measure the concentration of methane in the column, and (b) Installation of ports

In a real landfill, the waste has moisture content, so landfill gas includes some water vapor. Moreover, the air on the surface of the landfill contains moisture as well. So, it is necessary to include moisture in the design of gas transfer in the column test setup. As shown in Figures 3-21 and Figure 3-26, a humidifier was connected to the tubes through which gas passed from the cylinder to the column. The humidifier prevented the moisture loss of the soil in such a way that while gas was transferred through the tube, it picked up some moisture and took it to the soil. Therefore, the soil could hold enough moisture for microbial activity during the experiments, without gas transport drying it. A humidifier was connected to each column at the bottom to provide moisture for CH_4/CO_2 gas before entering the soil.

Figure 3-26 Humidifier connected to the gas cylinder and column

The flow rates of landfill gas (50% CH_4/50% CO_2) at the bottom of the columns were set based on the calibration and pressure correction factors by Matheson Trigas. Flow meters were adjusted to provide 12 mL min^{-1} landfill gas. This was equal to a gas flux of 0.55 m^3 m^{-2} day^{-1} and a methane flux of 179.4 g m^{-2} day^{-1}, which is within medium to high range of methane flux measured from landfills in previous studies. Although compressed air was initially set to flow at the top of the columns to simulate atmospheric air, it was turned off to ensure that the environment at the bottom of the columns was anaerobic. To make sure that there was no air at the bottom of the columns, samples were taken from the last two ports and injected to GC to measure oxygen content. Figure 3-27 shows all column reactors that were used in this study.

Figure 3-27 Four column reactors used in this study

Before the beginning of the column tests, we conducted a trial column (Figure 3-28) to check the column test equipment and detect any possible design flaw before the main tests. During the trial test, no problem was detected regarding the design of the columns; however, the soil was compacted to its 95% maximum density, which prevented substantial gas transfer inside the column. Therefore, in the main column tests, we tried to avoid problems such as high moisture content and high compaction ratio that could cause blocking of gas transfer pathways.

Figure 3-28 The trial column test

3.5.2 Running column reactors

After reaching equilibrium (equilibrium was reached when columns started to give constant readings), daily measurements were taken to analyze the vertical distribution of gas components (CH_4 and CO_2) for 10 days, and the average of readings was calculated to draw the graphs. During the tests, moisture content and temperature of the samples were also taken through the middle port of the columns.

According to the results of column tests, it was hypothesized that sulfate-reducing bacteria might be responsible for anaerobic oxidation of methane in column # 1 and 3, as discussed in Ch. 4, the presence of H_2S at the surface of the columns was evaluated. After running column tests, the gas accumulated in the headspace of the columns was analyzed using Landtec hydrogen sulfide analyzer. Figure 3-29 shows the process of collecting gas using Tedlar bags on top of the columns to collect gas samples and Figure 3-30 shows the measurement of gases using a Landtec GEM5000 that could measure the concentration of methane, carbon dioxide, oxygen, hydrogen sulfide, and carbon monoxide.

Figure 3-29 Collecting gas samples from the surface of the soil for gas analysis

Figure 3-30 Tedlar bag connected to Landtec hydrogen sulfide analyzer

After running the column tests, some accumulated water was observed at the bottom of the column with sulfate and the column with sulfate + hematite. Moreover, as mentioned before after almost a couple months, the samples inside these columns were clogged and no methane or carbon dioxide was transferring through these columns. After evaluating different parameters, it was hypothesized that microbes produced water in these samples and because of high water content, the soil was clogged, and some water was accumulated inside the tube connected to the bottom of these columns. Therefore, humidifiers were removed from the columns. However,

since the samples already had enough water content, removing the humidifiers did not have a negative effect on the reactors' performance.

3.6 Methane oxidation rate

By measuring methane concentrations at different depths of the soil, the amount of oxidation was obtained. Knowing methane flux to the soil sample and the flux at the top of the soil, we can calculate methane oxidation as follows:

$$Eff_{ox} = \frac{(Flux_{in} - Flux_{out})}{Flux_{in}} \times 100\% \qquad \text{Equation 3-14}$$

In which $Flux_{in}$ is the methane flux introduced to the column and $Flux_{out}$ is the methane flux from the topsoil.

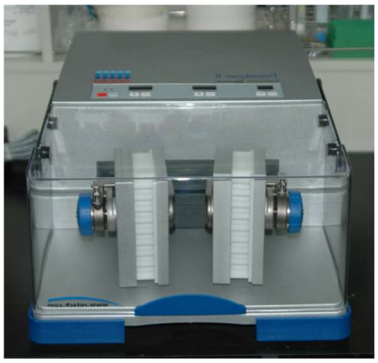
Typically, the methane oxidation rate is calculated using the "flux in" at the bottom of the column and "flux out" at the top of the column because methane oxidation rate refers to aerobic oxidation through the depth of the cover in all previous studies. However, since in this study we focused on anaerobic oxidation of methane, "flux in" was considered the methane flux from the bottom of the column (60.96 cm from the surface) and "flux out" was considered as methane flux at the depth of 45.72 cm of the cover. According to the previous studies (Scheutz et al., 2009) the aerobic methanotrophic zone is typically located higher 30-40 cm from the top of the column, with the highest oxidation at 15-20 cm from the surface, and aerobic methanotrophic activity is limited in the soil located at the depth below 60 cm from the surface, as shown in . According to Scheutz et al. (2009), oxygen availability in the depth lower than 40 cm is very limited and below the depth of 55 cm, the methane oxidation rate is almost zero. As mentioned

before, these graphs were obtained when the air flow rate on the surface of the soil was almost the same as the atmospheric condition. However, in our study, the anoxic zone extended up to an elevation higher than 60 cm from the surface, because we turned off air flow at the top of the columns. Therefore, the measured methane consumption in the last two ports located at the bottom of the column (45.72 cm and 60.96 cm from the surface) was the methane consumption in the absence of oxygen.

3.7 Methodology of microbe tests

In this test, deoxyribonucleic acid (DNA) of microorganisms was extracted, then PCR analysis was conducted to amplify the DNA, and spectrophotometer quantification was used to size the product of PCR.

DNA extraction is the method of extracting DNA from the cells or viruses in which DNA exists. Samples were stored at -20ºC until the DNA extraction, then the thawed samples were homogenized. QIAamp PowerFecal DNA Kit (Cat No. /ID: 12830-50) was used for DNA isolation. Samples were suspended with a Pasteur pipette and were then further homogenized by vortexing with 2 BB's. 250 µl of homogenized sample supernatant was harvested and DNA extraction was performed according to MoBio experimental protocol. Total genomic DNA was captured on a single silica spin column and DNA was then washed and eluted with 50 ul of solution C6. BBs, Qiagen vacuum manifold, and Qiagen Tissuelyser II used in this test are shown in Figure 3-31.



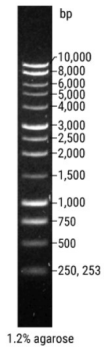
(a) (b) (c)

Figure 3-31 (a) Daisy bottle .177 cal zinc plated steel BBs, (b) Qiagen Vacuum Manifold used to process DNA samples, and (c) Qiagen Tissuelyser II used to disrupt sample bacteria

Polymerase Chain Reaction **(**PCR) is a technique to make many copies of a specific DNA region. PCR can be used to identify the small amount of sample because PCR amplifies the regions of DNA that it targets. It is a promising tool for community analysis in anaerobic processes, and has three steps; denaturation (yielding two single-stranded DNA molecules from a double-stranded DNA template), annealing (annealing of the primers to each of the single-stranded DNA templates to begins DNA formation), and extension (doubling the number of DNA target sequences, and finally exponential amplification of the specific DNA target region). The PCR protocols were followed from earth microbiome website (16S rRNA amplification protocol), and the primers that were used for DNA amplification, were the updated version of 515F/806R primers from Earth Microbiome Project. 300PE sequencing (300 bp paired-end-sequencing) was performed (the sample was sequenced 300 bp in one direction, then flipped and sequenced 300 bp from the other side). The sequencing reaction kit used in this test was maximally capable of sequencing 25 million reads. To check whether the PCR successfully generated the anticipated DNA target, agarose gel electrophoresis was used to quantitate the PCR products. Figure 3-32 shows the thermocycler used for PCR analysis.

Figure 3-32 Applied Biosystems Veriti thermocycler used for PCR analysis

DNAs were spectrophotometrically quantified by measuring the absorbance of UV light by the samples and the 260/230 ratio was used to determine the purity of the samples. A pure DNA sample which is relatively free from protein contamination has a 260/280 ratio of 1.8. DNA can be quantified by cutting the DNA, running it on an agarose gel, staining using ethidium bromide and comparing the size of DNA with DNA ladder. Figure 3-33 shows the DNA ladder and UV/White Light Transilluminator used for observing gel samples and quantifying the PCR product.



(a) (b)

Figure 3-33 (a) DNA ladder, and (b) UV/White Light Transilluminator

Chapter 4

Results

4.1 Results of the ESCA test

Electron Spectroscopy for Chemical Analysis *(*ESCA) test was carried out to find wastes that were high in iron content for use as electron acceptors. Table 4-1 shows the selected wastes, along with their elemental content based on ESCA test.

Table 4-1 Chemical composition of potential waste electron acceptors based on ESCA test

West Type	Al	C	Ca	Cu	Fe	K	Mg	Mo	Na	O	Si
Red brick 1	1.5	22.5	11.4	ND	ND	ND	1.4	ND	ND	58.3	4.9
Red brick 2	1.6	16.4	9.6	ND	0.5	ND	1.3	ND	0.6	61.3	8.6
Red brick 3	1.7	22.2	8.3	ND	0.5	ND	1.2	ND	0.3	57.5	8.2
Red mud	6.4	7.0	0.6	ND	**1.7**	0.3	0.8	0.1	ND	65.3	17.7
Granite powder	3.7	15.0	1.5	0.1	0.5	1.0	1.9	ND	1.9	58.8	15.6
Glass	ND	12.8	2.4	ND	ND	ND	1.3	ND	8.0	56.5	19.0

ND: Note Detected

Based on the test results, the iron content of all materials was low; however, among all materials, red mud had the highest iron content. Therefore, red mud was the only waste that was used as electron acceptor source in the batch tests.

4.2 Results of the soil tests

4.2.1 Soil compaction test

After the compaction test, the moisture content of the samples, and the dry soil density were calculated. The results of the compaction test are presented in Table 4-2.

Table 4-2 Summary of the results of the compaction test

Mass of empty Mold (kg)	4.246	4.246	4.246	4.254	4.254	4.254
Mass of mold + compacted Soil (kg)	5.823	5.912	6.011	6.11	6.089	6.054
Mold Volume (m^3)	0.00093	0.0009	0.0009	0.000935	0.000935	0.00093
Moist unit weight ($kg\ m^{-3}$)	1687.33	1782.6	1888.5	1985.851	1963.382	1925.93
Mass of empty container (kg)	0.213	0.209	0.203	0.213	0.209	0.203
Mass of compacted soil + Container (kg)	1.786	1.871	1.964	2.066	2.042	2.001
Mass of dry soil + container (kg)	1.651	1.682	1.718	1.735	1.678	1.629
Mass of soil (kg)	1.438	1.473	1.515	1.522	1.469	1.426
Mass of water(kg)	0.135	0.189	0.246	0.331	0.364	0.372
Moisture content (%)	9.38804	12.831	16.238	21.7477	24.77876	26.087
Dry unit weight ($kg\ m^{-3}$)	1542.52	1579.8	1624.7	1631.12	1573.49	1527.46

The compaction curve was also extracted based on the results. According to the curve shown in Figure 4-1 Compaction curve for the landfill cover soil, optimum moisture content and maximum dry density of the soil were 20% and 1632.0 $kg\ m^{-3}$, respectively.

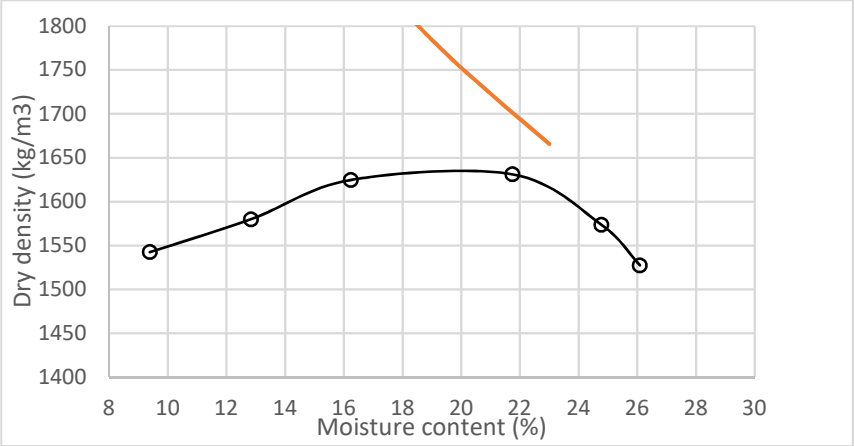


Figure 4-1 Compaction curve for the landfill cover soil

Typically, lower permeably would result in lower emissions from the landfill. However, in our study, if the soil is 100% compacted, gas transfer through the column will be very low, and it will take a long time for the gas to pass through the cover layer. Therefore, we needed to try samples with different compaction ratios to investigate the lowest compaction ratio that gives the required permeability by EPA. The first sample was compacted with a compaction ratio of 85% and the permeability of the sample was evaluated through the falling head permeability test.

4.2.2 Permeability test

Permeability test was conducted to measure the conductivity of the soil sample with 85% compaction. After substituting the obtained results, the permeability coefficient was obtained as follows:

$$k = 2.303 \frac{(\frac{\pi(3.175)^2}{4}) \times 8.14}{(\frac{\pi(9.80)^2}{4}) \times 636060} log \frac{46}{29.65} = 5.899 \times 10^{-7} cm/s$$

This value of permeability is lower than 10^{-5} cm sec^{-1}, which is required for landfill cover. So, we could use the soil with the same compaction ratio for column tests.

4.2.3 Size distribution test

4.2.3.1 Sieve analysis

The summary of sieve test results is presented in Table 4-3 and based on the results of the test, the percent finer versus particle size was drawn as shown in Figure 4-2. As shown in the graph, almost 80% of soil particles were smaller than 0.075 mm. So, hydrometer test was conducted to determine the size distribution of fine particles.

Table 4-3 The results of sieve analysis

Sieve No.	Sieve size (mm)	Mass of empty dish (kg)	mass of soil + dish (kg)	Mass retained on each sieve (kg)	% of mass Retained on each sieve (Rn)	Cumulative % Retained (∑ Rn)	% Finer
4	4.75	1.16	1.21	0.04	2.40	2.40	97.60
10	2	1.09	1.11	0.02	1.09	3.50	96.50
20	0.85	1.37	1.39	0.03	1.42	4.92	95.08
30	0.6	1.26	1.27	0.01	0.44	5.36	94.64
40	0.425	1.24	1.26	0.01	0.77	6.12	93.88
60	0.25	1.20	1.24	0.04	2.08	8.20	91.80
140	0.106	1.14	1.26	0.12	6.67	14.86	85.14
200	0.075	1.13	1.23	0.09	5.14	20	80

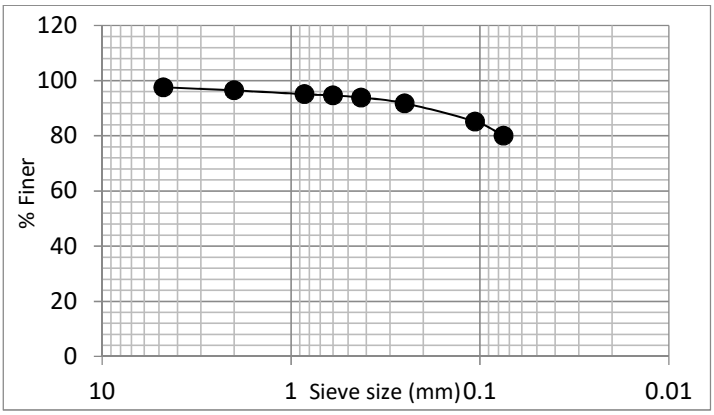


Figure 4-2 Size distribution of the soil obtained from sieve analysis

4.2.3.2 Hydrometer analysis

The results of the hydrometer test are presented in Table 4-4.

Table 4-4 Results of the hydrometer analysis

Time (min)	Reading	R_{CP}	% FINER	R_{CL}	L (cm)	A	D (mm)
0.25	56.7	51.6	82.56	57.7	6.8322	0.0135	0.070574
0.5	55.2	50.1	80.16	56.2	7.0782	0.0135	0.050794
1	54	48.9	78.24	55	7.275	0.0135	0.036412
2	52	46.9	75.04	53	7.603	0.0135	0.026322
4	50.2	45.1	72.16	51.2	7.8982	0.0135	0.01897
8	48.8	43.7	69.92	49.8	8.1278	0.0135	0.013607
15	47	41.9	67.04	48	8.423	0.0135	0.010116
30	44.9	39.8	63.68	45.9	8.7674	0.0135	0.007298
60	41.8	36.7	58.72	42.8	9.2758	0.0135	0.005308
120	39.6	34.5	55.2	40.6	9.6366	0.0135	0.003826
240	37.2	32.1	51.36	38.2	10.0302	0.0135	0.00276
480	35.2	30.1	48.16	36.2	10.3582	0.0135	0.001983
1440	33	27.9	44.64	34	10.719	0.0135	0.001165
2880	31.5	26.4	42.24	32.5	10.965	0.0135	0.000833
4759	29.5	24	38.4	30.5	11.293	0.0139	0.000677
8800	27.9	22.4	35.84	28.9	11.5554	0.0139	0.000504
11706	27	21.5	34.4	28	11.703	0.0139	0.00044
14726	26.8	21.3	34.08	27.8	11.7358	0.0139	0.000392
18894	26.3	20.8	33.28	27.3	11.8178	0.0139	0.000348
20504	26.2	20.7	33.12	27.2	11.8342	0.0139	0.000334
23462	25.8	20.3	32.48	26.8	11.8998	0.0139	0.000313
30226	25.8	20.3	32.48	26.8	11.8998	0.0139	0.000276

Usually hydrometer test measurements stop after 48 hours; however, since the curve did not reach zero slope after 48 hours, measurements continued after 48 hours.

By drawing the results of the hydrometer analysis and the results of sieve analysis on the same graph, we can evaluate the size distribution of the soil, as shown in Figure 4-3.

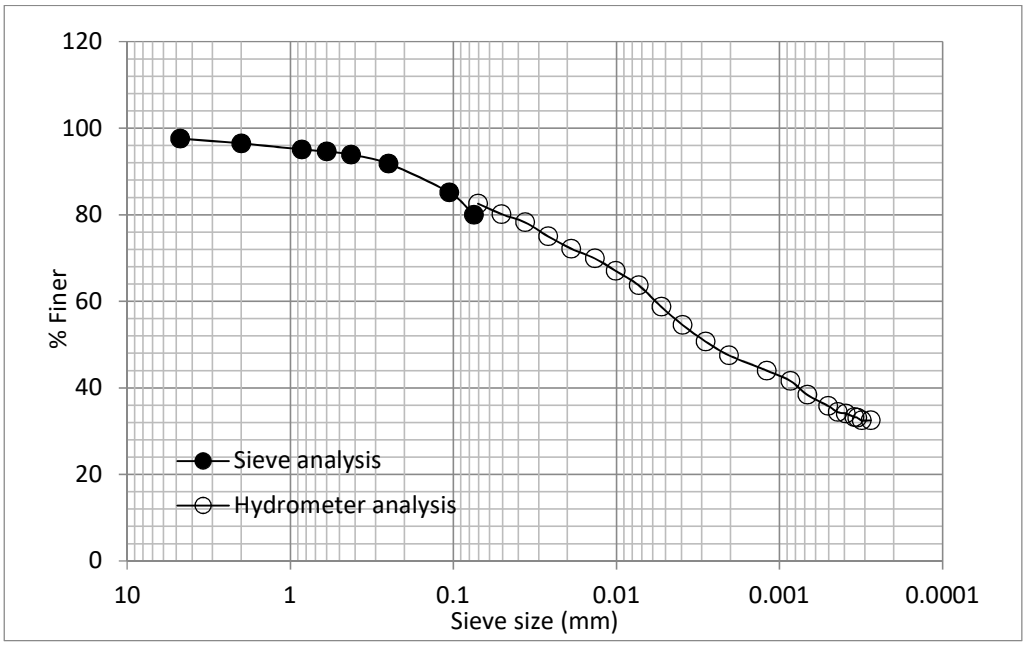


Figure 4-3 Distribution of particle size of the soil

4.2.4 Liquid limit

Table 4-5 shows the number of blows for which soil comes to contact along 13 mm of the groove, along with the moisture content for each sample. Figure 4-4 shows the number of blows versus the moisture content. Liquid limit, which is the moisture content of the soil for which 25 blows were needed to cover 13 mm groove, was obtained as 35.7% according to the graph, and the R^2 value for the regression was 0.9835.

Table 4-5 Data for determining the liquid limit

Number of blows	Mass of wet soil + container (gr)	Mass of dry soil + container (gr)	W (%)
16	24.34	18.236	37.6
25	15.02	11.578	35.9
29	18.05	13.81	34.4
35	18	13.756	33.3

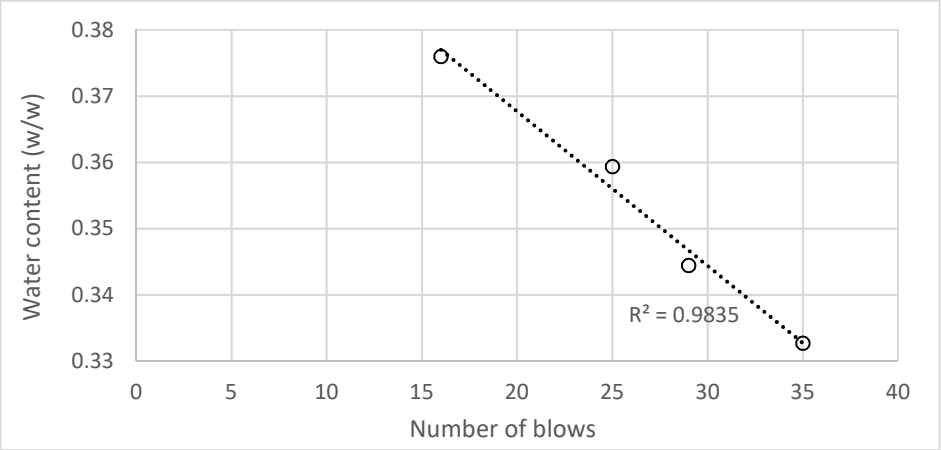


Figure 4-4 Graph of the number of blows versus moisture content for determining liquid limit

4.2.5 Plastic limit

Table 4-6 shows the results of the plastic limit test. The average value of plastic limit was 17.75%, and a plasticity index value of 17.95 was obtained. The soil was classified as CL, according to Figure 3-16.

Table 4-6 Moisture content of samples in the plastic limit test

Container number	Mass of empty container (g)	Mass of wet soil + container (g)	Mass of dry soil + container (g)	W (%)
1	1.02	3.55	3.169	17.7
2	1.00	4.77	4.2	17.8
Average				17.75

4.3 Results of the batch reactors

4.3.1 Adsorption test

Methane removal in the batch reactors could be due to adsorption and oxidation. As mentioned before, a reactor was designed to evaluate the amount of methane that can be adsorbed to the soil. Figure 4-5 shows the removal of methane over time in this adsorption reactor, and Figure 4-6 shows methane adsorption as the percentage of initial methane concentration in the headspace of the reactor.

As shown in the graph, almost 0.5 nmole/g soil was the maximum soil that was in the sample. Therefore, in the batch tests, methane removal of 0.5 nmole/ g soil will be considered as the amount of methane adsorbed to the soil and if methane removal is higher than 0.5 nmole/ g soil, it will be attributed to AOM.

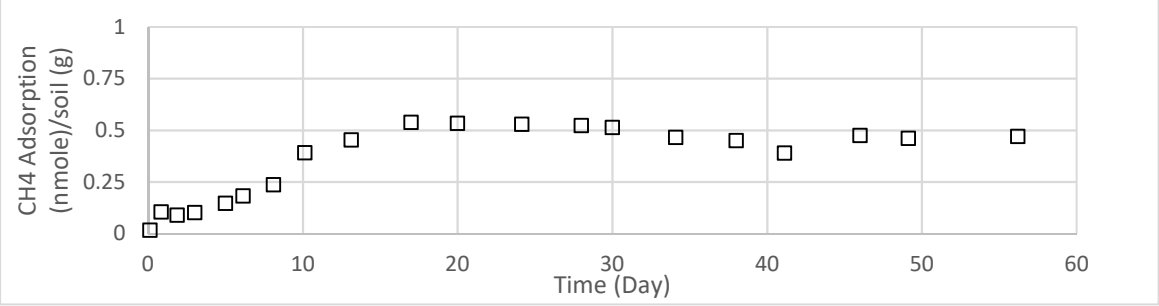


Figure 4-5 Methane adsorption over time in the adsorption reactor

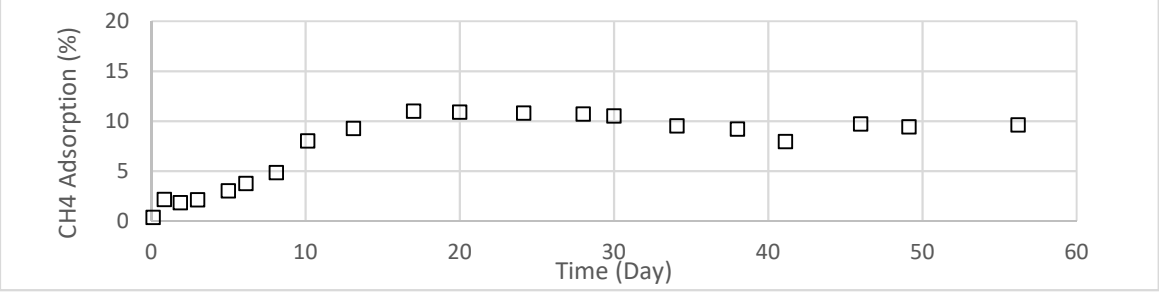


Figure 4-6 Percentage of methane adsorption over time in the adsorption reactor

4.3.2 Methane removal in batch reactors

4.3.2.1 Initial trials: Lessons learned

The first and second set of batch tests were conducted first, and then the remaining four sets were set up. In the first and second set, the equilibrium was reached after 40 days. Therefore, for the other sets, samples were taken from the reactors for 40 days. According to a study by Orit et al. (2014), the samples from reactors could show changes in the concentration of methane after 6 months. Therefore, samples were taken from the reactors after 6 months from the beginning of the tests. The data obtained based on the samples taken from the reactors in both first 40 days and after 6 months are shown in the appendix. According to the results, the methane concentration changed inside the reactors after 6 months, which is compatible with the previous research works due to the slow reaction rate of anaerobic oxidation of methane.

Figure 4-7 shows the average methane removal in the reactors over the long-term (first and second sets in nmoles methane removed/g soil) for soil with 3 electron acceptors (nitrate, sulfate, and iron), and soil by itself, with BES (second set) and without BES (first set). Figure 4-8 shows the average methane consumption as a percent of the initial concentration of methane in the sample taken from the headspace of reactors over the long-term. The amount of methane on the first day was considered as the initial amount of gas, and the removal of the gas was calculated by subtracting the remaining amount of gas from the initial value.

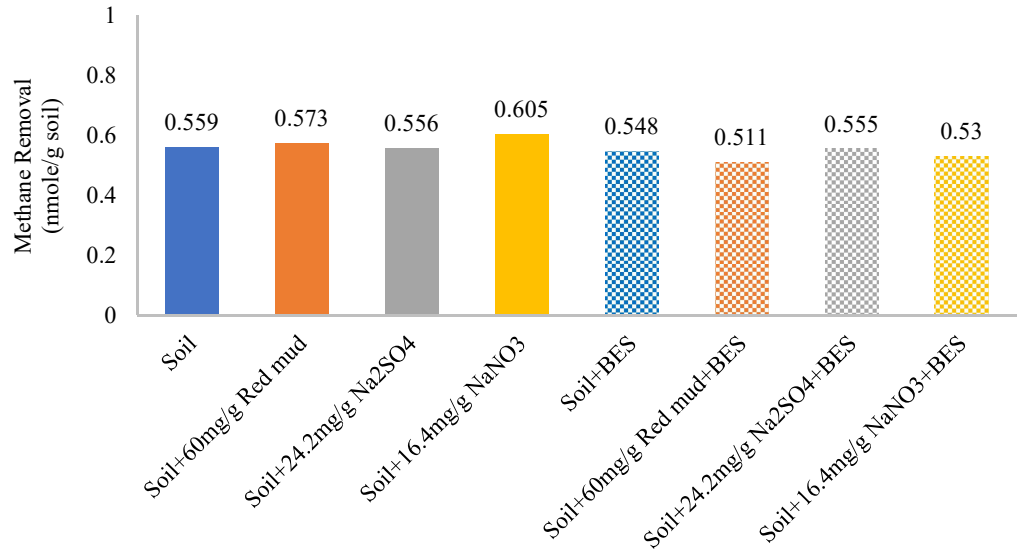


Figure 4-7 Average methane removal for soil with nitrate, sulfate, and iron (red mud) vs. soil by itself, with and without BES inhibitor

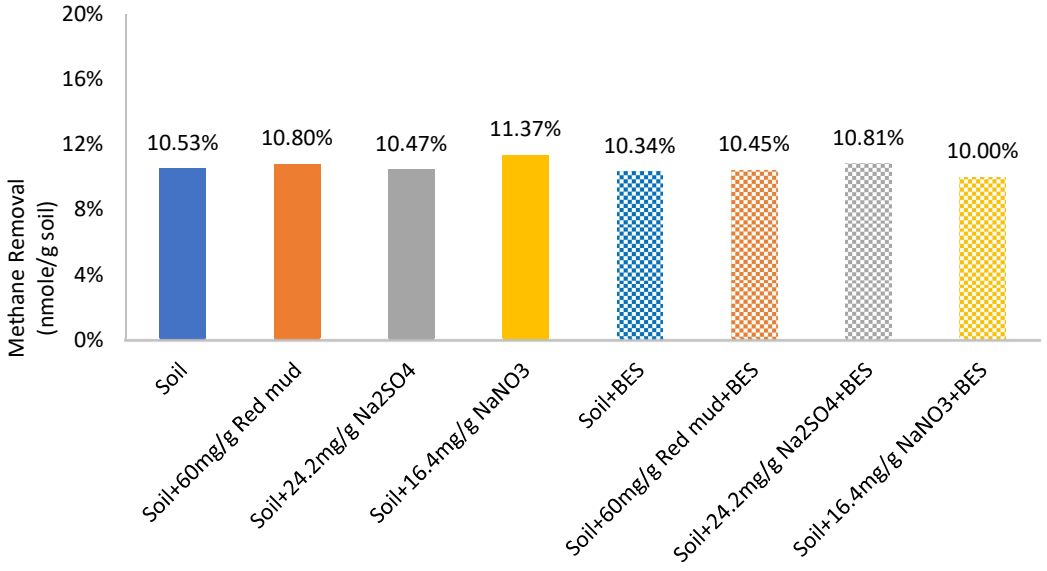


Figure 4-8 Average percentage of methane removal for the soil with nitrate, sulfate, and iron (red mud) with and without BES inhibitor

As shown in Figure 4-7 and Figure 4-8, samples with electron acceptors had similar average methane removal compared to the control test (plain soil), which was similar to the adsorption test (0.5 nmole/g soil). Therefore, in the first two sets, methane adsorption was the major

methane consumption mechanism. In general, methane removal in all reactors of the first and second set was low. There were no consistent trends in methane removal with and without BES.

A reason for low methane removal for the initial tests (Sets 1 and 2) was probably low water content in the reactors. In previous studies that focused on the anaerobic oxidation of methane, AOM was evaluated in the aquatic phase and water content was much higher than the water content in our samples. High water content provides more nutrient availability for microorganisms. Therefore, even if the soil in the first and second set contained enough nutrients, microbial activities would still be inhibited without enough moisture content to provide availability of these nutrients for microorganisms,. High moisture content was considered for the next batch reactors to solve this issue.

Another reason for low methane consumption in the first and second set may have been the low solubility of methane in water. Mole fraction solubility of methane in water is 2.552×10^{-5} (Gevantman, 2013). Therefore, methane may not be available for microbes in the liquid phase for oxidation.

In the case of $FeCl_3$, low methane removal in the first and second was likely due to the high concentration of chemicals that was toxic for microorganisms. The pH of the reactor that contained $FeCl_3$ was 2 at the end of the batch tests, which is very acidic for microorganisms and showed that methane removal in this reactor was due to chemical reactions rather than methane oxidation. This reactor was removed due to the acidic environment and generation of a significantly high amount of carbon dioxide.

To address issues of low methane removal in the first and second set of batch experiments, subsequent experiments were conducted with RAMM nutrient solution, higher initial concentration of methane, and higher water content (increased from 20% to 47%).

4.3.2.2 Impact of alternative electron acceptor chemicals on methane removal

In the next set of tests, using RAMM nutrient solution and higher water content, different electron acceptors were added to the soil samples to increase AOM. The effect of different electron acceptors can be evaluated as follows.

4.3.2.2.1 Nitrate as an electron acceptor

Figure 4-9 shows tests of soil with 2 concentrations of nitrate and 2 concentrations of sulfate compared to the plain soil control. Average methane consumption in the control reactor (containing soil) was 0.962 nmole/g soil. Since the maximum methane adsorption obtained was 0.5 nmole/g soil, 0.462 nmole/g soil of methane was removed due to AOM. In the sample with 0.84 mg/g nitrate, methane removal was slightly higher than the control soil. However, in the sample with higher nitrate concentration (1.64 mg/g), methane removal over the long-term was substantially higher.

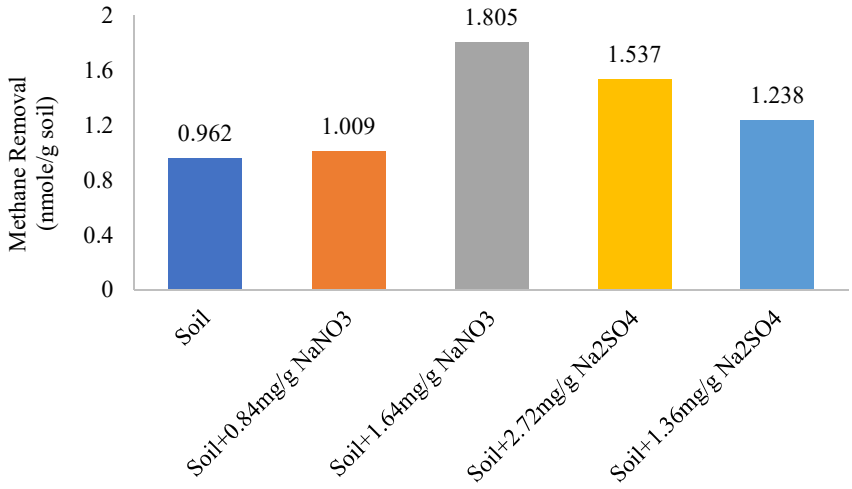


Figure 4-9 Average methane removal of soil amended with nitrate and sulfate

Methane oxidation by reducing nitrate ions is plausible and favorable (Smemo and Yavitt, 2011). In soil with enough available nitrate, methane can be removed based on Equation 2-23. ANME-2d are capable of oxidizing methane in the presence of nitrate (Bar-Or et al., 2017). Gibbs free energy for this reaction is equal to -372.8, -337.1, and -362.0 for nutrient-rich soil, poor/intermediate nutrient, and nutrient-poor soils correspondingly (Smemo and Yavitt, (2011), which shows the feasibility of the reaction. However, NO_2^-, which is an intermediate during the denitrification process, can suppress methane generation and consequently AOM. When NO_2^- is reduced, N_2 and O_2 will be produced along with the oxidation of CH_4 (Smemo and Yavitt, 2011).

4.3.2.2.2 Sulfate as an electron acceptor

Figure 4-9 also shows the results of the samples with 2 concentrations of sulfate compared to the plain soil in the control test. Both samples (1.36 and 2.72 mg sulfate/g soil) had higher average methane removal compared to the control reactor. In Figure 2-17 presented in Chapter 2, AOM increases with increase in the concentration of sulfate in the sample. This is compatible

with our finding, since increasing the sulfate concentration increased methane removal over time. In the reactor with 2.72 mg/g sulfate, the concentration of sulfate was 20 mM and the concentration of methane in the headspace was 14.7 mM, which probably falls in the combination of the concentrations on the right side of the curve presented in Figure 2-17.

Therefore, considering the fact that higher sulfate concentration will result in higher AOM, and also higher methane removal (according to Figure 4-9 Average methane removal of soil amended with nitrate), the reactor with higher sulfate concentration was selected for column experiments.

Methanotrophic archaea ANME and sulfate-reducing bacteria use sulfate to oxidize methane according to Equation 2-20, in which Gibbs free energy is -16.6, which is much lower than Gibbs free energy of the reactions in which nitrate or iron act as electron acceptors.

4.3.2.2.3 Iron as an electron acceptor

Figure 4-10 shows tests of soil with various chemicals used to add iron (III) as an electron acceptor, compared to the plain soil control. The reactor that contained soil sample with sulfate and hematite had the highest oxidation rate among the iron batch tests, and higher average methane removal compared to the control test, so it was considered for column tests. Average methane removal in the reactor amended with hematite by itself was lower than the control test, and the reactor that contained $Fe(OH)_3$ removed about the same methane as the control test.

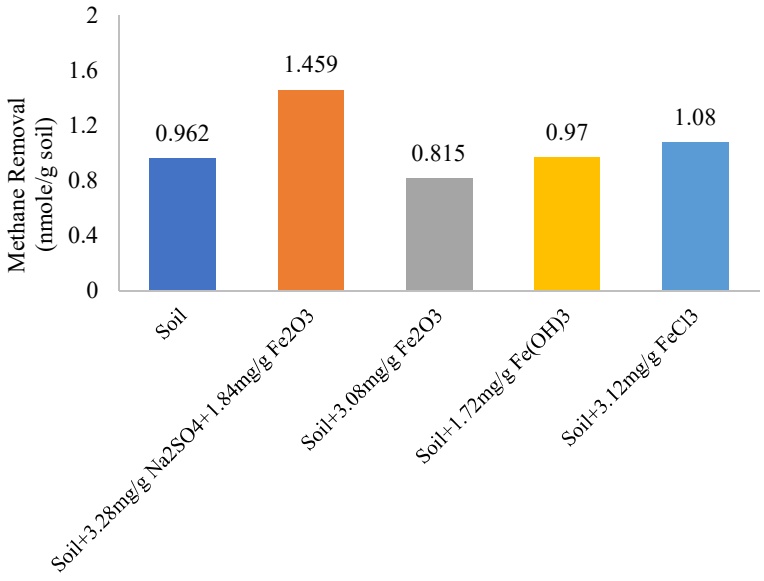


Figure 4-10 Average methane removal of soil amended with iron

Our results for sulfate + hematite and $FeCl_3$ are compatible with previous studies in which iron was found to stimulate sulfate-dependent AOM, as well as previous findings in which anaerobic oxidation of methane has been also linked to Fe (III) (Ettwig et al., 2016; Bar-Or et al., 2017). An increase in anaerobic oxidation of methane due to the availability of Fe (III) happens when methanogens are responsible for methane consumption according to Equation 2-22, and the Gibbs free energy for this reaction is equal to -572 kJ/mole.

The lack of removal by hematite by itself and $Fe(OH)_3$ may be because of higher methane generation that canceled out high methane consumption in the reactor. According to previous studies, iron nanoparticles can increase methane production (Wang et al., 2016). In an environment with a high amount of methane, however, iron reduction coupled with methane oxidation can overcome methanogenesis.

4.3.2.2.4 Other electron acceptors

Figure 4-11 shows tests of soil with other electron acceptors (NO_2^-, Mn^{+4}, and NH_4^+). As shown, the soil amended with other electron acceptors had similar average methane removal over the long-term to the control soil.

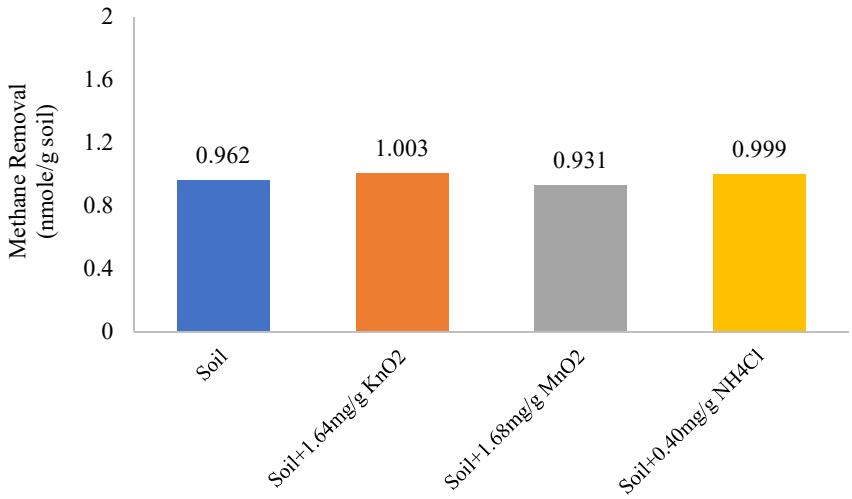


Figure 4-11 Average methane removal of soil amended with other electron acceptors

4.3.2.2.5 Summary – Impact of alternate electron acceptors

Samples amended with sulfate, nitrate, and sulfate + hematite had greater average methane removal over the long-term compared to the control test with no electron acceptor. Figure 4-12 shows methane removal in the reactors with different electron acceptors and the control test with 47% moisture content, 36% methane concentration, and added nutrient.

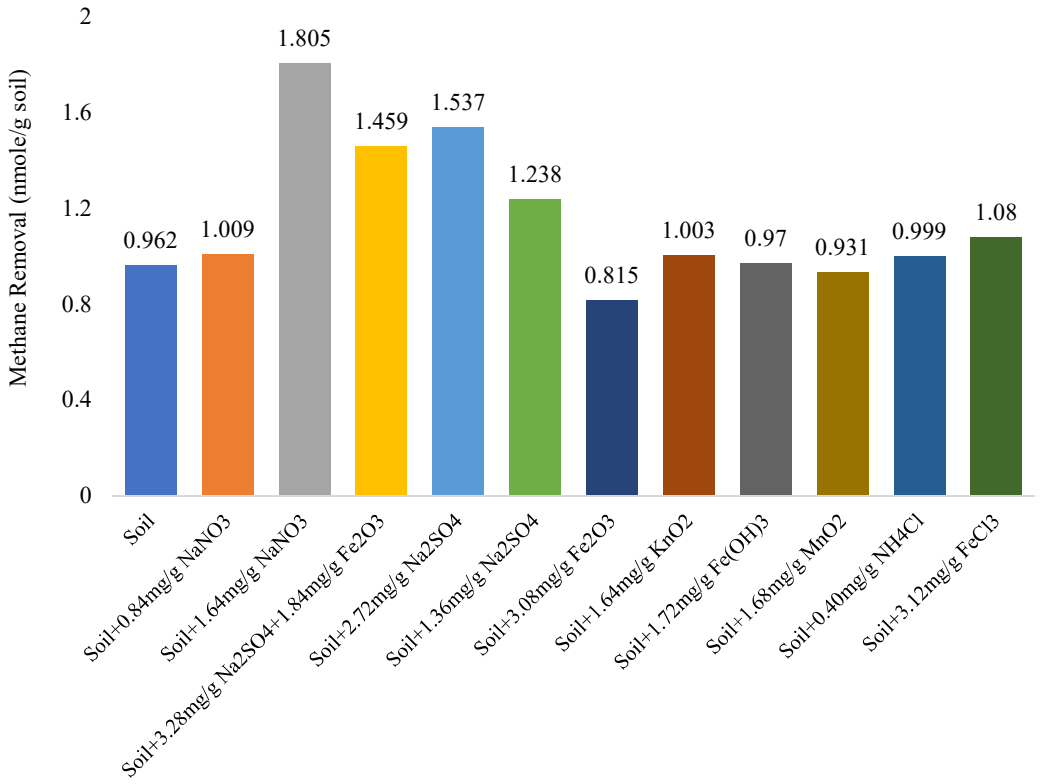


Figure 4-12 Average methane removal in the reactors with different electron acceptors and the control test (47% moisture content, 36% methane concentration, added nutrient)

4.3.2.3 Impact of inhibitor

According to previous studies, BES can inhibit both methane generation and oxidation at the same time. If methane oxidation is coupled with methanogens' activity, BES can inhibit methane oxidation as well as generation. However, if electron acceptor's reduction is not coupled with methane generation pathway, BES does not inhibit AOM (Bar-Or I. et al. 2017).

According to Figure 4-13, average methane removal over the long-term for plain soil sample with and without BES was similar. According to Figure 4-14, adding inhibitor increased average methane removal in the reactor with nitrite. It also slightly increased average methane removal in the reactor amended with iron. The reason for the increase in methane removal could be the

involvement of microorganisms other than methanogens in the mechanism of AOM; the inhibitor did not suppress these microorganisms.

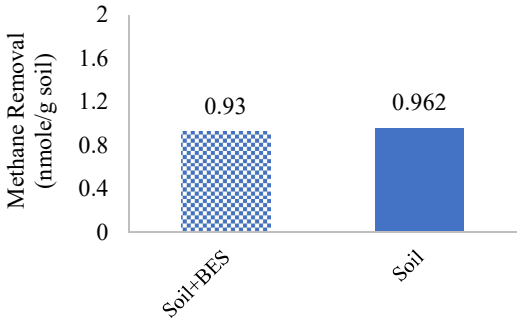


Figure 4-13 Average methane removal in the control reactor with and without inhibitor

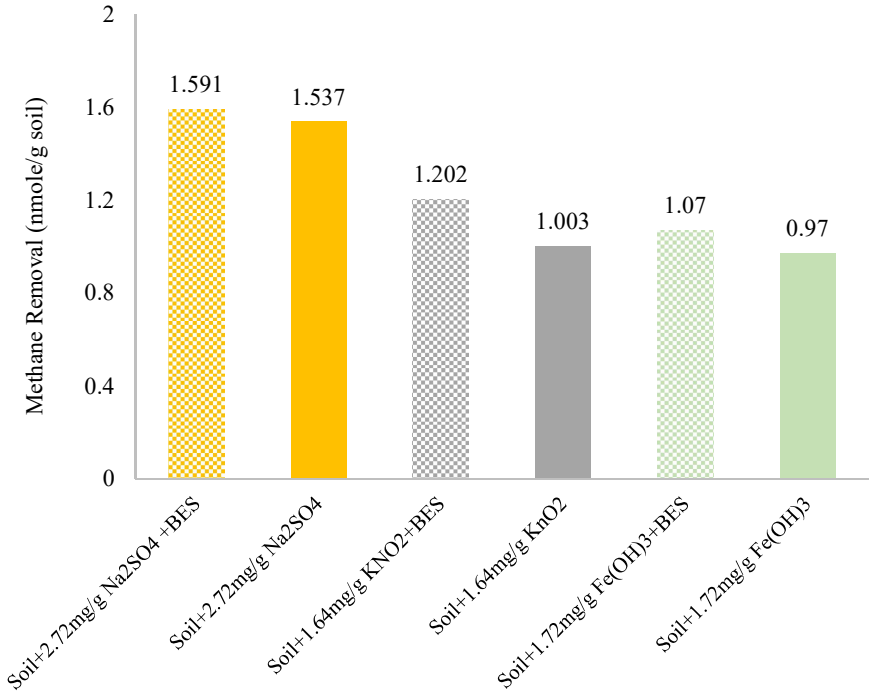


Figure 4-14 Average methane removal in the reactor that contained KNO_2 with and without inhibitor

Average methane removal was slightly greater over the long-term for the sulfate reactor with BES compared to that without inhibitor, according to Figure 4-14. Acetogenesis is the mechanism involved in methane consumption by sulfate-reducing bacteria, in which

hypothetically methane-consuming bacteria produce H_2 and acetate acid that is consumed by sulfate-reducing bacteria (Caldwell S.L. et al., 2008). Therefore, if sulfate-reducing bacteria are responsible for anaerobic oxidation of methane in the presence of sulfate, the inhibitor may not affect AOM. As a result, methane consumption slightly increased in this reactor.

4.3.2.4 Impact of nutrient solution

By comparing the graphs shown in Figure 4-15, we can conclude that average methane consumption in almost all samples was higher in the reactors with RAMM solution, compared to average methane removal in the samples without RAMM solution over the long-term.

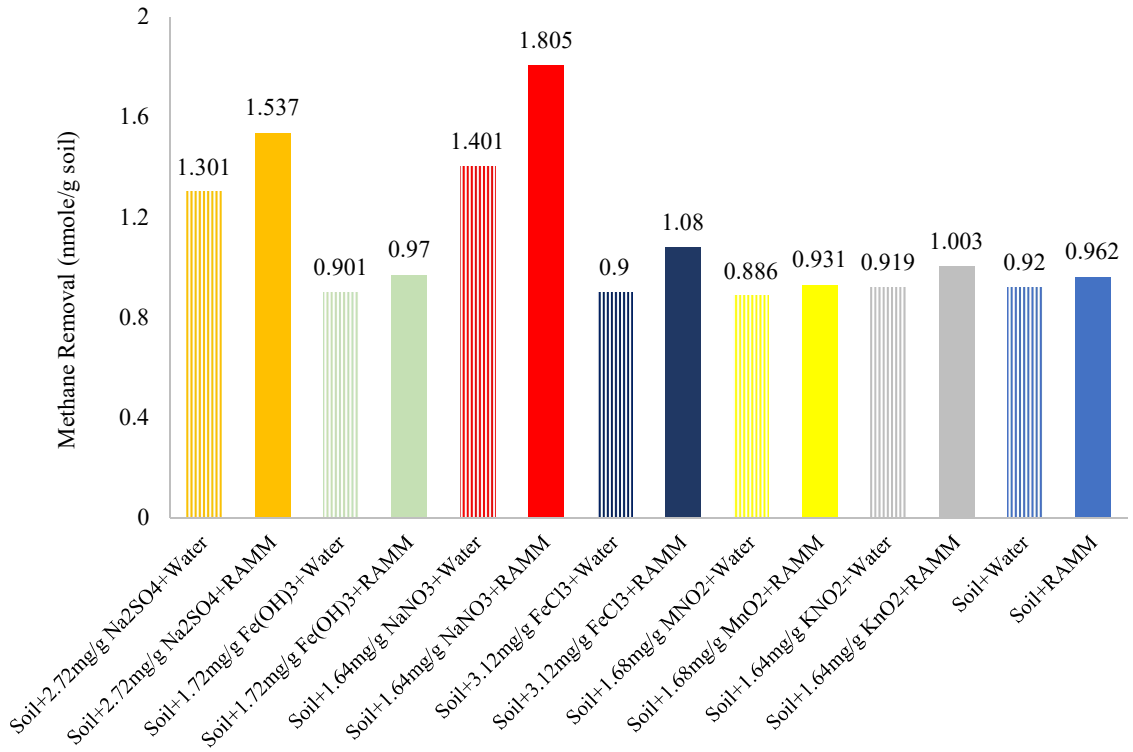


Figure 4-15 Comparing average methane removal in the reactors with and without RAMM solution

As shown in **Error! Reference source not found.**, for the reactors amended with iron hydroxide, nitrite, manganese, and the control test, average methane removal over the long-term was just slightly higher for the samples with RAMM solution compared to the similar reactors without RAMM solution. This low difference could be because of the fact that average methane removal for the reactors amended with these electron acceptors was too low to distinguish their lower methane removal in the absence of RAMM solution. However, for other electron acceptors, average methane removal was high enough to highlight the differences in the presence or absence of RAMM solution.

In general, the average methane consumption showed lower values for the reactors with no added nutrient compared to that of the reactors with RAMM solution. These results show that adding RAMM solution could provide nutrients and a more favorable environment for microbial activities, so AOM increased in the samples with RAMM solution.

4.3.2.5 Impact of initial concentration of methane

By comparing graphs presented in Figure 4-16, we can conclude that average methane removal in the reactors with 12% initial methane concentration is lower than the average methane removal in the reactors with 36% initial methane concentration. According to the previous studies, methane oxidation increases by increasing the initial concentration of methane in the headspace to some level, and then it decreases.

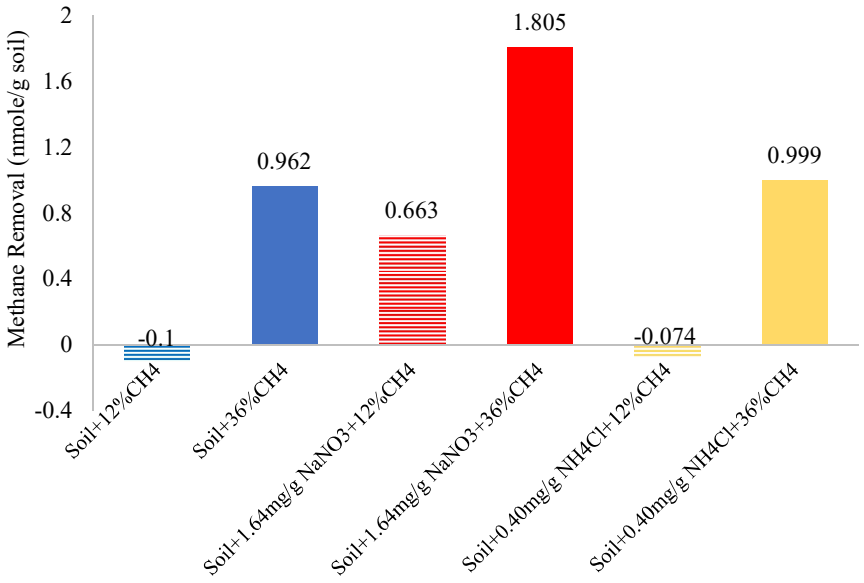


Figure 4-16 Average methane removal in the reactors with different concentration of LFG

4.3.3 Michaelis-Menten constant and oxidation rate for batch reactors

Kinetics of methane oxidation shows how fast oxidation occurs. The Michaelis-Menten equation is used to express the rate of oxidation according to $V = \frac{V_{max}}{1+K_m/C} = \frac{V_{max} \times C}{C+K_m}$ Equation 2-14, in which V_{max} is the maximum reaction rate, and Michaelis-Menten constant K_m is the half-saturation constant (concentration of methane when the oxidation rate is half of the maximum oxidation rate). For low concentrations of methane, the oxidation equation will be first-order. In this case, the maximum methane concentration is below the saturation level; however, for high concentrations of methane, the reaction will be zero-order.

Low methane affinity and a high oxidation rate lead to a high Michaelis-Menten constant and high maximum oxidation rate (a condition that favors microorganisms living in an environment with low methane and high oxygen concentration). High methane affinity and low oxidation rate lead to a low Michaelis-Menten constant and low maximum oxidation rate (a condition that

favors microorganisms living in an environment with high methane and low oxygen concentration).

Maximum oxidation rates and Michaelis-Menten constants were calculated for all sets of batch reactors and the results are presented in Table 4-7. As shown in Table 4-7, all reactors that were amended with electron acceptors had higher maximum oxidation rates compared to the control reactor in each set. However, for each set in general, maximum oxidation rate values for the samples with different electron acceptors were not significantly different from that of the control reactor.

Table 4-7 Reaction kinetics for the batch reactors in all sets

Set No.	Reactor No.	Added chemical	Concentration of Chemical (mg/g sample)	CH_4/CO_2 (mL)	Water/ RAMM	(w%)	V_{max} (nmoles/day)	K_m (nmoles)	R^2	Average Methane Removal (nmole/g)
1	1	-	-	30	Water	20%	97.21	-0.0039	0.9998	0.559
	2	Red mud	60.00	30	Water	20%	97.26	-0.0069	1	0.573
	3	Na_2SO_4	27.20	30	Water	20%	97.74	-0.0039	1	0.556
	4	$NaNO_3$	16.40	30	Water	20%	97.09	-0.0146	1	0.605
	5	$FeCl_3$	31.20	30	Water	20%	-	-	-	-
2	6	-	-	30	Water	20%	96.49	-0.0048	1	0.548
	7	Red mud	60.00	30	Water	20%	97.53	-0.0078	0.999	0.511
	8	Na_2SO_4	27.20	30	Water	20%	98.2	-0.0066	1	0.555
	9	$NaNO_3$	16.40	30	Water	20%	97.0	-0.0065	1	0.53
	10	$FeCl_3$	31.20	30	Water	20%	-	-	-	-
3	11	-	-	90	RAMM	47%	140.41	0.005	0.9983	0.962
	12	$NaNO_3$	0.84	90	RAMM	47%	140.92	-0.0018	0.9995	1.009
	13	$NaNO_3$	1.64	90	RAMM	47%	145.34	-0.0271	1	1.805
	14	Na_2SO_4	3.28	90	RAMM	47%	145.0	-0.0385	1	1.459
		Fe_2O_3	1.84							
	15	Na_2SO_4	2.72	90	RAMM	47%	145.05	-0.0459	1	1.537
	16	Na_2SO_4	1.36	90	RAMM	47%	146.44	-0.0036	1	1.238
	17	Fe_2O_3	3.08	90	RAMM	47%	145.52	-0.0055	1	0.815
	18	KnO_2	1.64	90	RAMM	47%	144.89	-0.0366	0.9998	1.003
	19	$Fe(OH)_3$	1.72	90	RAMM	47%	144.29	-0.0212	1	0.97
	20	MnO_2	1.68	90	RAMM	47%	144.02	0.0002	1	0.931
	21	NH_4Cl	0.40	90	RAMM	47%	142.92	-0.0087	1	0.999
	22	$FeCl_3$	3.12	90	RAMM	47%	144.47	-0.0168	1	1.08
4	23	-	-	90	RAMM	47%	141.26	0.0024	1	0.93
	24	Na_2SO_4	2.72	90	RAMM	47%	146.36	-0.0087	0.9999	1.591
	25	KNO_2	1.64	90	RAMM	47%	145.73	-0.0011	0.9999	1.202
	26	$Fe(OH)_3$	1.72	90	RAMM	47%	143.31	-0.0017	0.9999	1.07
5	27	-	-	90	Water	47%	137.09	0.0121	0.998	0.92
	28	Na_2SO_4	2.72	90	Water	47%	138.17	0.0026	0.9992	1.301
	29	KNO_2	1.64	90	Water	47%	139.14	0.0004	0.9999	0.919
	30	$Fe(OH)_3$	1.72	90	Water	47%	145.42	-0.004	0.9999	0.901
	31	MNO_2	1.68	90	Water	47%	141.45	-0.0266	0.9967	0.886
	32	$NaNO_3$	1.64	90	Water	47%	143.98	-0.0502	0.9997	1.401
	33	$FeCl_3$	3.12	90	Water	47%	143.74	-0.0036	0.9999	0.90
6	34	-	-	30	RAMM	47%	92.41	-0.0023	1	-0.1
	35	$NaNO_3$	1.64	30	RAMM	47%	96.59	-0.103	0.9999	0.663
	36	NH_4Cl	0.40	30	RAMM	47%	100.19	-0.0006	0.9998	-0.074

According to Table 4-7, the maximum oxidation rate is low for the reactors of the first and second sets, which was consistent with the overall low average methane removal for these sets, as shown in Figures 4-7 and 4-8. Maximum oxidation rates were significantly higher in Set 3, due to the nutrient solution, higher methane concentration and higher water content, which is compatible with the average methane removal results obtained from the graphs. Overall, the maximum oxidation rate was for soil amended with 1.36 mg/g sulfate. In Set 3, the control test with no electron acceptor had the lowest maximum oxidation rate.

For some reactors in Set 4 with inhibitors, the maximum oxidation rate increased compared to the corresponding reactors in the Set 3 without inhibitor, while it decreased for other reactors in the Set 4. In this set also control test with no electron acceptor had the lowest maximum oxidation rate.

In Set 5 without RAMM solution, the maximum oxidation rate decreased for most of the reactors compared to Set 3 with RAMM solution, as shown in Table 4-7. These results are compatible with the findings in the graphs presented in Figure 4-15. In this set also, all reactors that were amended with electron acceptors had a higher maximum oxidation rate compared to the control reactor.

According to Table 4-7, in Set 6 with lower initial methane concentration, the maximum oxidation rate significantly decreased compared to Set 3. Probably lower concentration of methane caused the decrease in maximum oxidation rate as well as a decrease in methane removal. In this set, as with previous sets, batch reactors with electron acceptors had higher maximum oxidation rates than that of the control reactor.

4.4 Results of the column tests

4.4.1 Moisture content and temperature

Figure 4-17 and Figure 4-18 show the variation of moisture content and temperature over time in columns #1, 2, and 3 correspondingly. Temperature and moisture content were not recorded in column #4 due to a technical error. As shown in the graphs, temperature and moisture content were almost constant during the tests.

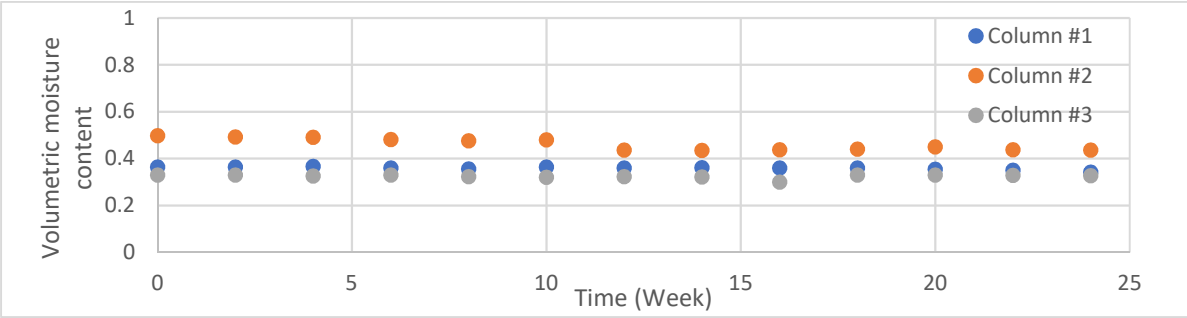


Figure 4-17 Volumetric moisture content of the soil inside the columns over time

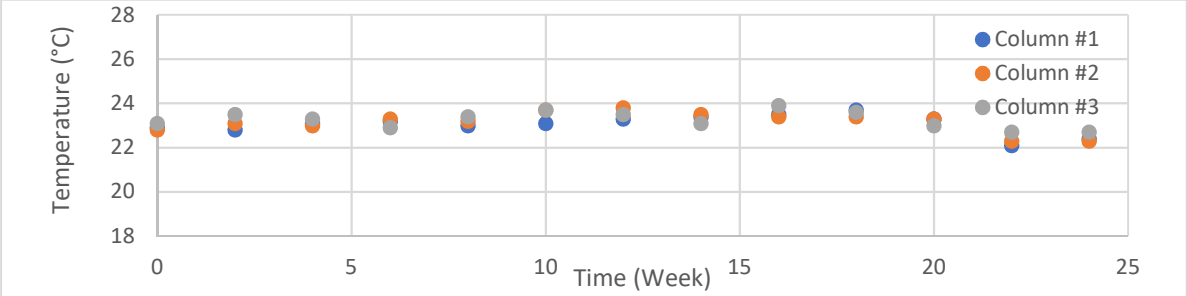


Figure 4-18 Temperature of the soil inside the columns over time

4.4.2 Methane removal for higher LFG flow rate (12 ml/min)

Figure 4-19 shows sampling results from the 4 ports of each column for a methane flow rate of 12 ml/min. The environment of the soil placed at the level of the first and second ports from the bottom was considered anaerobic, according to the previous studies. Therefore, methane

oxidized in the first two ports at the bottom of columns was likely due to anaerobic oxidation, while methane oxidation in the two top ports (that were in the aerobic zone, confirmed via oxygen measurements) was due to aerobic oxidation. The green arrows on the graphs show the anaerobic zone inside the reactors.

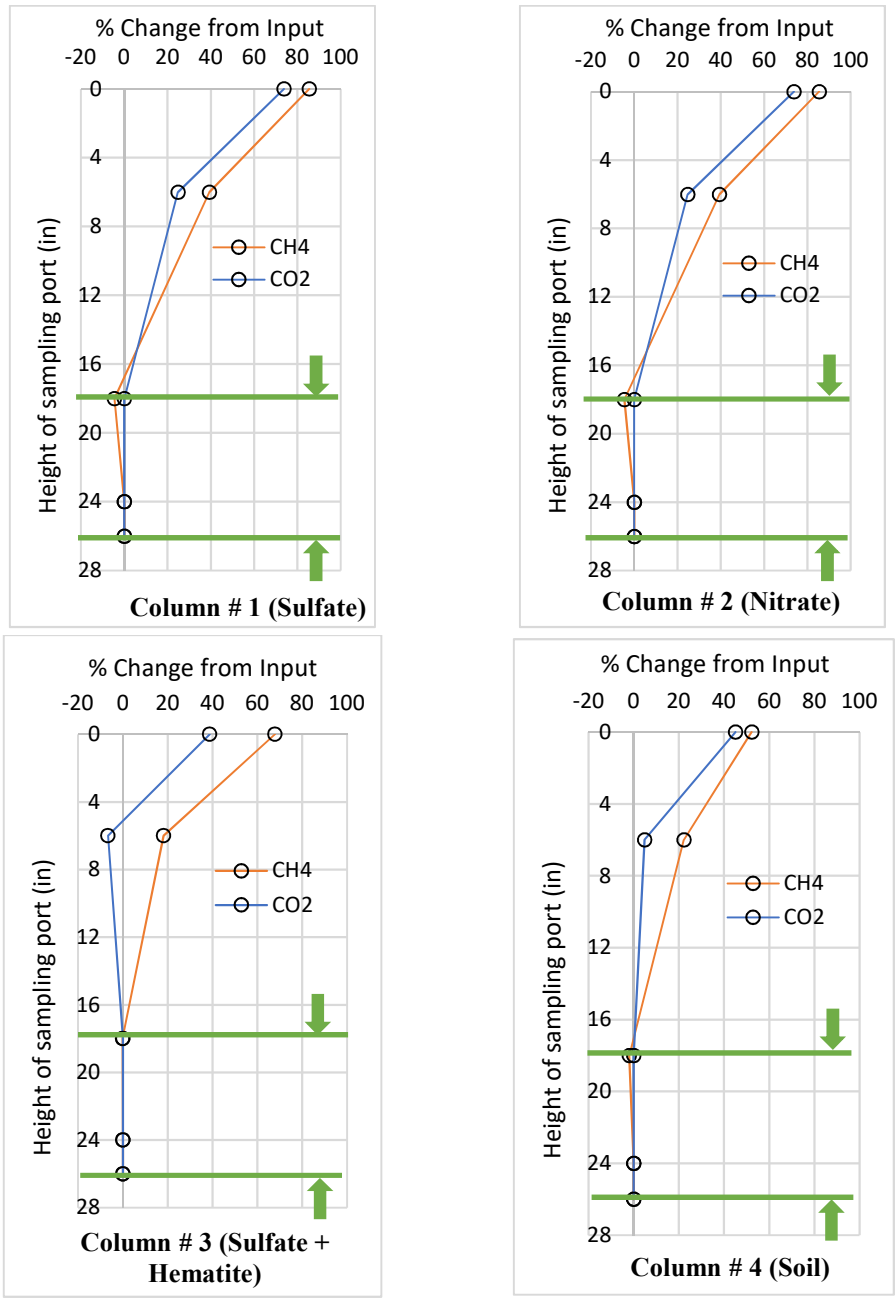


Figure 4-19 Distribution of methane and carbon dioxide in the columns for 12 ml/min LFG flow rate

As shown in the graphs, there is almost no net anaerobic oxidation of methane in two ports at the bottom of the columns. In column #1, methane generation was greater than the oxidation, so the amount of methane increased from port #1 to port #2. In column # 2, there was less methane generation compared to column # 1.

In column # 3, methane generation was less than the first two columns; therefore, either anaerobic oxidation of methane was higher, which counterbalanced the generated methane in the bottom of the column, or the environment of the column was less favorable for methane generation. In column #4, methane generation was slightly higher than the oxidized methane, so net methane generation was observed.

After analyzing the samples, the methane oxidation rate was also calculated for the anaerobic zone for different columns. As shown in Table 4-8, methane oxidation rates are negative for the anaerobic zone of all columns.

Table 4-8 Methane oxidation rate in the bottom two ports (anaerobic zone) for 12 ml/min LFG flow rate

Column No.	**1 (Sulfate)**	**2 (Nitrate)**	**3 (Sulfate + hematite)**	**4 (Soil by itself)**
Flux in (g CH_4 m^{-2} day^{-1})	179.37	179.37	179.37	179.37
Flux out (g CH_4 m^{-2} day^{-1})	187.99	188.24	180.06	189.01
Oxidation rate (g CH_4 m^{-2} day^{-1})	-4.806	-4.945	-0.385	-5.374

In the higher parts of the columns, where oxygen is available, the methane oxidation rate was positive, which is compatible with previous studies. However, since in the anaerobic zone of the columns methane oxidation and generation occur simultaneously, which is different from the

aerobic zone of the columns, methane generation could occur at a higher rate compared to anaerobic oxidation of methane.

Since there was no net anaerobic oxidation of methane in the anoxic zone of any of the columns, the value of anaerobic oxidation rate of methane in the columns was either very low or at least less than the methane generation rate. Therefore, we decided to lower the LFG flow rate into the columns from 12 ml min^{-1} to 6.0 ml min^{-1}. The lower methane flow rate had no effect on methane concentration; however, it provided anaerobic microbes more time to oxidize methane, and since anaerobic oxidation of methane has a very low oxidation rate, lowering methane flow rate may favor the AOM.

4.4.2 Methane removal for lower methane flow rate (6 ml/min)

After dropping the methane flow rate, we waited for a week, so microorganisms acclimated to the new flow rate and reached an equilibrium condition. Then, the samples were taken from the ports for ten days and the average of the readings are shown in Figure 4-20.

According to the graphs, Column # 3 which contained sulfate and hematite had the highest anaerobic oxidation of methane in the anoxic zone. In this column, methane removal was highest at the depth of 18 in from the surface. In Column # 1 almost no net methane generation or oxidation was observed at a depth of 18 in. Columns # 2 and 4 had more methane generation than methane oxidation in the anoxic zone. So, no net methane oxidation was observed in these columns in the anoxic zone.

In the batch tests, soil amended with nitrate had higher average methane removal compared to the control reactor. However, in the column tests, the same sample removed no more methane compared to the control test in the anaerobic zone. According to the previous studies, during the process of methane removal, generation of some intermediate products can inhibit methane oxidation by nitrate.

In the batch tests, methane was injected into the reactors just once at the beginning of the tests, so probably the concentration of the produced toxic intermediates was low. However, in the column tests, there was a continuous flow of methane into the columns, which could generate a high concentration of toxic intermediates that inhibited methane removal by nitrate-reducing bacteria.

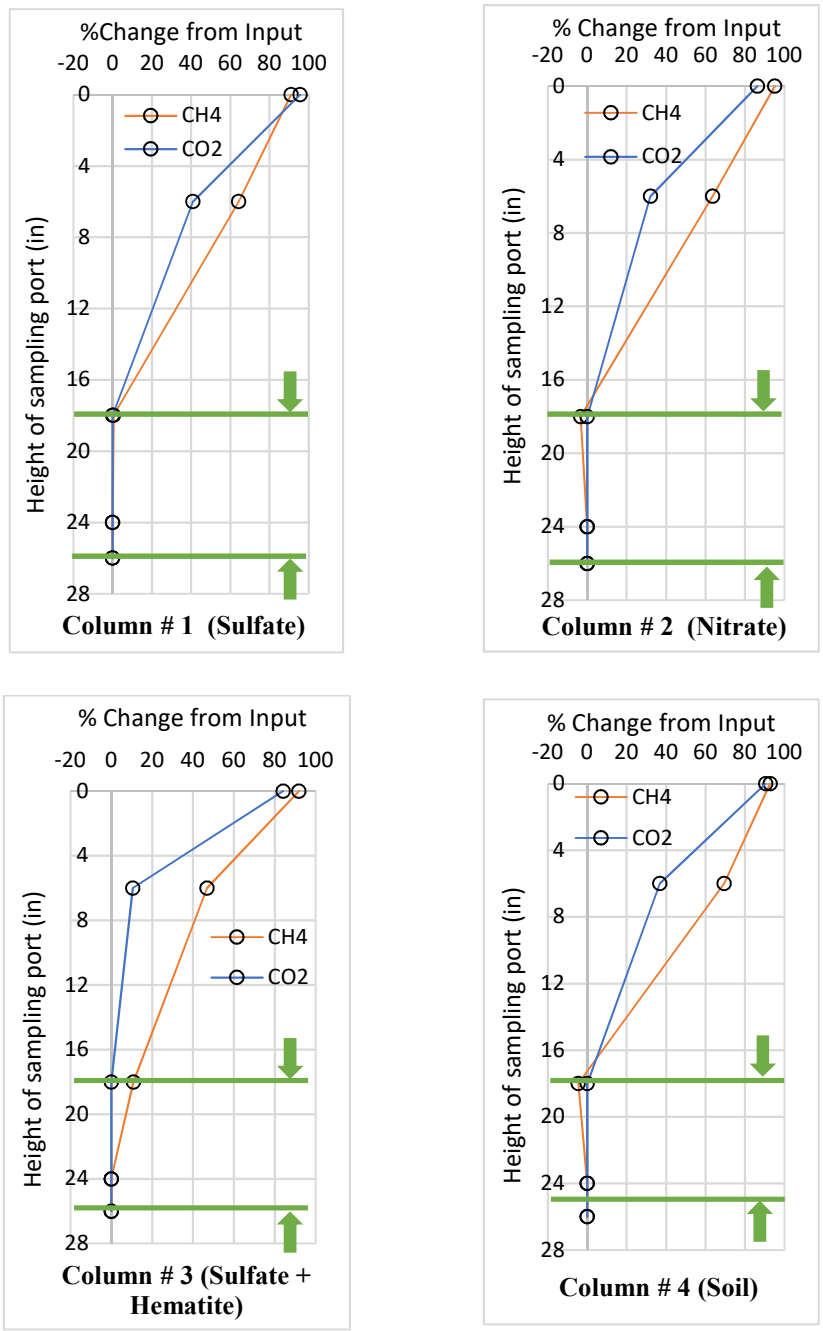


Figure 4-20 Distribution of methane and carbon dioxide in the columns for 6 ml/min LFG flow rate

To further compare the AOM in the columns, the oxidation rate of methane was calculated based on the readings in the anaerobic part of the columns, and the results are presented in Table 4-9.

Table 4-9 Methane oxidation rate in the bottom 2 ports (anaerobic zone) for 6 ml/min LFG flow rate

Column No.	**1 (Sulfate)**	**2 (Nitrate)**	**3 (Sulfate + Hematite)**	**4 (Soil by itself)**
Flux in (g CH^4 m^{-2} day^{-1})	89.69	89.69	89.69	89.69
Flux out (g CH^4 m^{-2} day^{-1})	88.82	93.03	80.11	93.77
Oxidation rate (g CH^4 m^{-2} day^{-1})	0.970	-3.724	10.681	-4.549

As shown in Table 4-9, methane oxidation rate had the highest value for column # 3 and was followed by column #1 as the column with the second highest methane oxidation rate in the anoxic zone. According to the graphs, in column # 2 and 4, methane generation was higher than methane oxidation at the bottom of columns, and the obtained negative values for oxidation rate probably indicate that.

In both column # 1 and 3 sulfate was added to the soil, so probably sulfate-reducing bacteria were responsible for higher anaerobic oxidation in these columns, which is compatible with previous studies in which addition of sulfate increased anaerobic oxidation of methane. Moreover, in column # 3 iron oxide was also added to the soil which probably increased anaerobic oxidation of methane by sulfate-reducing bacteria. In previous studies, iron oxides increased AOM by stimulating sulfate-dependent anaerobic oxidation of methane. Therefore, the presence of hematite in this column probably favored AOM by sulfate-reducing bacteria. Our result is compatible with the previous study by Sivan et al. (2014), in which the addition of hematite increased the activity of sulfur cycling microorganisms by 40%.

According to the results of the gas analysis presented in Table 4-10, hydrogen sulfide was observed at the surface of column # 1 and 3, which shows the presence of sulfate-reducing

bacteria (SRB). Since there was the emission of H_2S from these two columns, it was concluded that sulfate was consumed by SRB. Therefore, probably SRB oxidized methane to reduce sulfate.

The concentration of H_2S gas was lower in the headspace of column #3 compared to column #1, which could be because of either lower concentration of sulfate or the presence of iron, since the previous studies have shown that soil amended with iron oxides can act as a non-organic filter and remove H_2S gas.

Table 4-10 percent gas components found at the headspace of the columns

Column No.	**Gas component (%)**					
	CH_4	**CO_2**	**O_2**	**H_2S**	**CO**	**Balance**
1	17.4	24.4	2.8	3	1	51.4
2	15.4	23.3	4.5	0	2	56.8
3	16.7	23.4	5.4	1	1	52.5
4	15.1	19.1	9.1	0	1	56.7

It should be mentioned that the percentages shown in Table 4-10 were not stable for a long time. For example, the percent of hydrogen sulfide dropped over time, which is probably due to the consumption of sulfate by SRB. As a result, the concentration of H_2S could have been higher at the beginning of the column tests.

To further investigate the activity of SRBs, soil samples were taken from the anoxic zones of columns to conduct microbe tests and study the microbial community of the samples.

4.5 The Results of Microbe Tests

The microbial communities of the column reactors were analyzed. Figure 4-21 shows the results. Bacteria comprise 99% and archaea comprise only 1% of the microorganisms. Among the bacteria, proteobacteria which is a major phylum of gram-negative bacteria, was observed as the highest population in all columns. Among the classes of proteobacteria, Gammaproteobacteria had the highest percentage. The order of the majority of gammaproteobacteria was Methylococcales, which are methanotrophs. Since we had collected the sample from the cover soil of Arlington landfill, the presence of methanotrophs was expected. However, our samples were exposed to the anaerobic environment in the bottom of column reactors, so the population of methanotrophs might have been decreased due to DNA biodegradation.

Alphaproteobacteria, which are aerobic anoxygenic phototrophic bacteria, had the second highest population of proteobacteria phylum and Betaproteobacteria had the third highest population. Since Betaproteobacteria can use nitrate as a terminal electron acceptor, their highest population in column #2 was expected.

Deltaproteobacteria had the third highest population from the proteobacteria phylum. In the class of Deltaproteobacteria, Desulfarculales order, which is sulfate-reducing bacteria, were observed. Desulfobacterales and Desulfovibrionales are orders of sulfate-reducing bacteria that existed in our samples in column #1, 3, and 4 (0.35%), and column #2 (0.092%).

Desulfuromonadales is also an order in the Proteobacteria phylum, and members of this order are capable of anaerobic respiration utilizing compounds such as sulfur, Mn(IV), Fe(III), and nitrate, as electron acceptors. Desulfuromonadales had the highest population in column #2

(0.055%), and traces of this order was also observed in other columns. Desulfuromonadales probably used nitrate in column #2 for anaerobic respiration.

As we expected, sulfate-reducing bacteria were observed in the samples. Since we tested the microbial community of the samples after 9 months, DNA of some bacteria and archaea might have been biodegraded by other microorganisms. This could be the reason for the low population of these bacteria. As mentioned before, the concentration of H_2S gas declined in the headspace of the columns, which can be due to a decrease in the population of some microorganisms like sulfate-reducing bacteria.

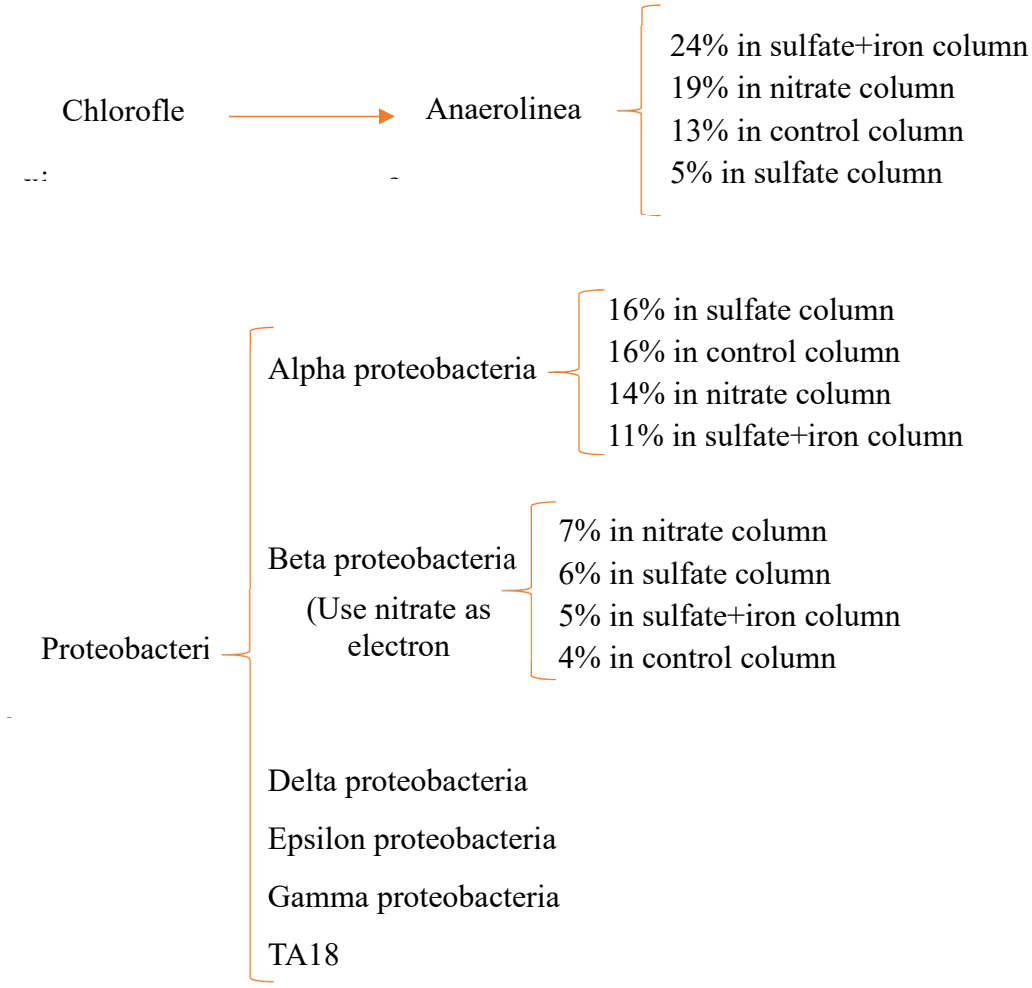


Figure 4-21 Microbial population in the soil samples from the column reactors

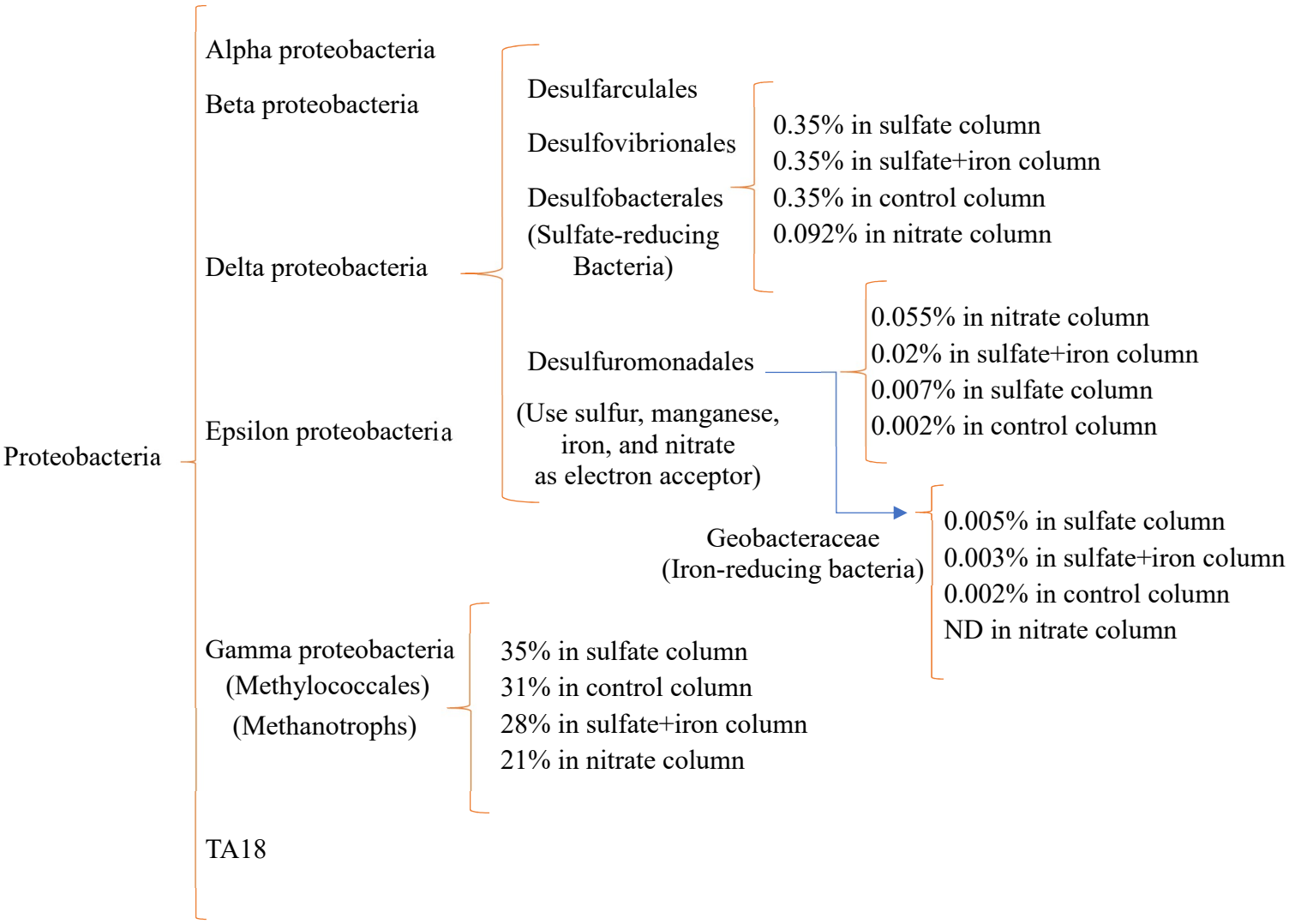


Figure 4-21 (continued) Microbial population in the soil samples from the column reactors

Chapter 5

Conclusions and Suggestions for Future Studies

5.1 Conclusions

Batch tests results showed that sulfate (2.72 mg Na_2SO_4/g soil), nitrate (1.64 mg $NaNO_3$/g soil), and a combination of sulfate (3.28mg Na_2SO_4/g soil)+iron (1.84 mg Fe_2O_3/g soil) could remove more methane compared to the control test over the long-term and these reactors also had higher maximum oxidation rate. Moreover, according to the batch tests, methane removal decreased in the reactors with no added nutrients, lower moisture content, and low initial concentration of methane. The results also showed that adding inhibitor increased methane removal in some reactors while it lowered AOM in other reactors.

The results of column tests showed that at a higher landfill gas flow rate, there was no significant difference in methane removal in the anoxic zone of the columns; however, at a lower flow rate, methane removal in the column amended with sulfate + iron had the highest (around 10%) removal of methane in the anoxic zone, followed by the column that contained sulfate. The results showed H_2S gas at the headspace of these two columns, which indicated that sulfate-reducing bacteria were likely responsible for methane removal in the anoxic zone of the columns.

5.2 Suggestions for future studies

The measurement of the Oxidation Reduction Potential (ORP) of the samples is suggested to have a better understanding of the tendency of different samples to accept electron, since a

sample with higher ORP will have higher tendency to obtain electron while oxidizing other species.

Our goal was to find an electron acceptor that promotes AOM in the cover soil, and it was shown that adding electron acceptor like iron and sulfate can stimulate anaerobic oxidation of methane in the soil. However, sulfate-dependent AOM also produced hydrogen sulfide gas on the surface of the cover soil sample. In the future, we can evaluate the ways to remove H_2S gas in the cover amended with iron and sulfate.

At the next step, it is suggested to find wastes that are high in sulfate and iron content and evaluate the AOM in the mixture of soil and wastes. We used chemicals as the source of electron acceptors in our study, however, chemicals are expensive to be added to the landfill soil. If we can find a waste that promotes anaerobic oxidation of methane, it would help not only to save energy (by adding no chemicals) but also to reduce the volume of the landfills that is used for landfilled waste.

Column tests simulate gas transfer through the landfill cover soil and there is a continuous flow of LFG. Though column tests are a better model of a landfill cover comparing to the batch tests, they have some limitations compared to the real field measurements. For example, there is no simulation of different temperatures, rainfalls, wind, and other climate conditions in the column tests. Some column tests are conducted outdoors to simulate the climatic condition, but even in those tests, lateral LFG transfer is not modeled. Therefore, the next step in this research could be the field measurements. After finding the waste that promotes AOM in the cover, the waste could be mixed with the soil and be used as landfill cover. Filed measurements for a couple years from the proposed cover will be a good representation of the effect of waste on

AOM over the long-term and consequently total methane oxidation (aerobic and anaerobic) in the landfill cover.

In our study, we quantified the rate of anaerobic oxidation of methane by measuring the concentration of methane. What we obtained in our research was, in fact, the net anaerobic oxidation rate. For example, if methane generation was higher than the oxidation rate in one of the samples, no AOM was observed. Since methane generation and oxidation happen simultaneously in an anaerobic environment, it is not possible to quantify the gross AOM rate. Therefore, it is suggested to evaluate the microbial carbon flow. If we inject ^{13}C-enriched CH_4 (instead of LFG) into the batch reactors and measure the concentration of ^{13}C-enriched CH_4 over time, we can find the gross AOM, since methanogens produce mostly $^{12}CH_4$ and $^{12}CO_2$ (Miller et al., 2019). So, it would be possible to differentiate between the methane that has been generated by methanogens and the methane that was injected into the reactors, to calculate gross AOM. Moreover, during the column tests, real methane consumption by AOM could be evaluated by measuring ^{13}C-enriched CH_4 in the anoxic section of the columns. If ^{13}C-enriched CH_4 enter the column from the bottom, and we measure the concentration of ^{13}C-enriched CH_4 at the end of the anoxic zone, we can measure gross AOM, and clarify how much methane was removed in the cover due to AOM.

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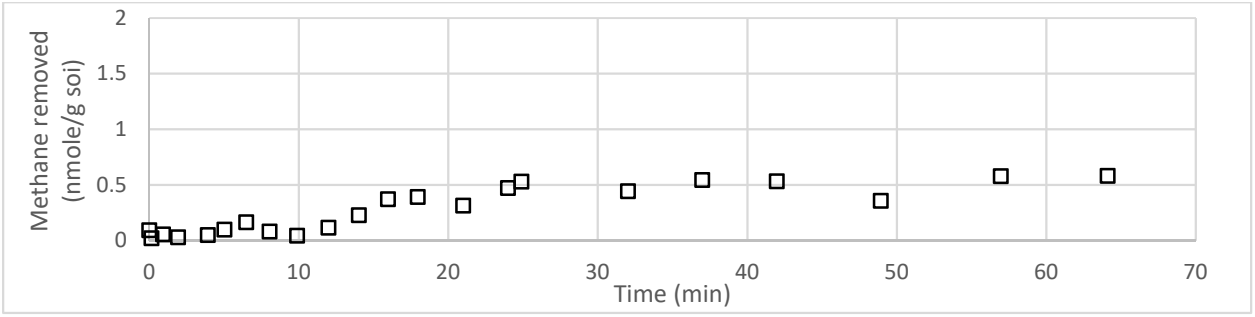
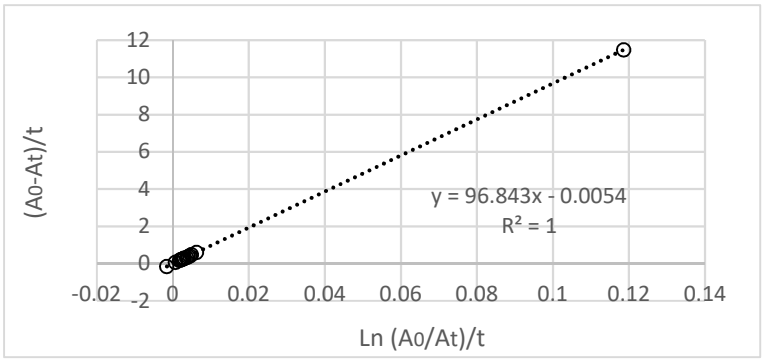
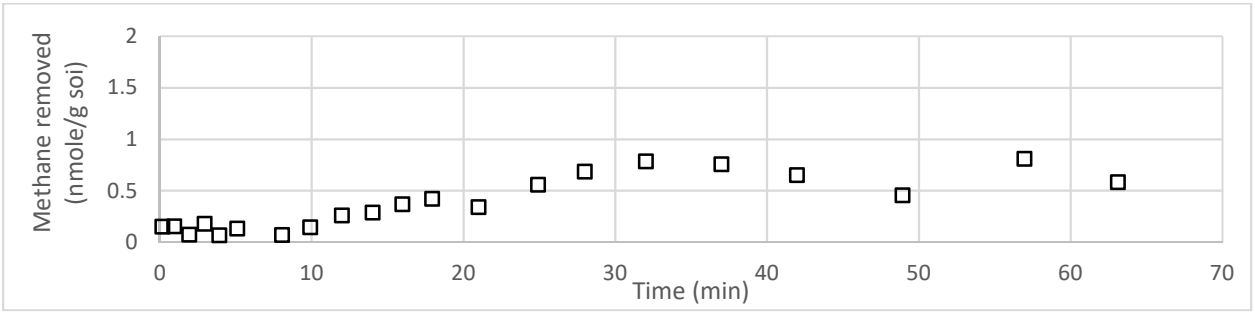
Appendix

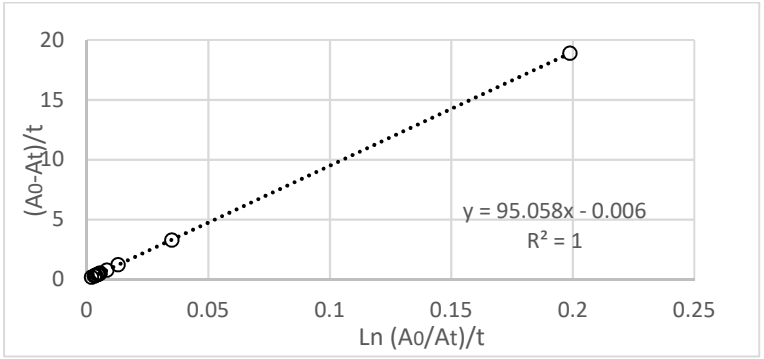
Raw Data Obtained from Batch Reactors



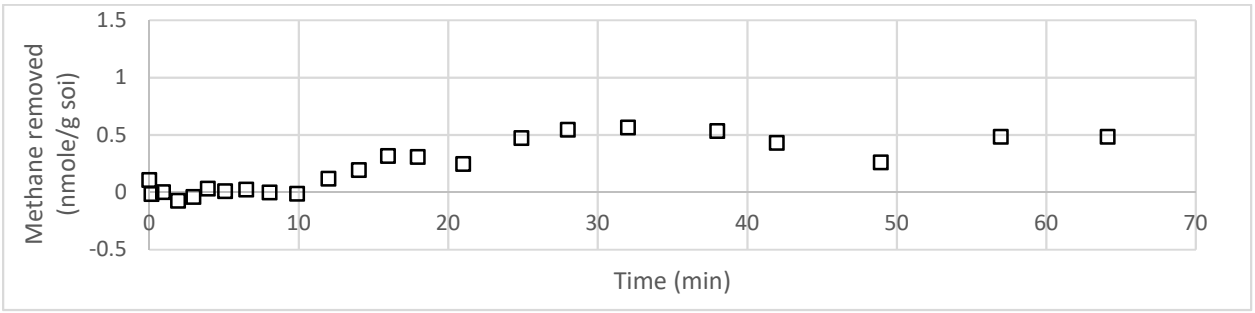


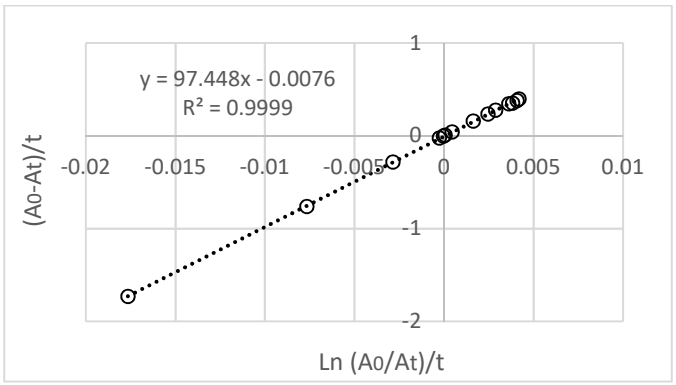
Reactor #1, Soil



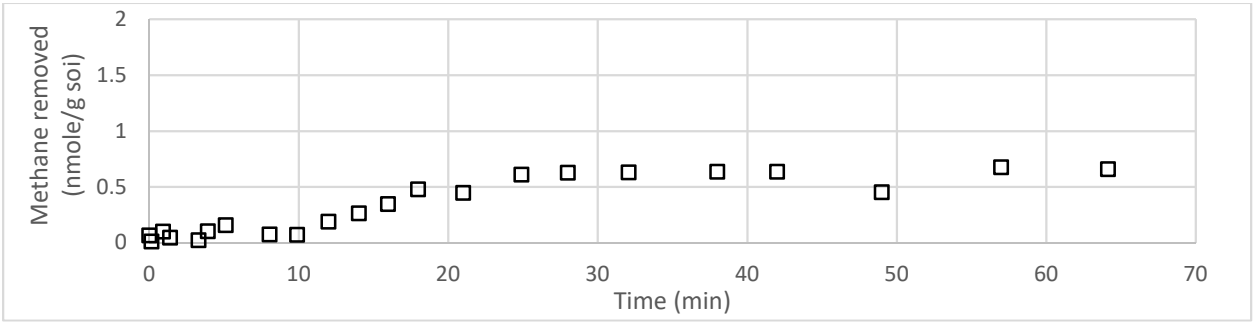


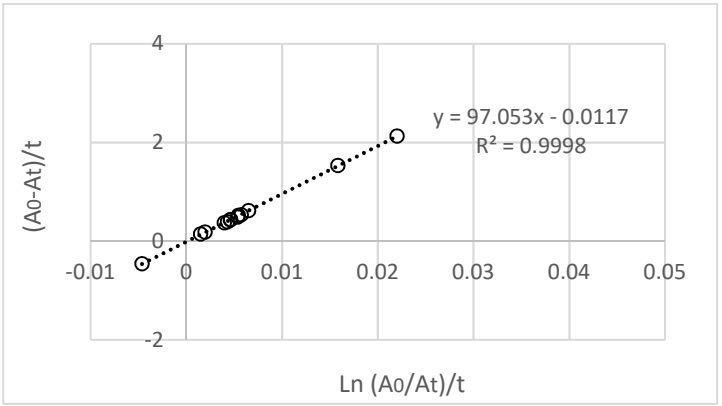
Reactor #2, Soil + 1.26 g Red Mud



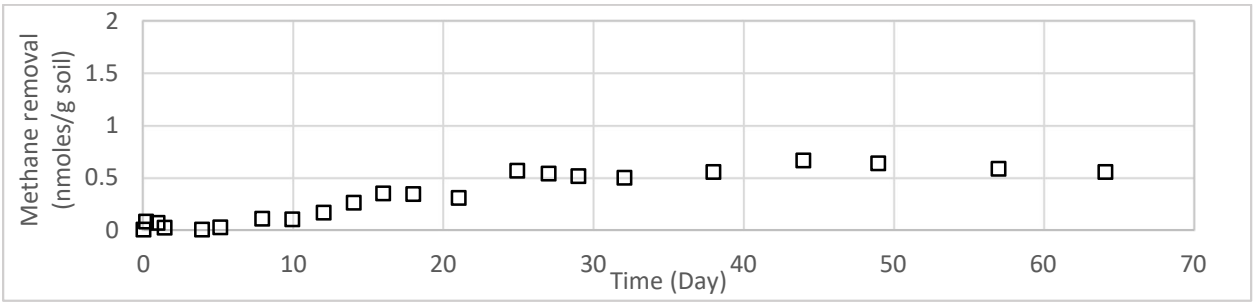


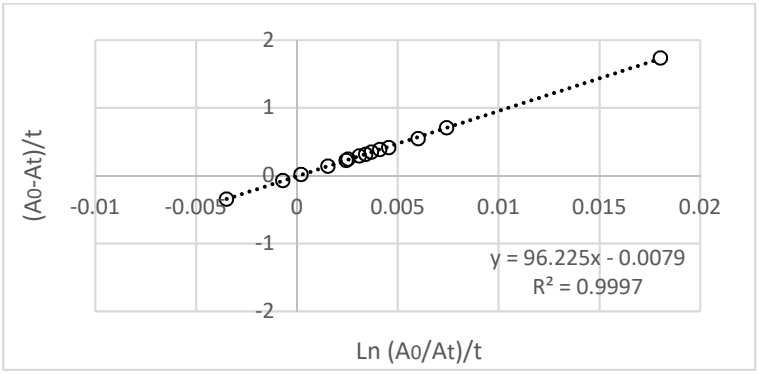
Reactor #3, Soil + 0.57 g Na_2SO_4



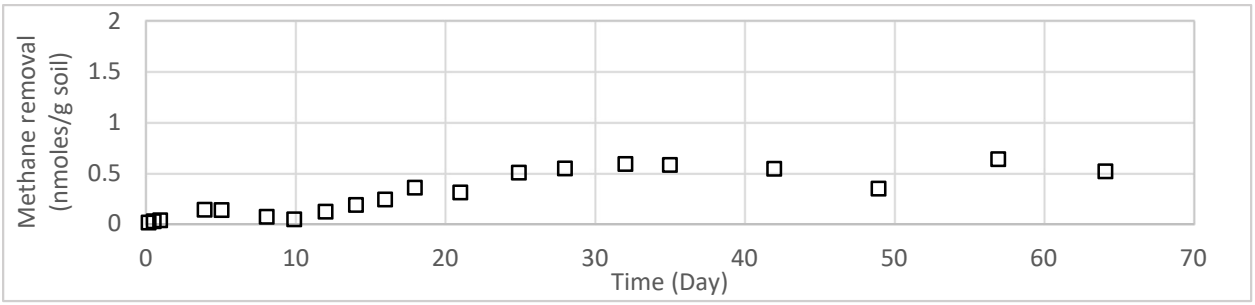


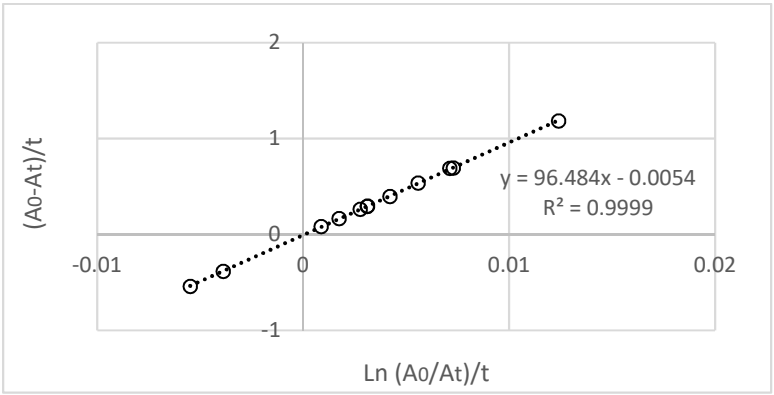
Reactor #4, Soil + 0.34 g $NaNO_3$



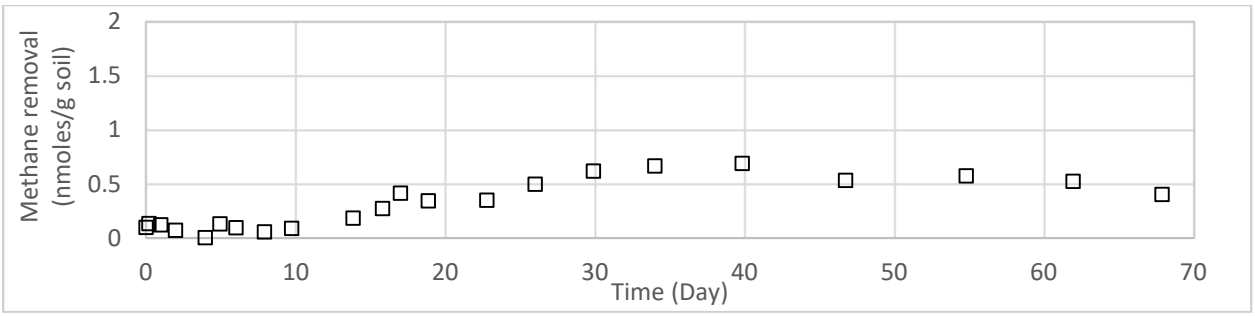


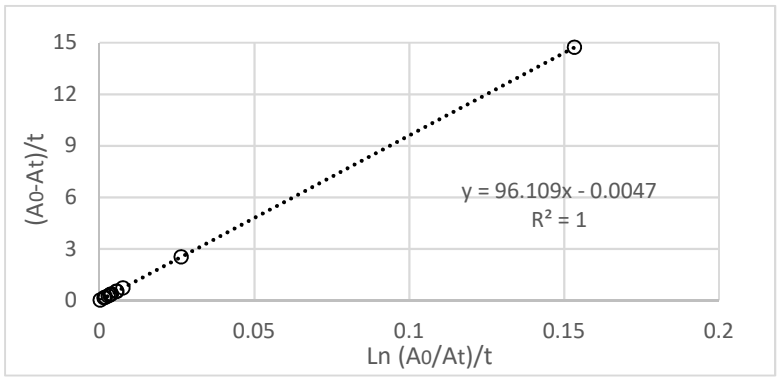
Reactor #6, Soil + BES



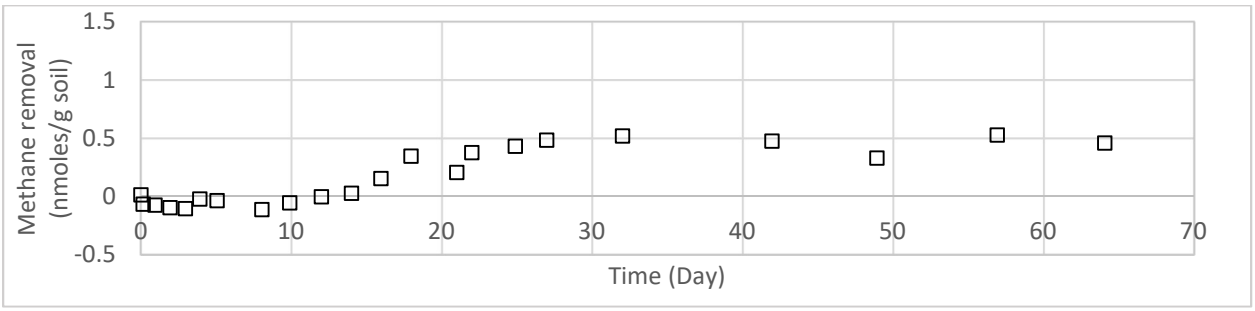


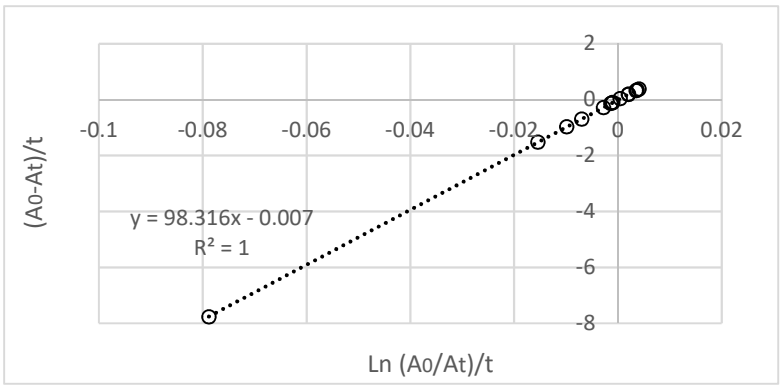
Reactor #7, Soil + 1.26 g Red Mud + BES



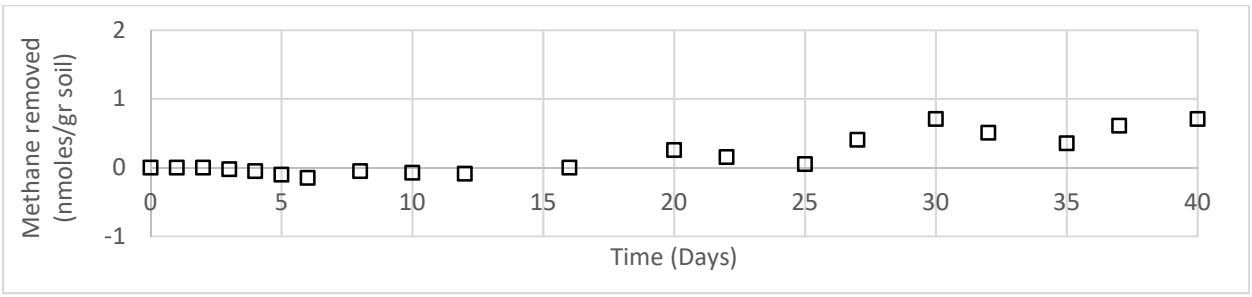


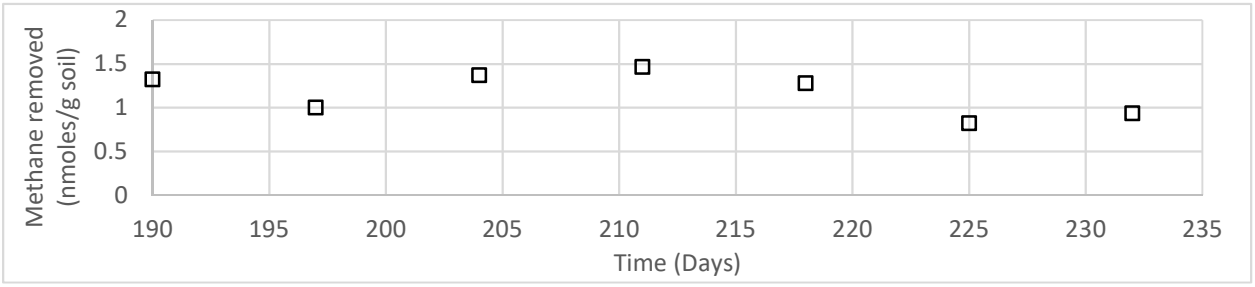
Reactor #8, Soil + 0.34 g $NaNO_3$ + BES

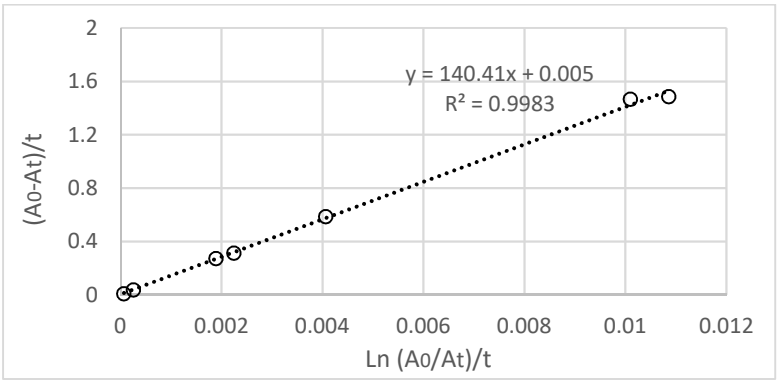




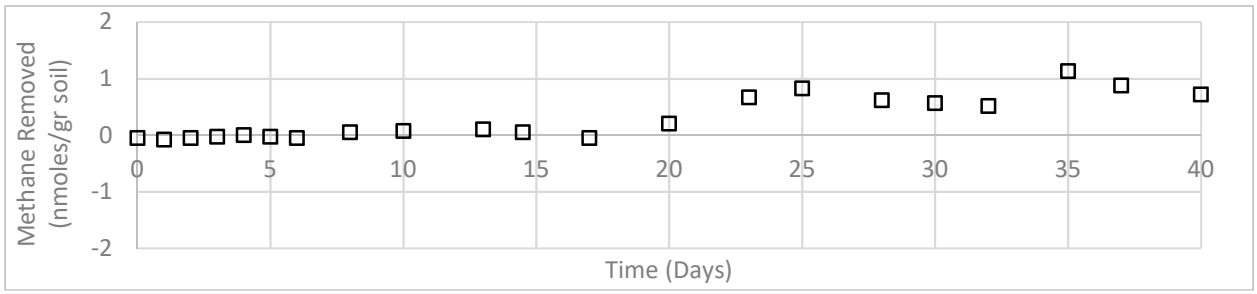
Reactor #9, Soil + 0.57 g Na_2SO_4 + BES

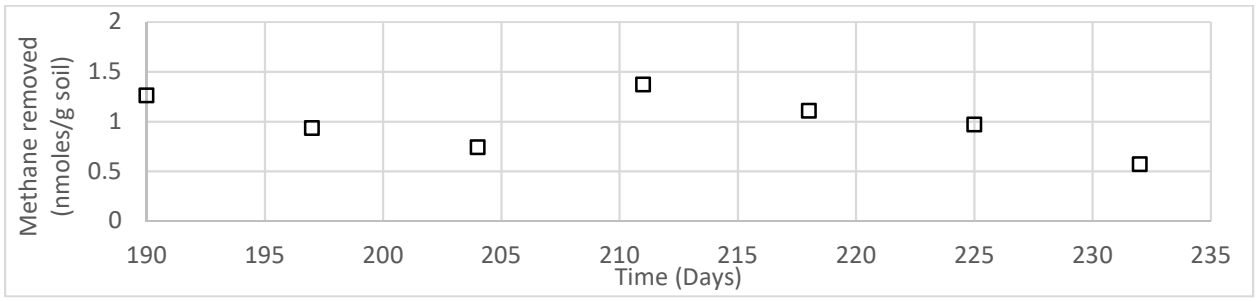


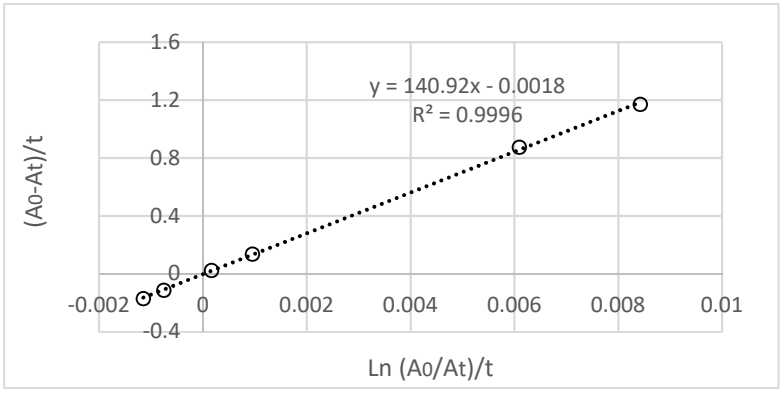




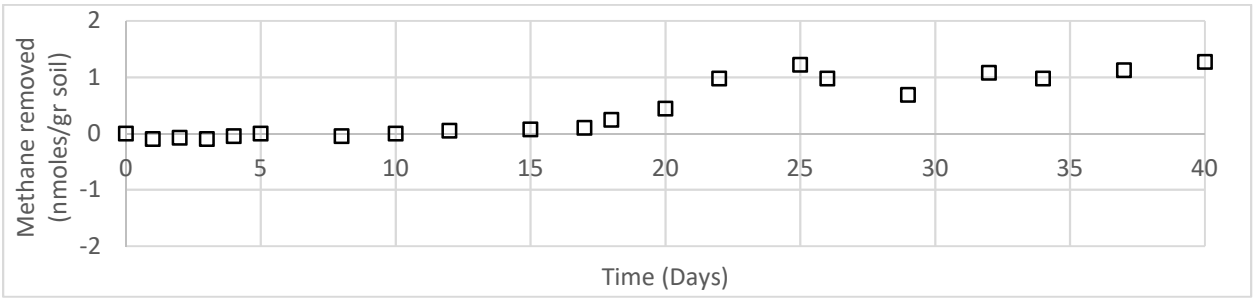
Reactor #11, Soil

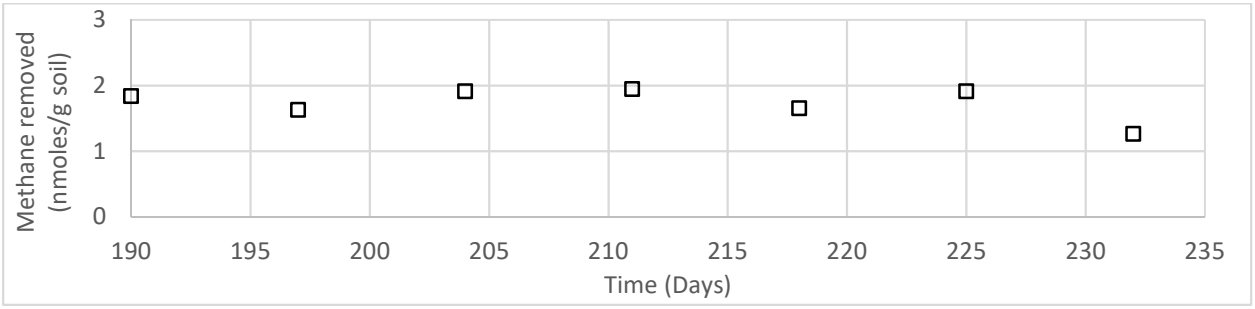


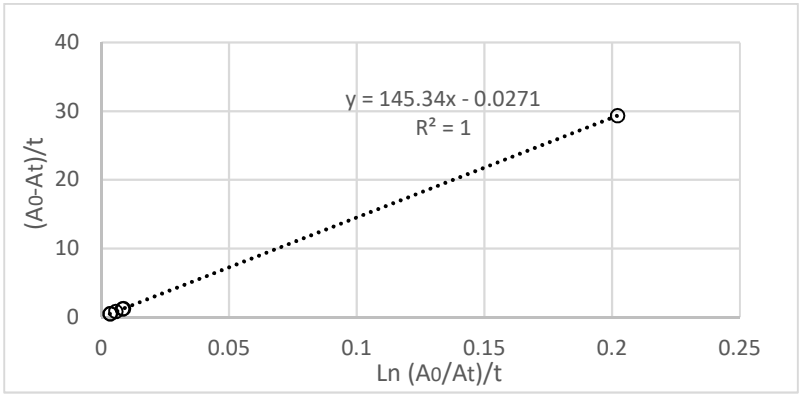




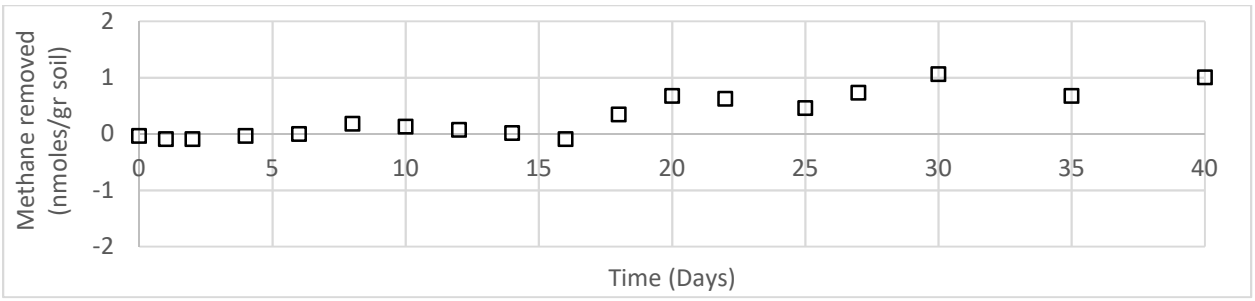
Reactor #12, Soil + 0.021 g $NaNO_3$

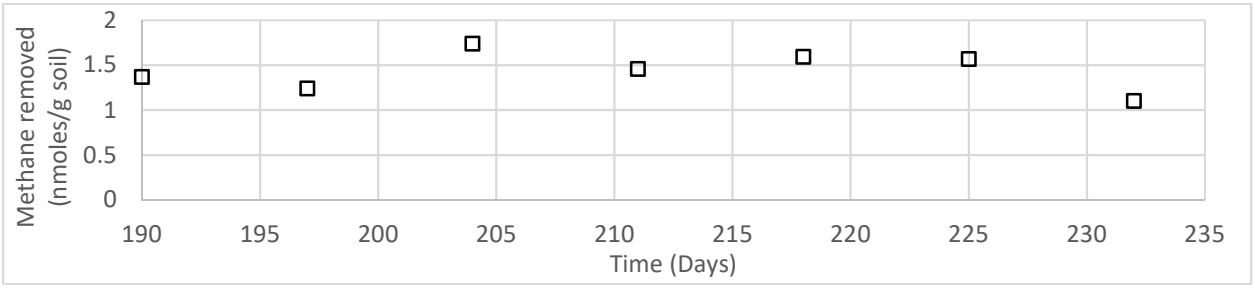


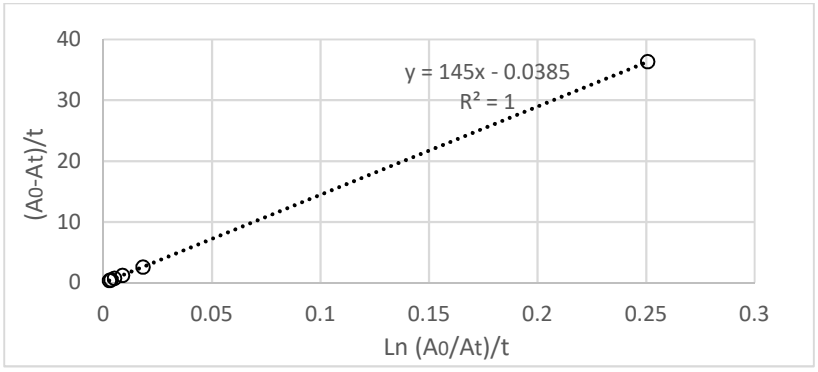




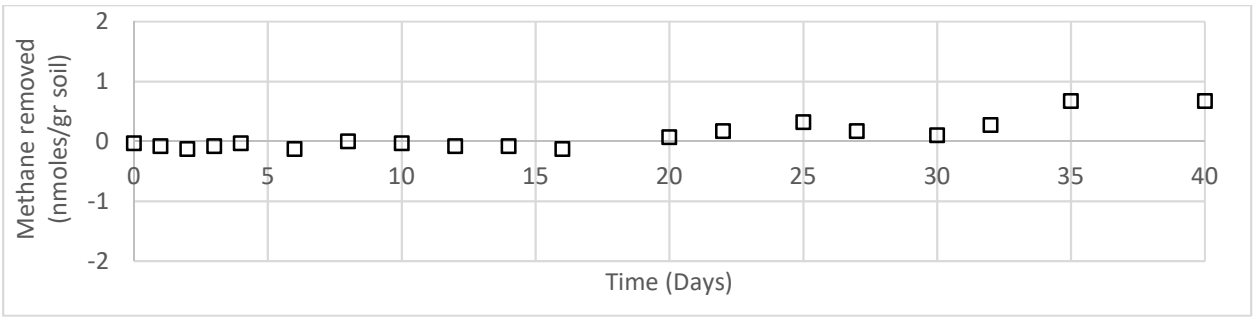
Reactor #13, Soil + 0.042 g $NaNO_3$

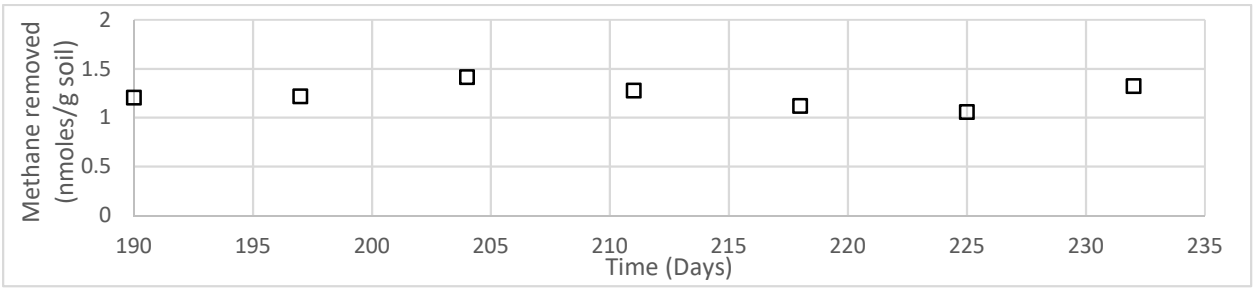


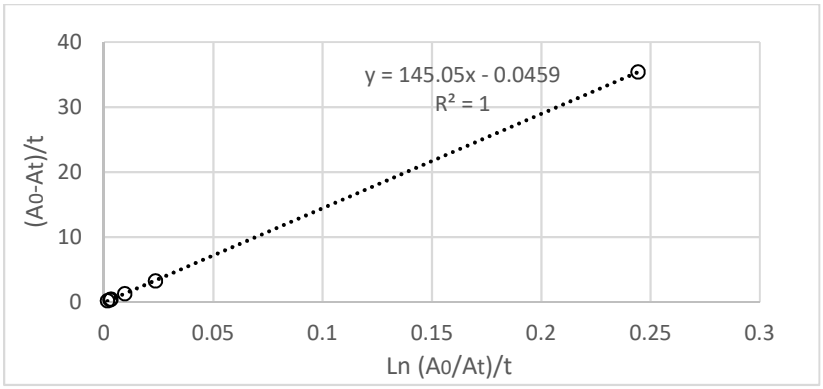




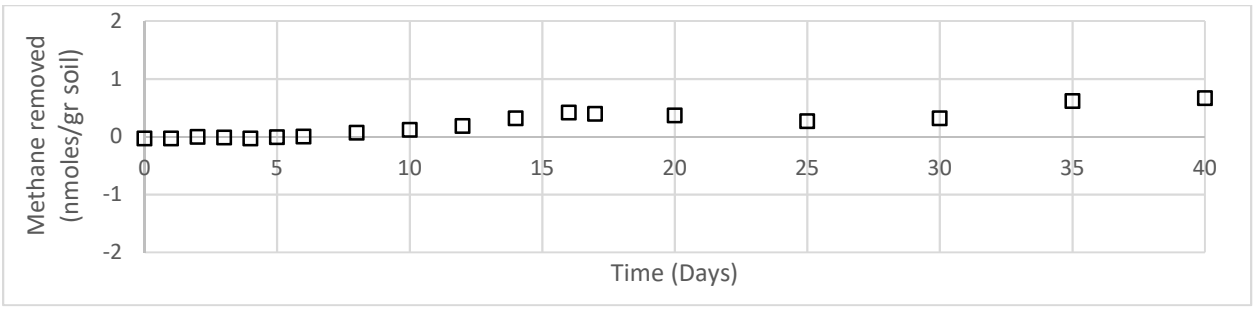
Reactor #14, Soil + 0.082 g Na_2SO_4 + 0.046 g Hematite

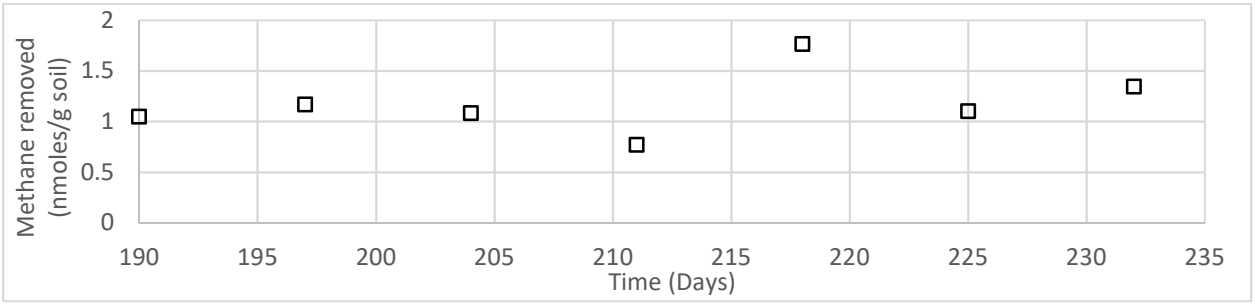


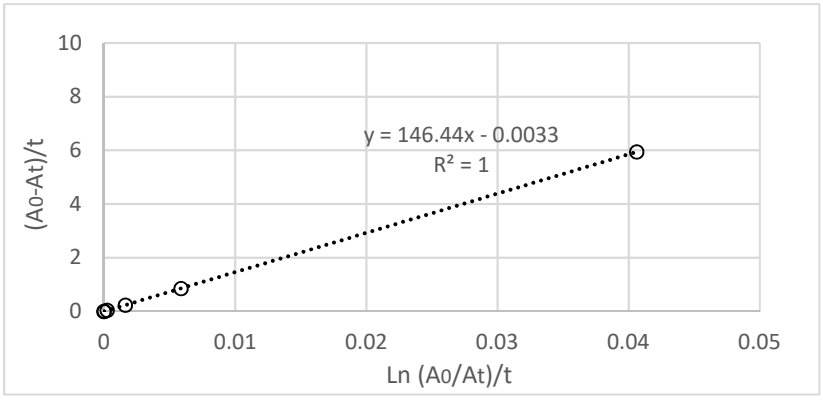




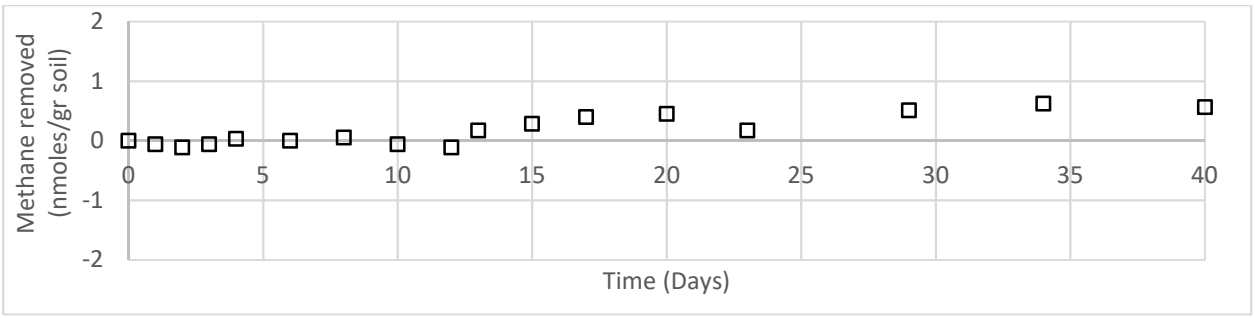
Reactor #15, Soil + 0.068g Na_2SO_4

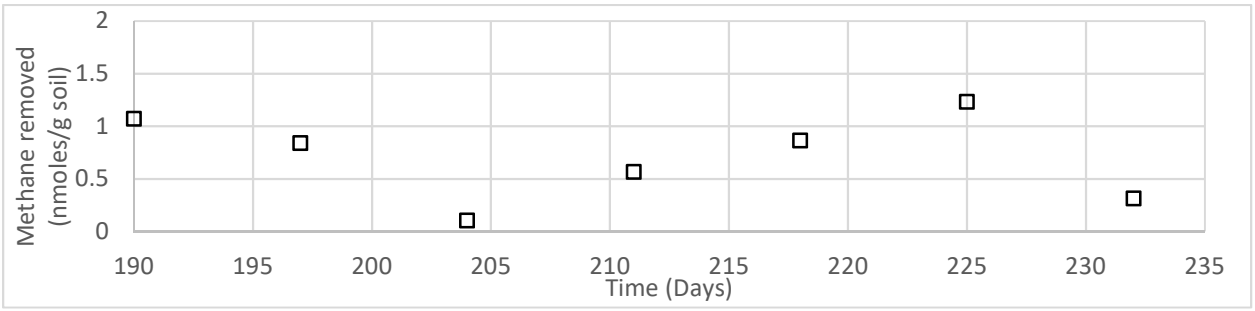


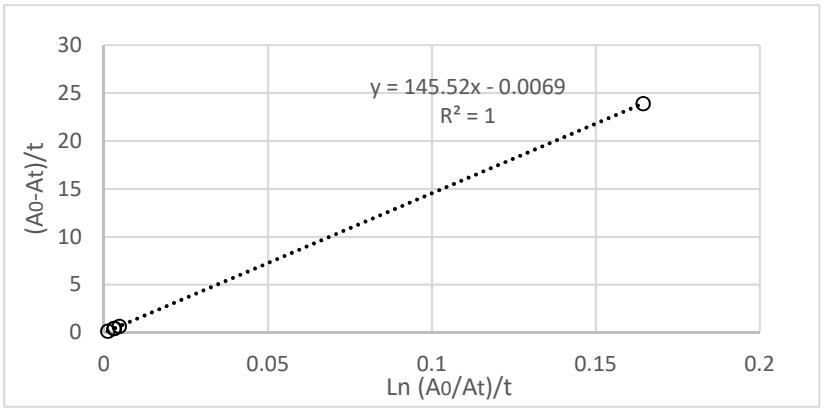




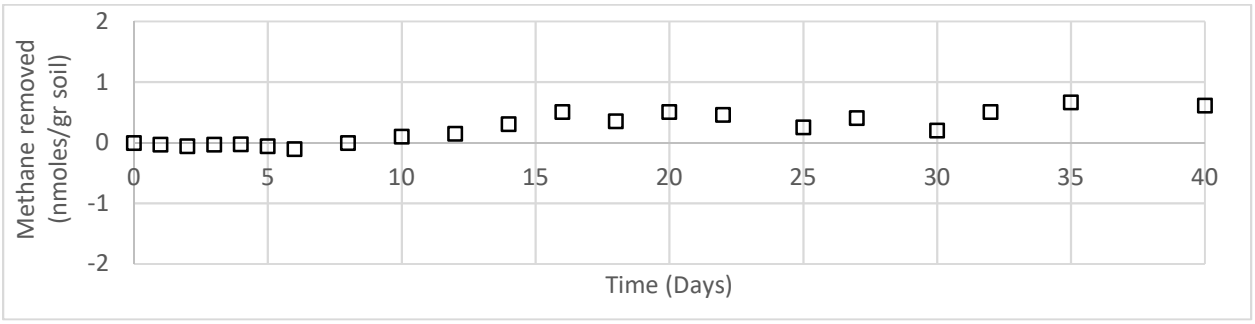
Reactor #16, Soil + 0.034g Na_2SO_4

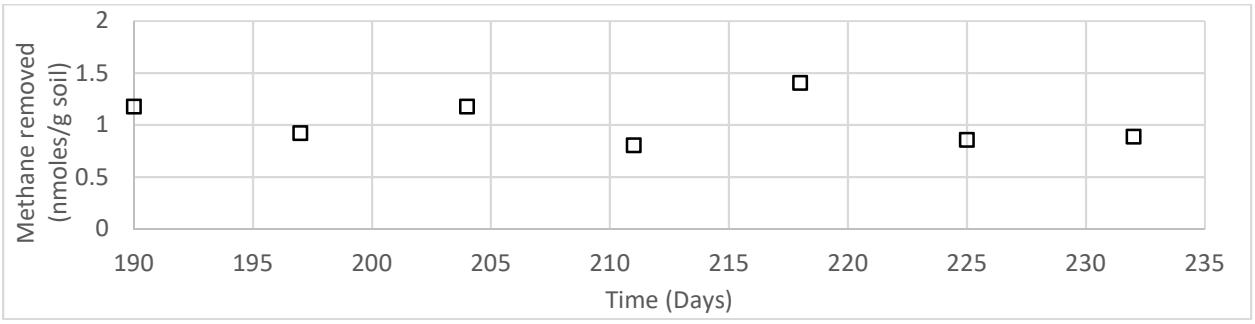


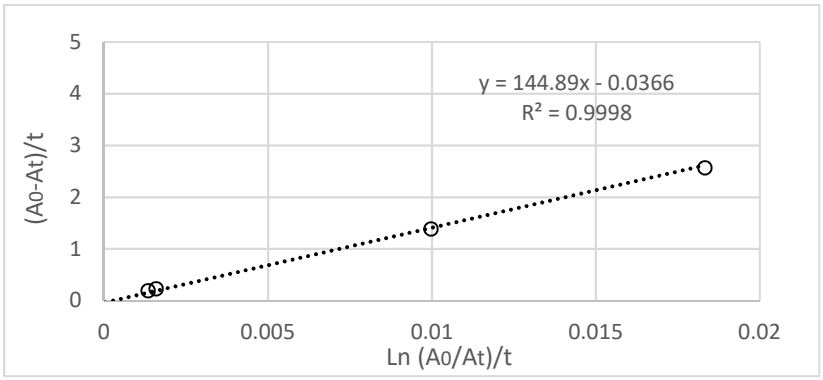




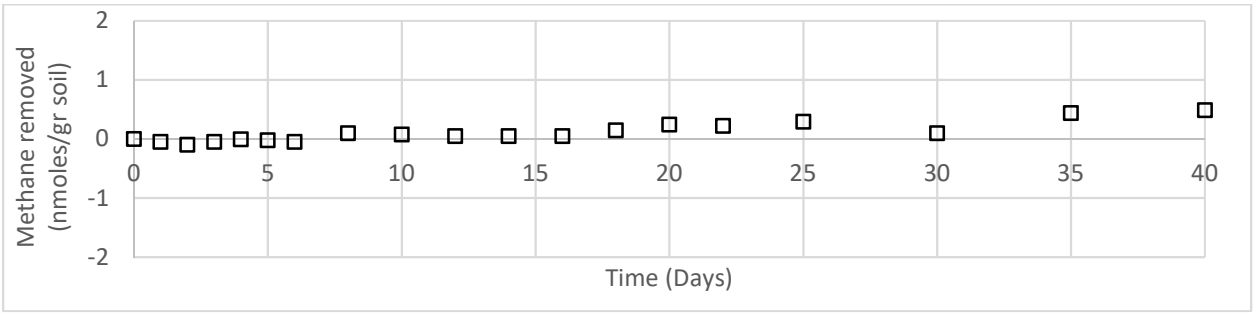
Reactor #17, Soil + 0.077g Hematite

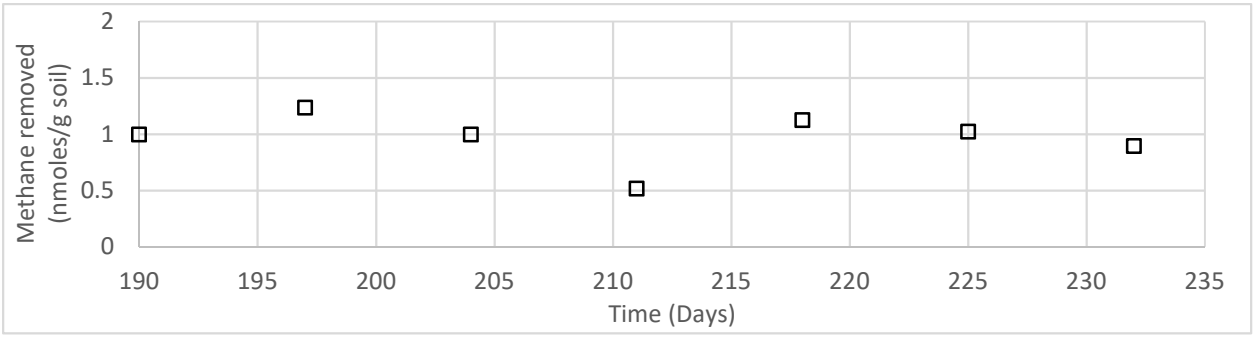


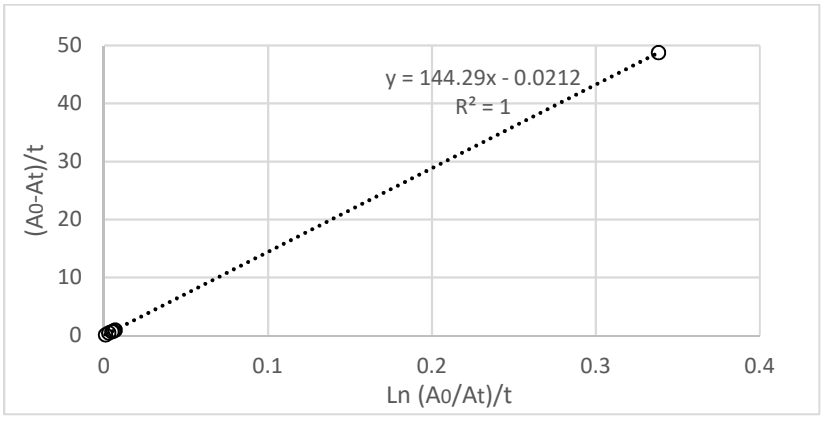




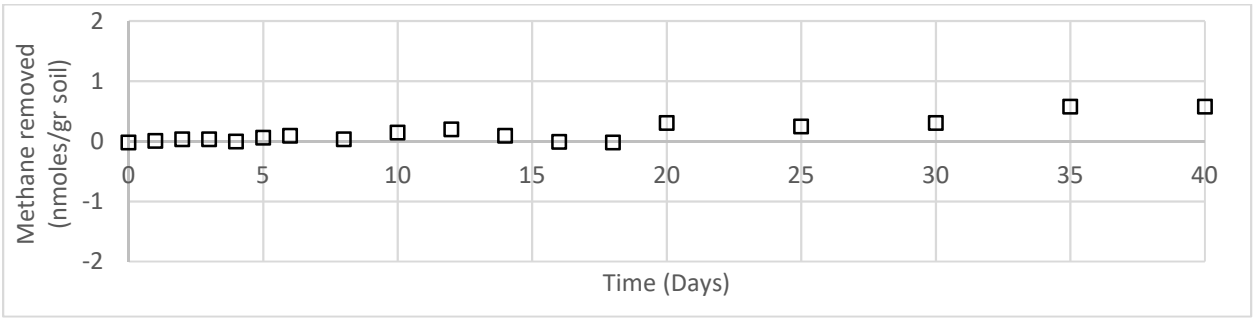
Reactor #18, Soil + 0.041g KNO_2

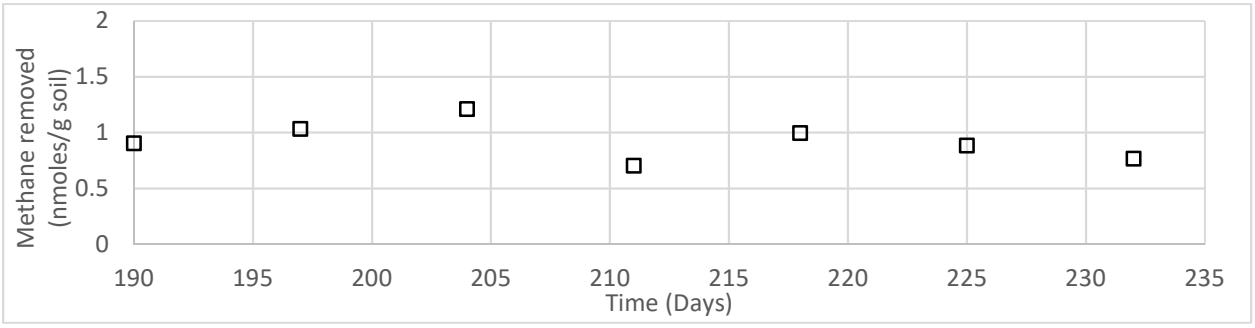


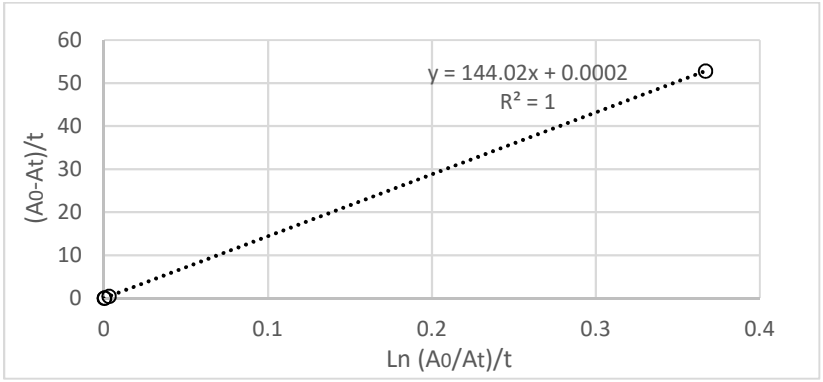




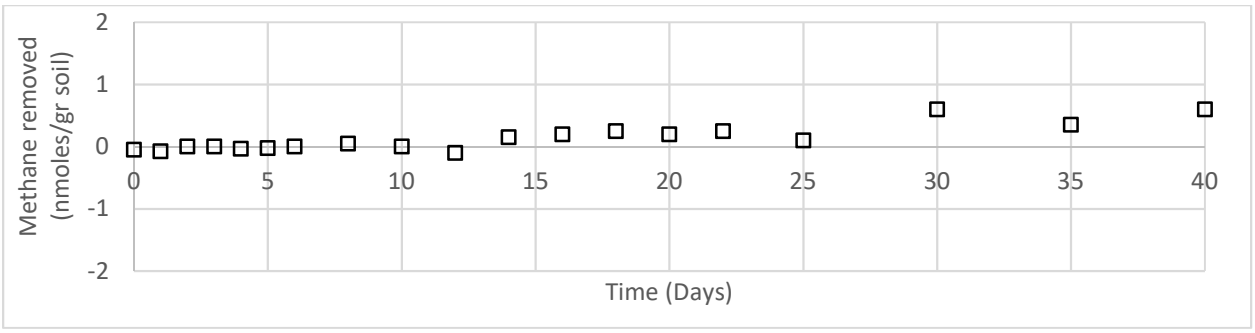
Reactor #19, Soil + 0.043g $Fe(OH)_3$

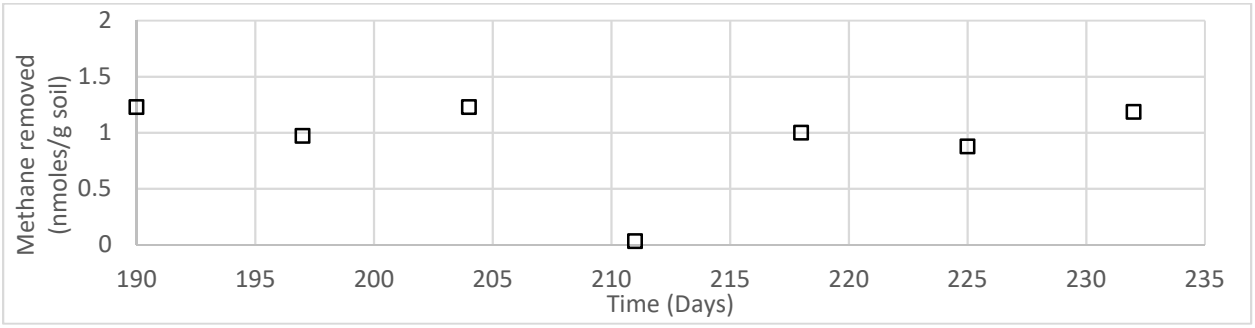


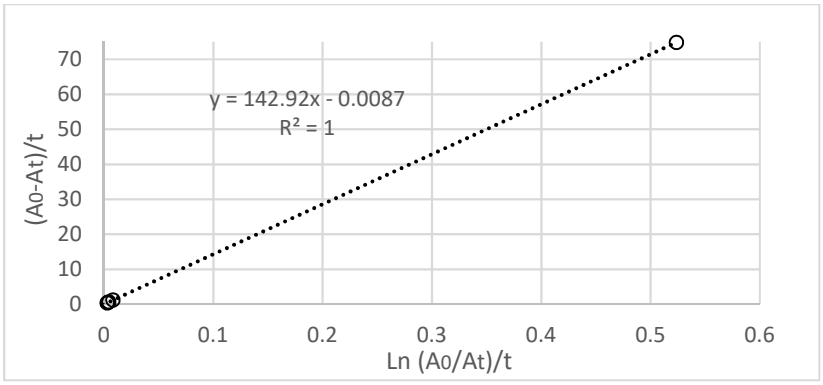




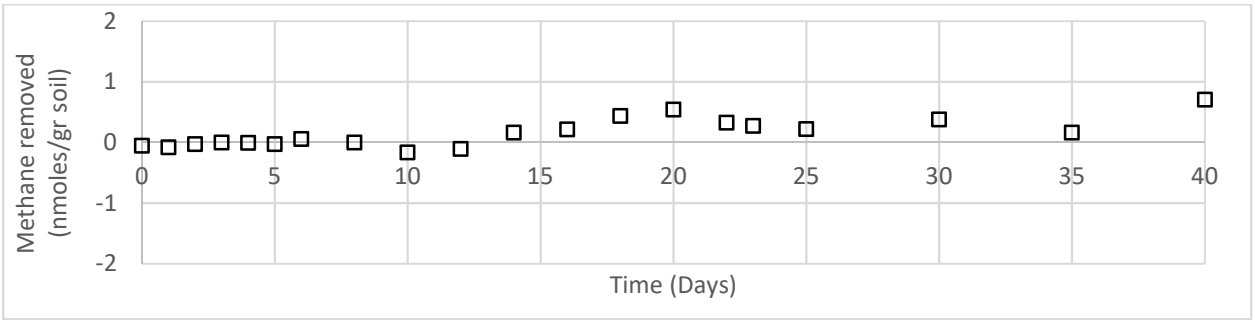
Reactor #20, Soil + 0.042 g MnO_2

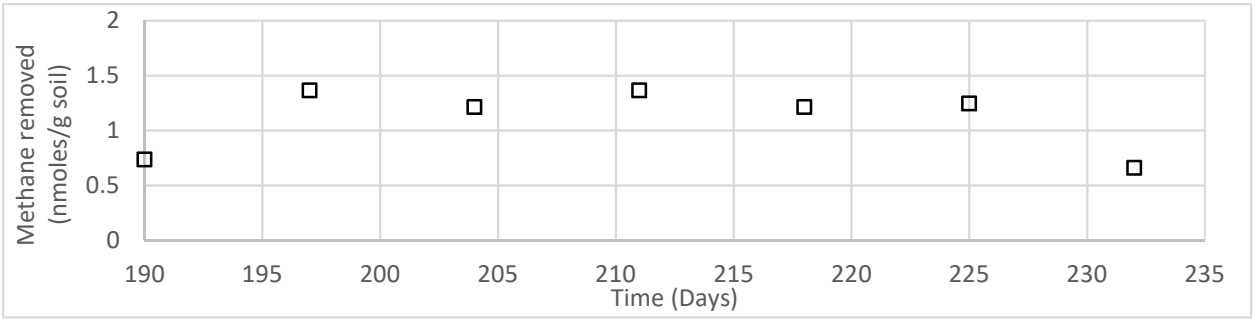


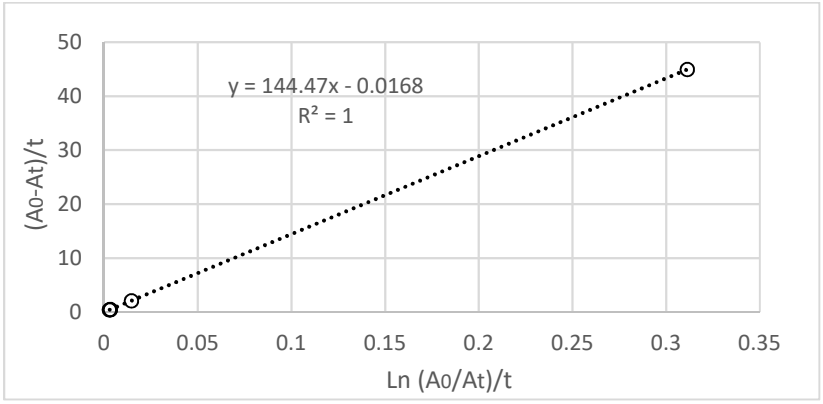




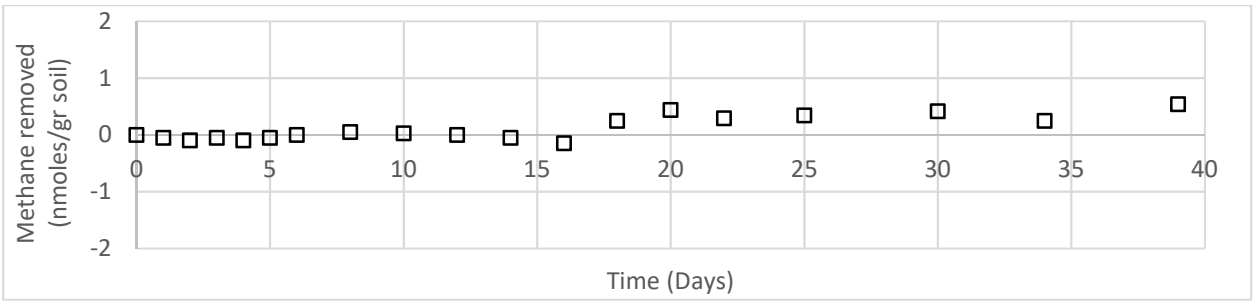
Reactor #21, Soil + 0.01g NH_4Cl

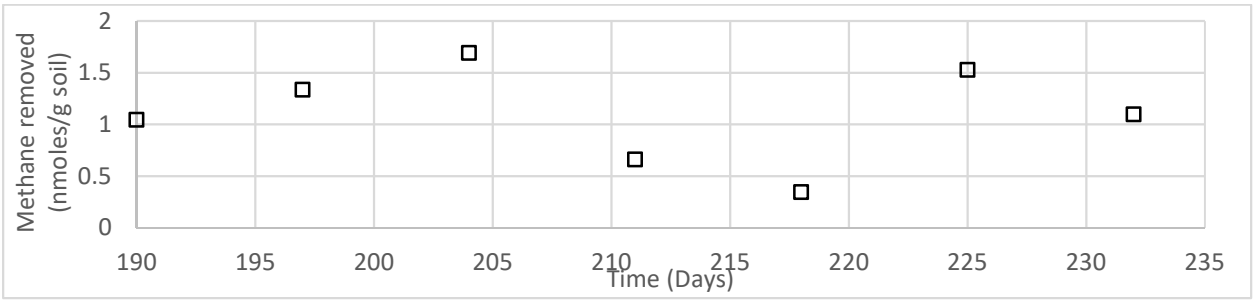


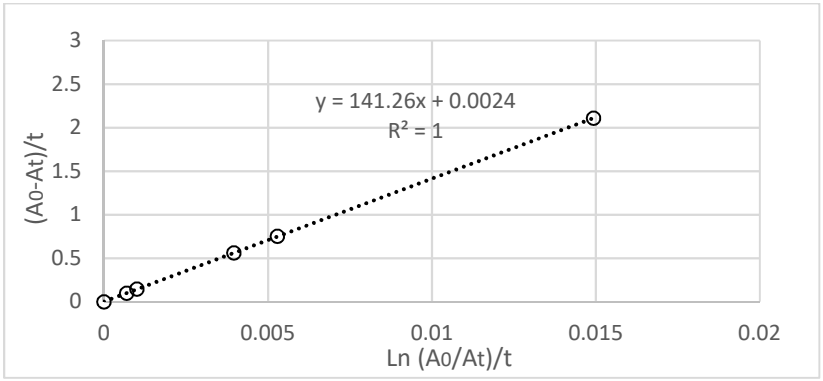




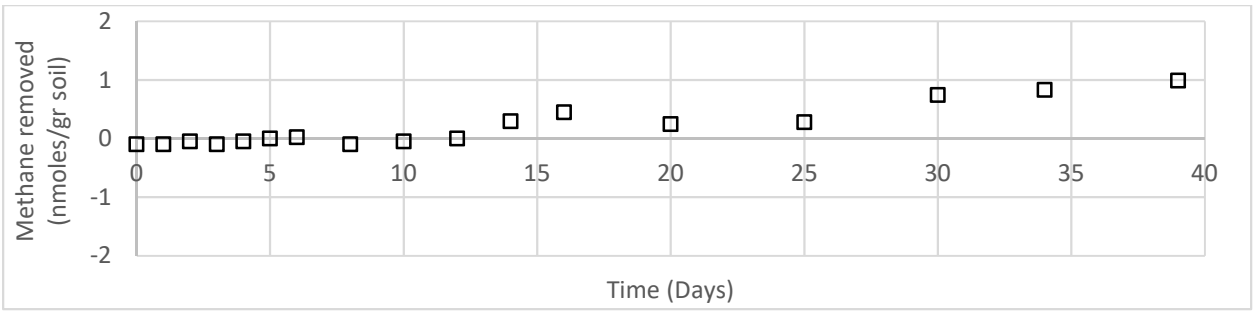
Reactor #22, Soil + 0.078 g $FeCl_3$

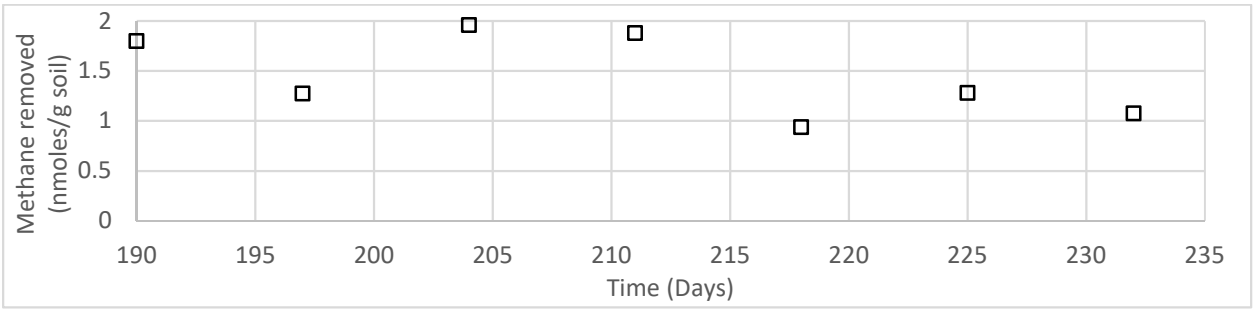


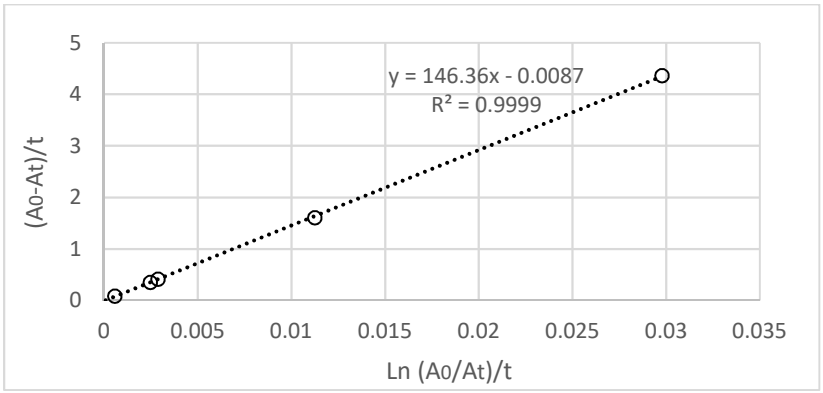




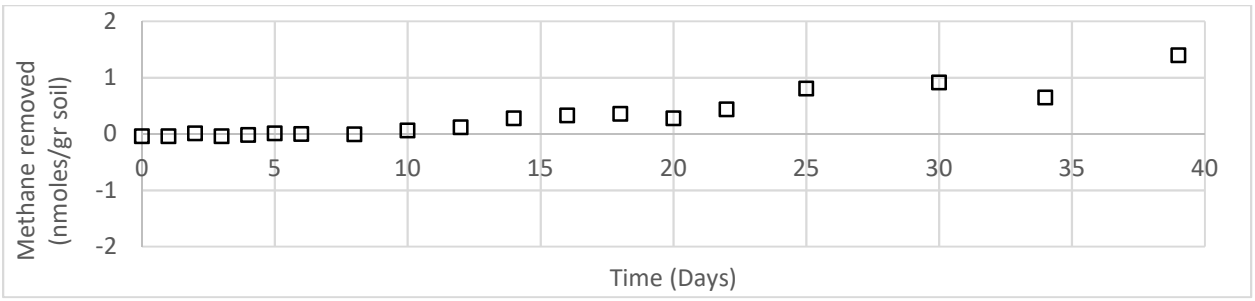
Reactor #23, Soil + RAMM + Inhibitor

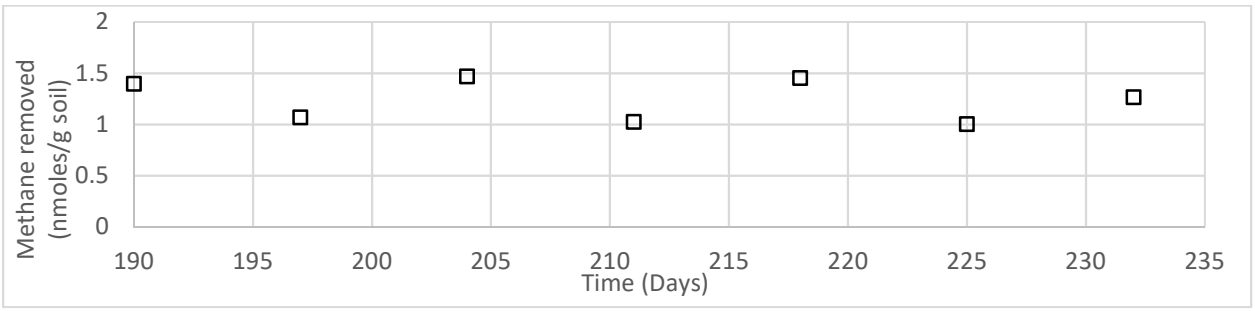


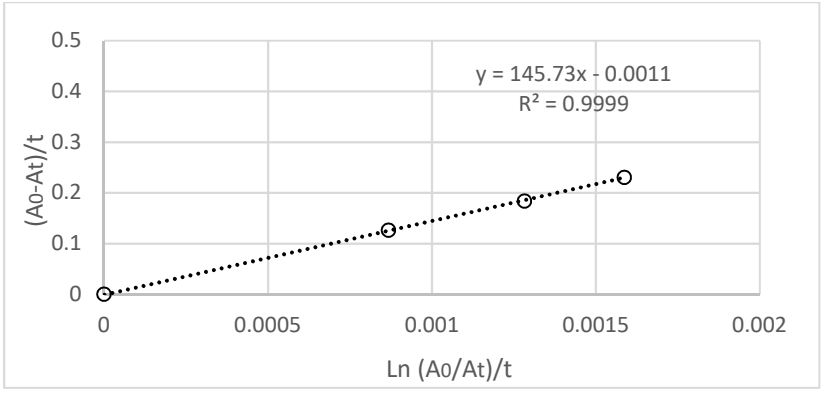




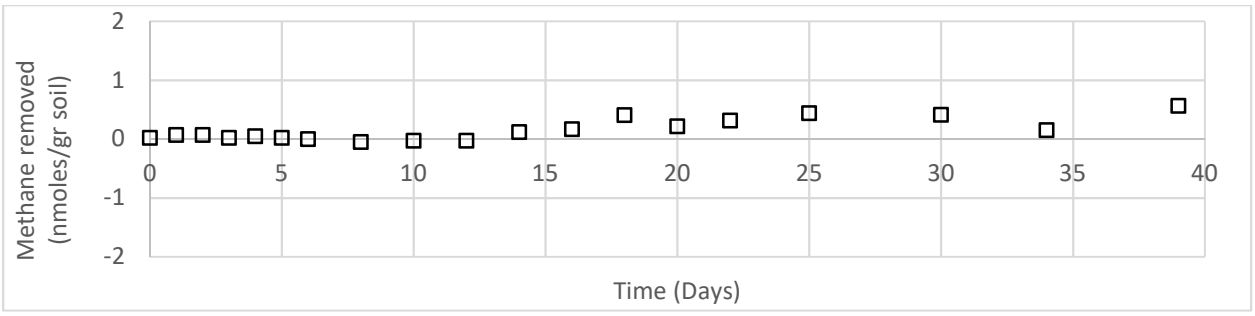
Reactor #24, Soil + RAMM +0.068g Na_2SO_4 + Inhibitor

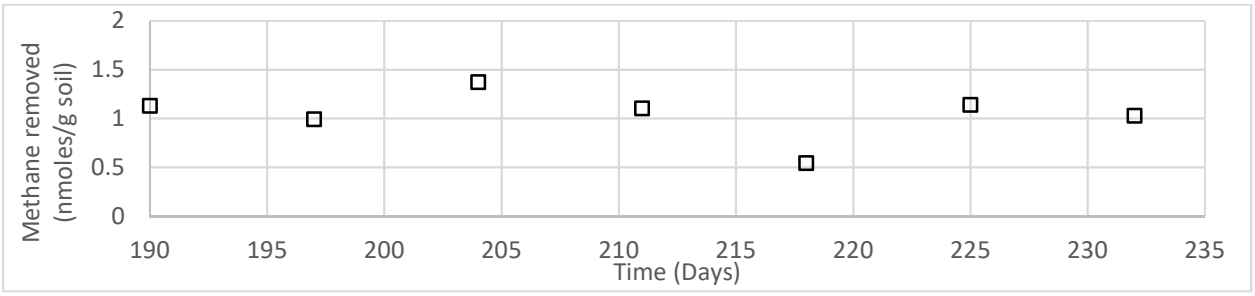


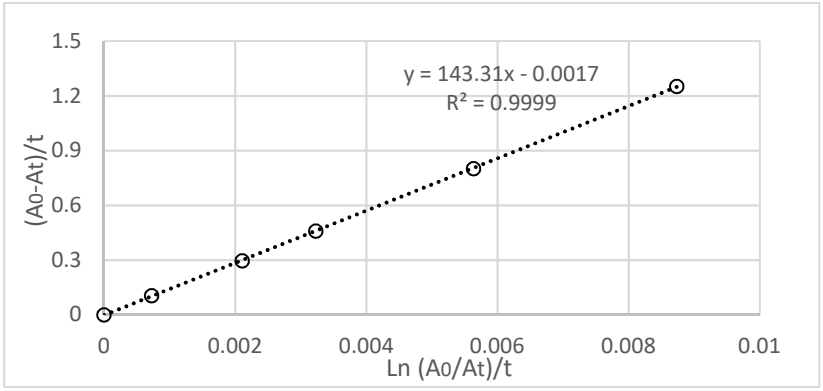




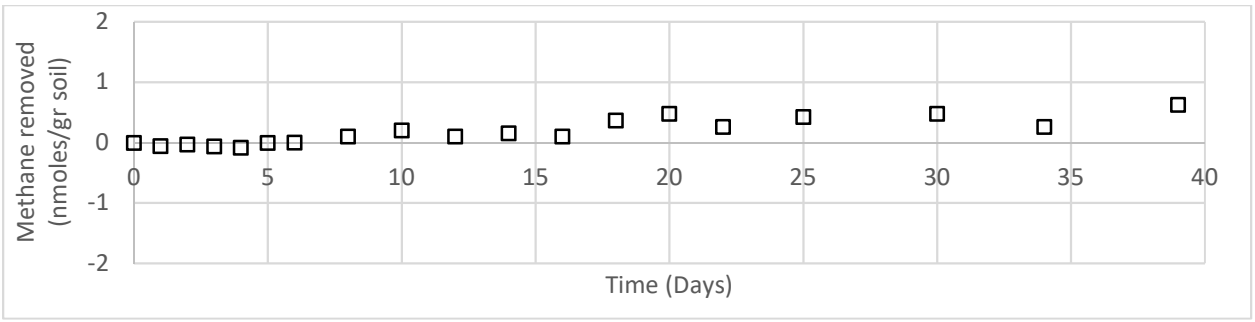
Reactor #25, Soil+RAMM+0.041g KNO_2 + Inhibitor

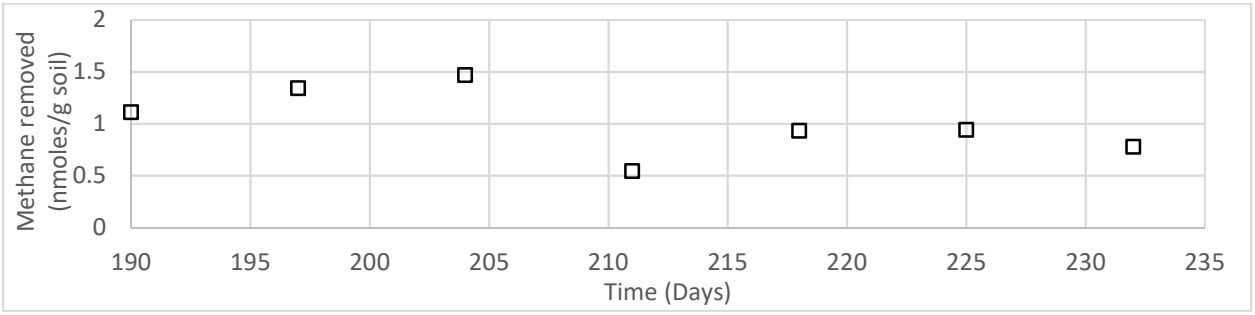


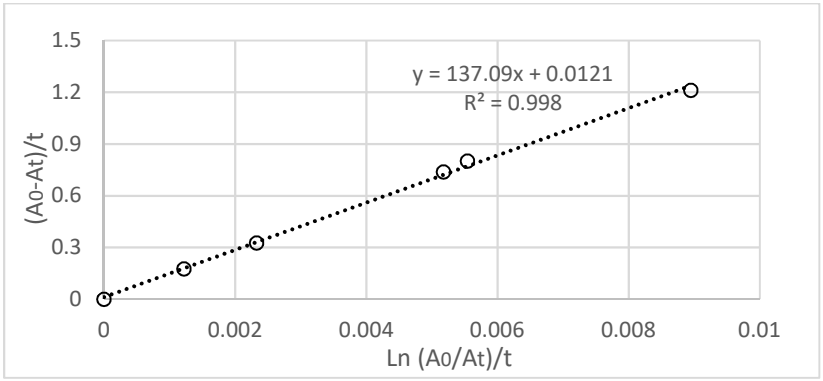




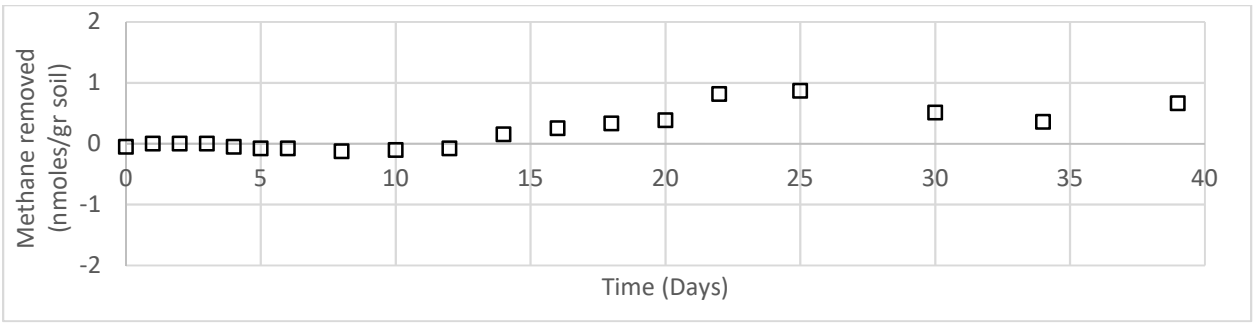
Reactor #26, Soil + RAMM+ 0.043g $Fe(OH)_3$ + Inhibitor

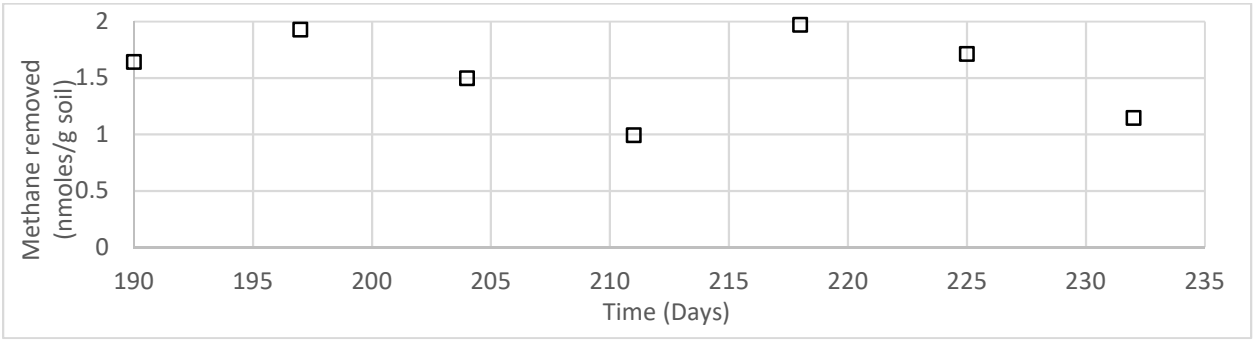


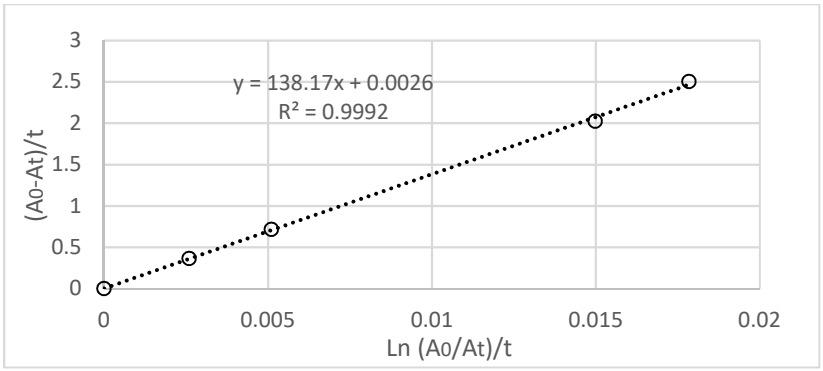




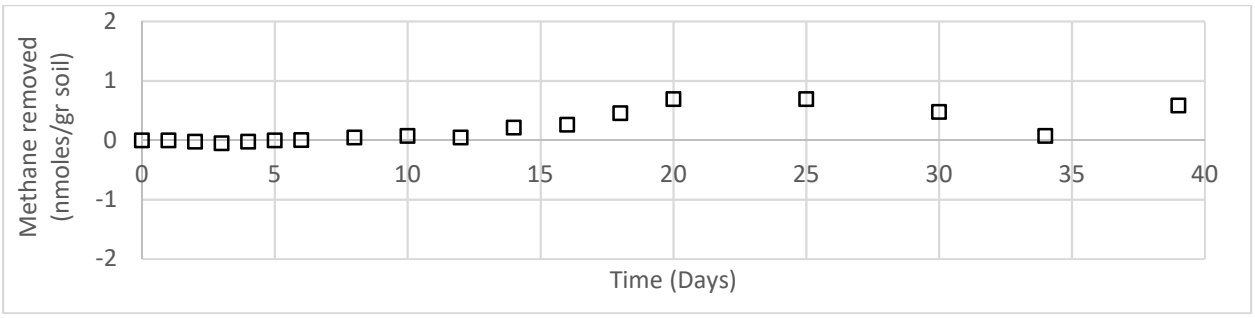
Reactor #27, Soil

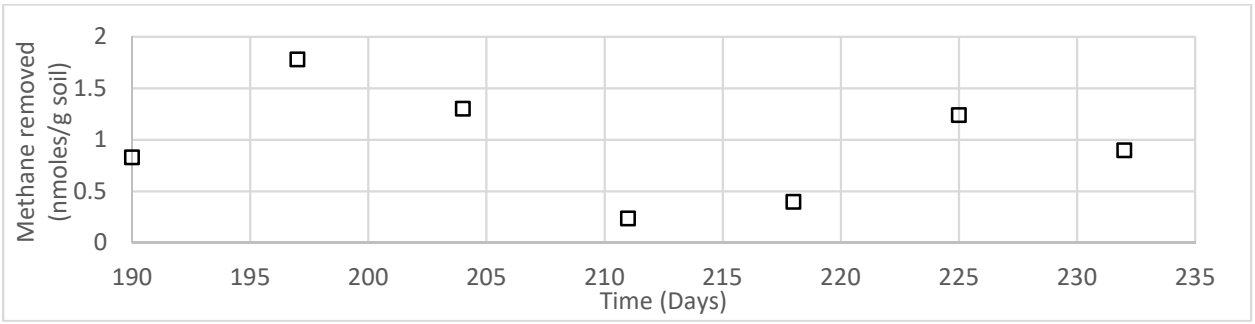


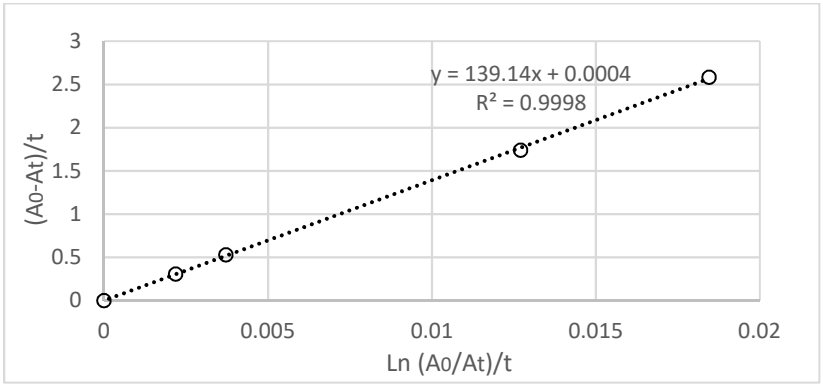




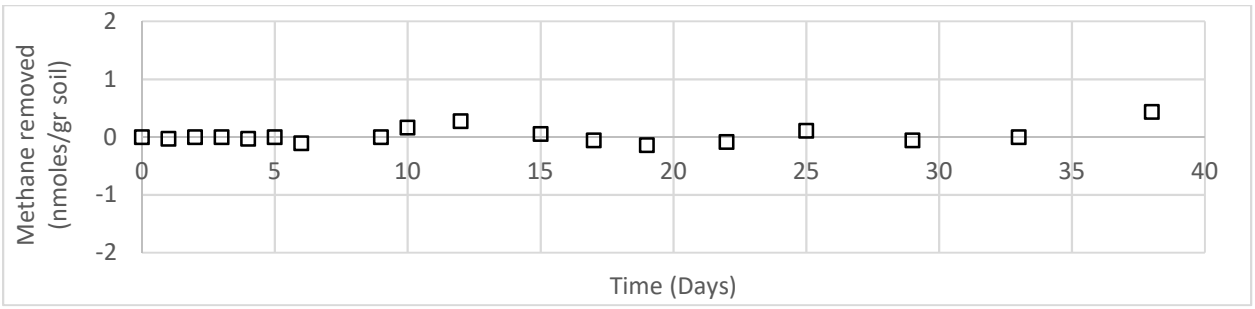
Reactor #28, Soil + 0.068 g Na_2SO_4

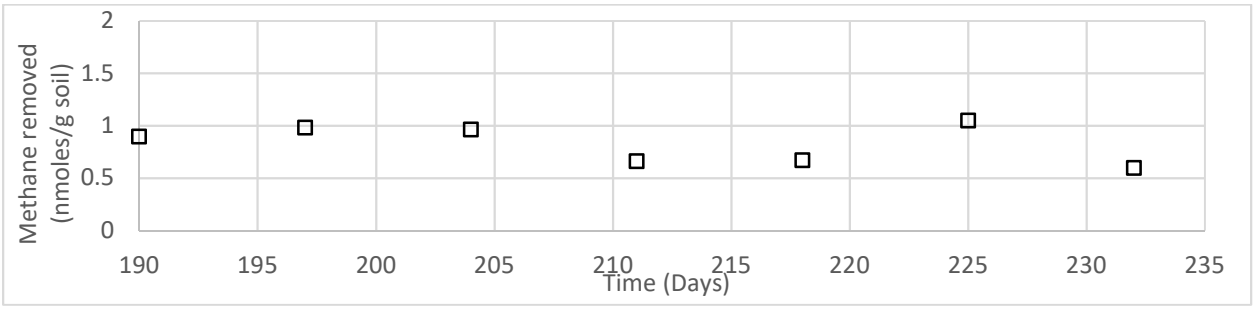


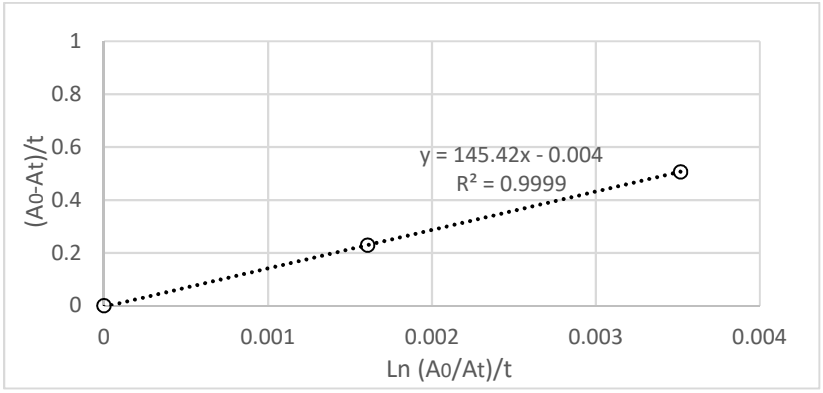




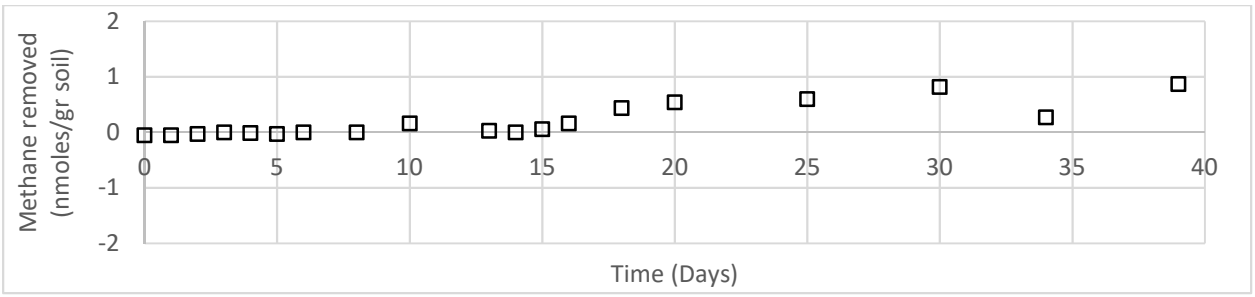
Reactor #29, Soil + 0.041 g KNO_2

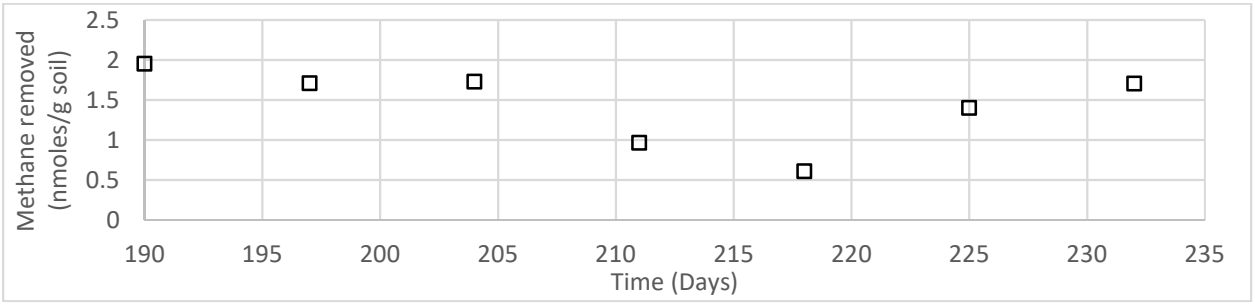


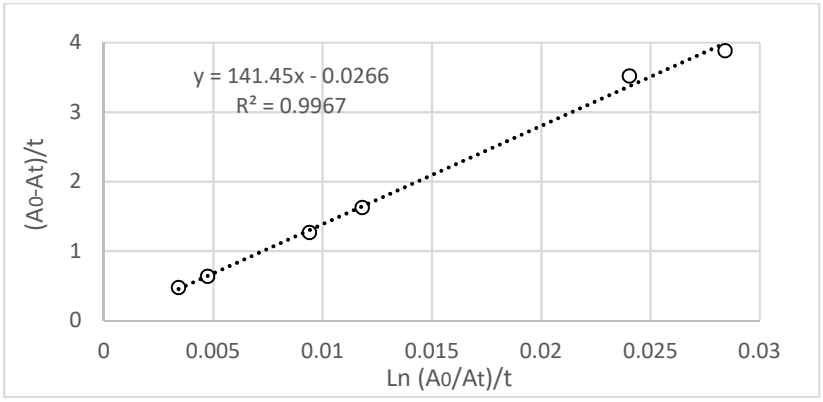




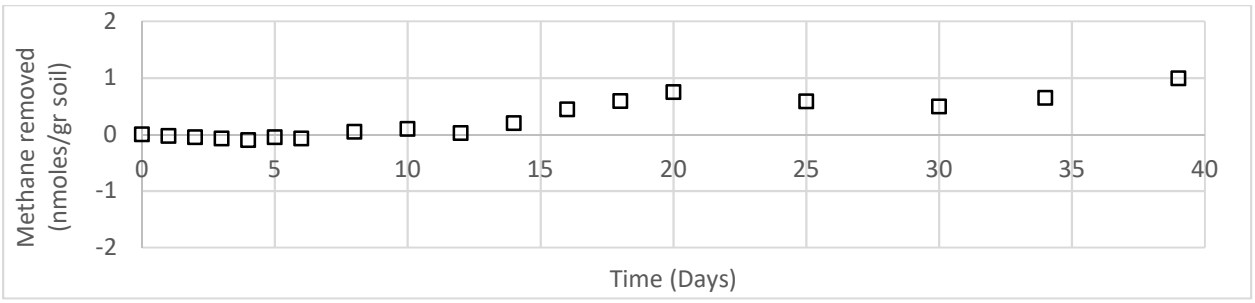
Reactor #30, Soil + 0.043 g $Fe(OH)_3$

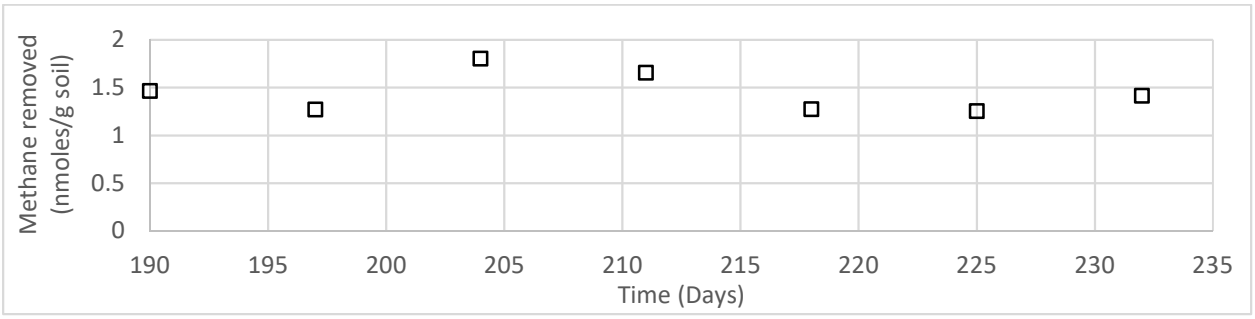


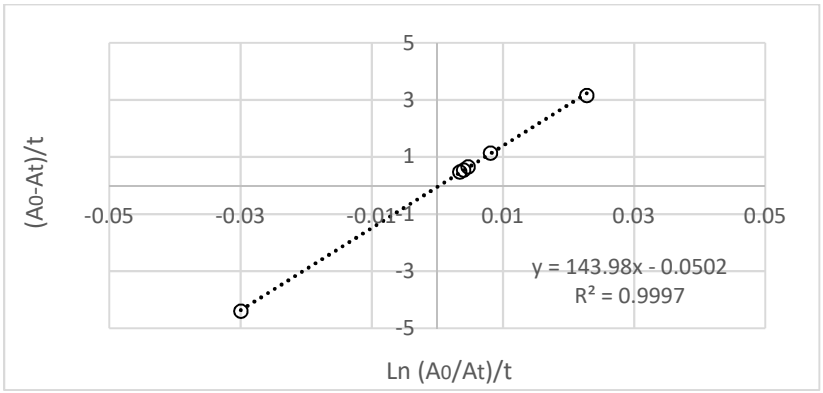




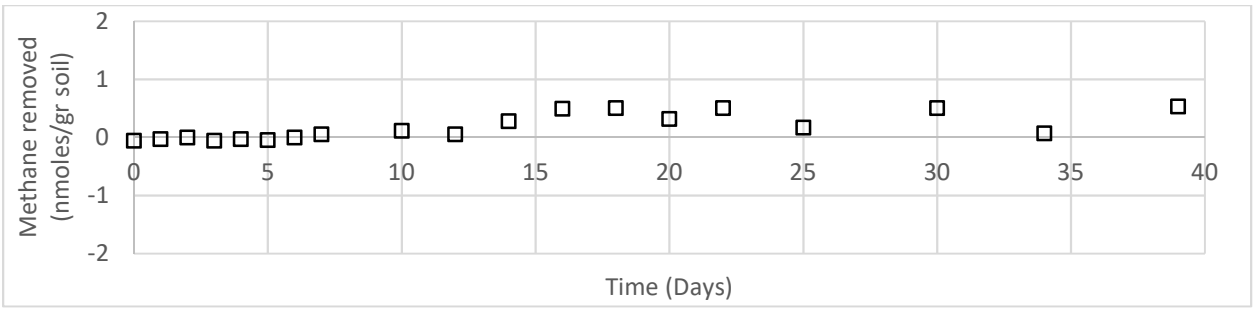
Reactor #31, Soil + 0.042 g MNO_2

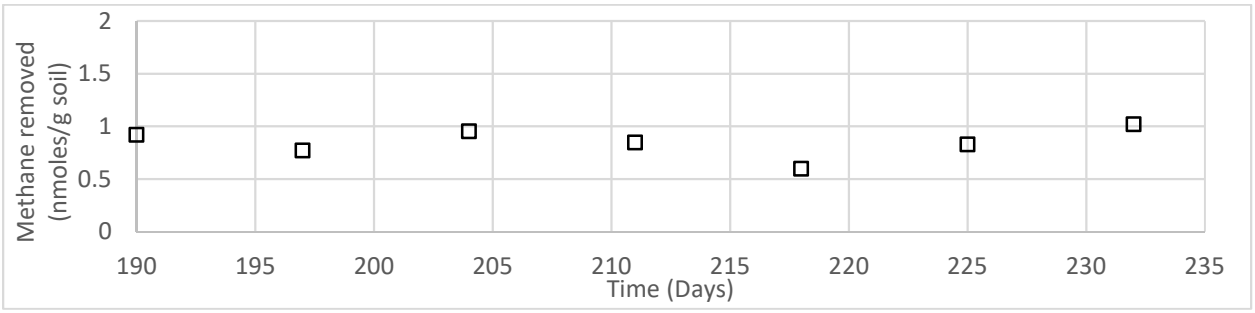


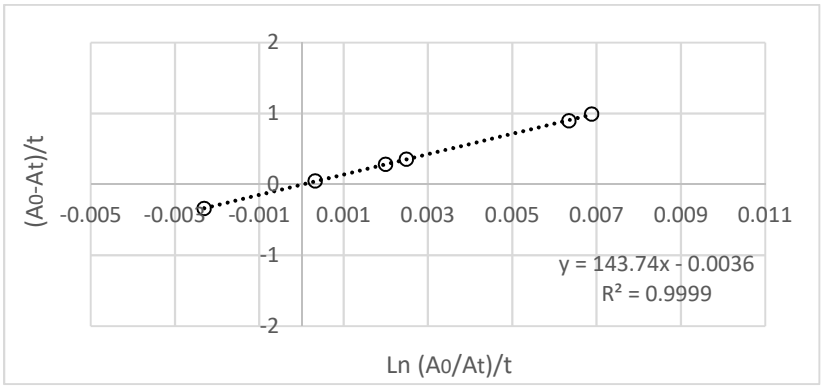




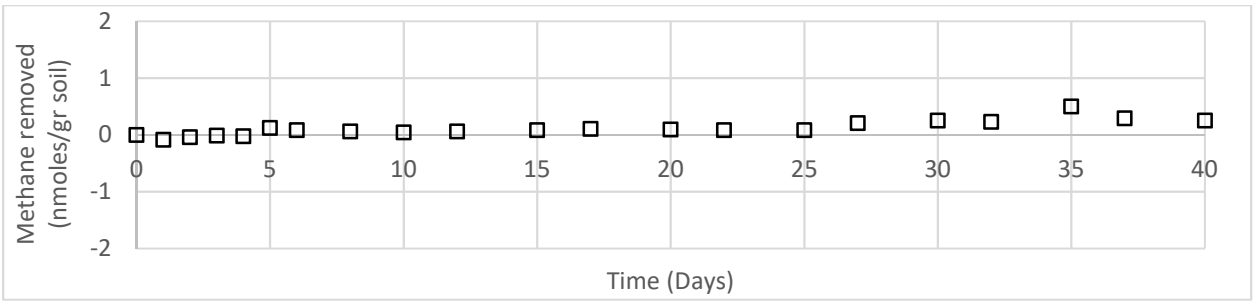
Reactor #32, Soil + 0.041 g $NaNO_3$

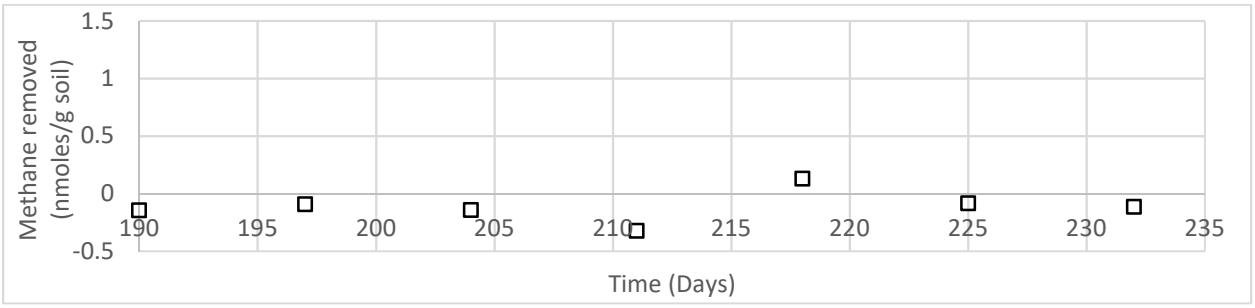


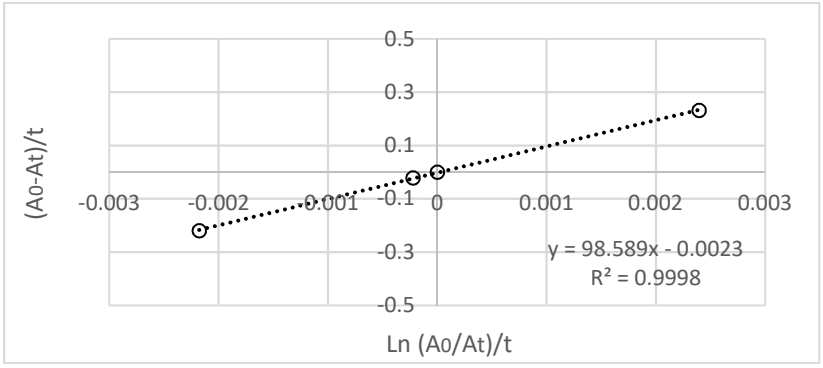




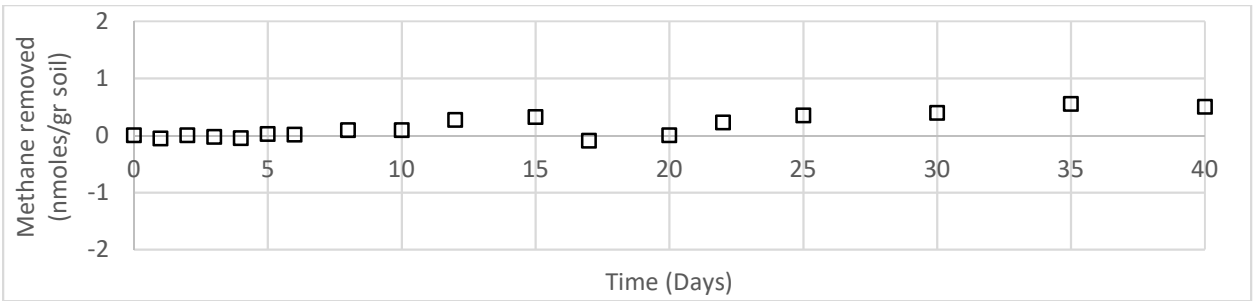
Reactor #33, Soil + 0.078 g $FeCl_3$

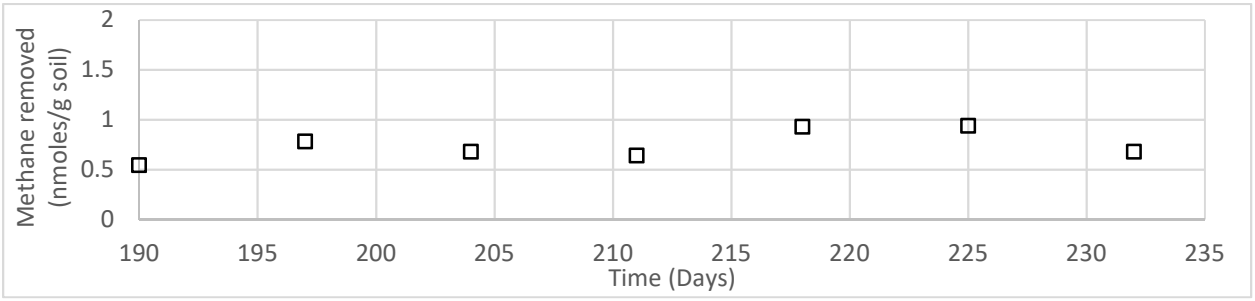


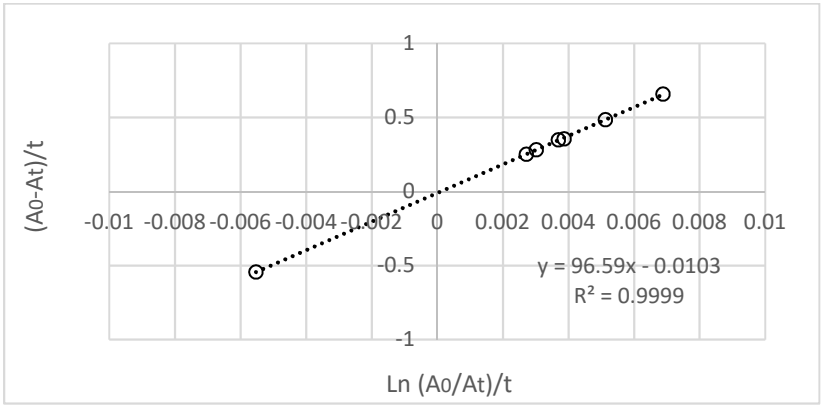




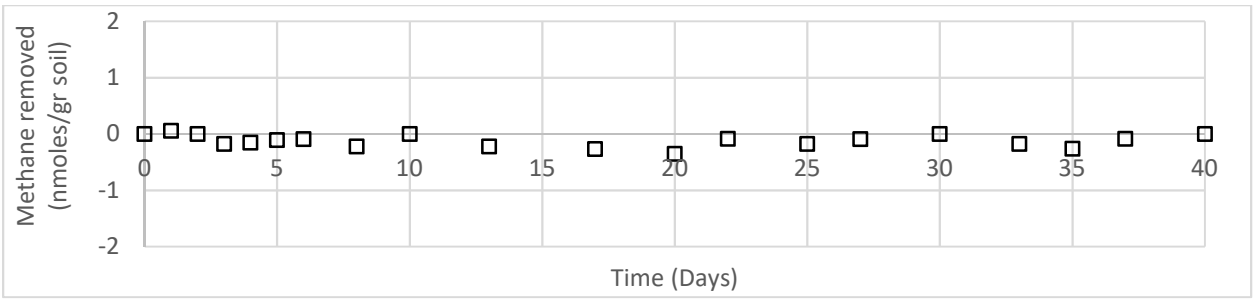
Reactor #34, Soil

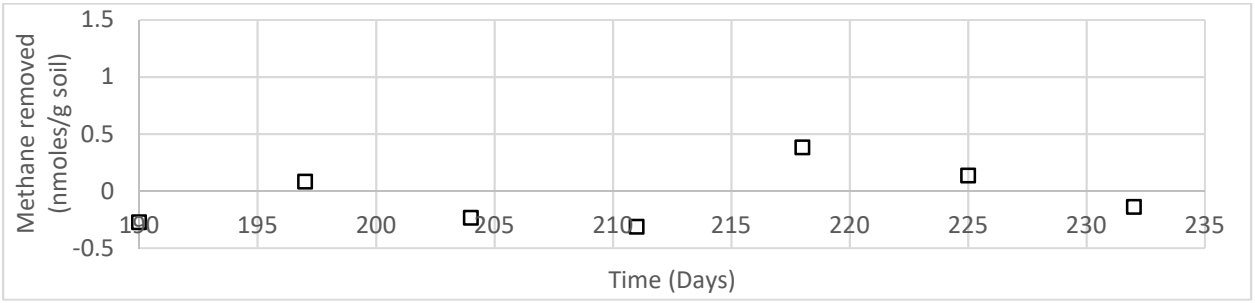


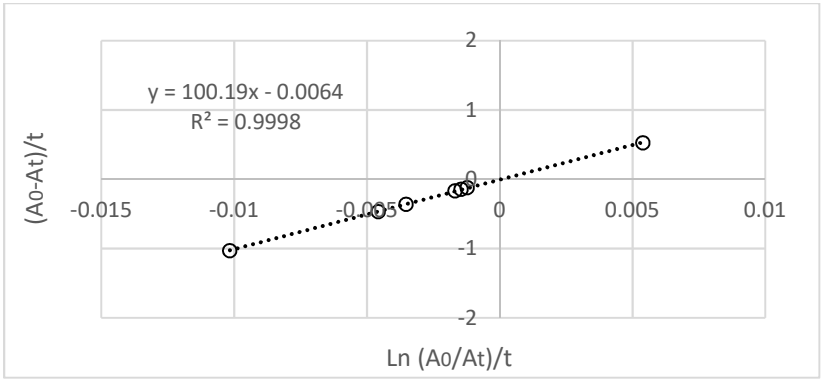




Reactor #35, Soil + 0.041 g $NaNO_3$







Reactor #36, Soil + 0.01g NH_4Cl