Methane Emission Treatment and Potential Upcycling using Methylomicrobium alcaliphilum

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Methane Emission Treatment and Potential Upcycling using *Methylomicrobium alcaliphilum*

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M.S. Plan B Report
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Public Abstract

Anthropogenic methane emissions are harmful to the environment and can be difficult to treat. Use of *M. alcaliphilum* for bioremediation of methane looks to be one of the most promising methods of reducing the negative environmental impacts of methane emissions while simultaneously providing an economic incentive. Successful bioreactor design for *M. alcaliphilum* based methane treatment requires biokinetic constants and microbial growth information. Methods were established for determining biokinetic constants, including growth rate, biomass yield (*Y*<sub>x/s</sub>), specific methane consumption rate (*q*<sub>CH<sub>4</sub></sub>), and a correlation between methane oxidation and carbon dioxide production for *M. alcaliphilum*. Values determined for these constants were used to provide a preliminary engineering design for treating methane emissions from the North Valley Landfill in Cache County, Utah.

Technical Abstract

*M. alcaliphilum*, an obligate methanotroph, was evaluated for use in bioreactors purposed for oxidizing anthropogenic methane emissions. Bench scale culture of *M. alcaliphilum* yielded biological constants including growth rate (μ=0.13 h<sup>-1</sup>), biomass yield on substrate (*Y*<sub>x/s</sub> = 1.19 gDCW/gCH<sub>4</sub> ± 0.02), apparent yield on substrate (*Y*<sub>x/sA</sub> = 0.49 gDCW/gCH<sub>4</sub> ± 0.03), and specific methane degradation rate (*q*<sub>CH<sub>4</sub></sub> = 0.13-0.26 gCH<sub>4</sub>/gDCW-h). Using the EPA LandGEM tool to generate emission predictions for the North Valley Landfill, in Cache County, Utah, a preliminary engineering design model for treatment of methane emissions was developed. Using the model, treatment of over 800000 m<sup>3</sup> methane emitted from approximately 280000 Mg of waste was estimated using 250 kg of *M. alcaliphilum* contained in reactors sized at a total liquid phase volume of 50000 L.
1. Background
1.1. The Importance of Methane

1.1.1. Methane as a Greenhouse Gas

Increasing atmospheric greenhouse gas (GHG) concentrations have necessitated
development of methods to not only reduce GHG emissions, but to also increase GHG treatment.
Because of their large quantity, carbon dioxide (CO$_2$) emissions receive the majority of attention
given to GHGs. However, CO$_2$ is not the most potent GHG. Pound for pound, methane (CH$_4$) is
over twenty-five times more effective than CO$_2$ at trapping heat in the atmosphere over a 100-
year period.$^1$ CH$_4$ is the second largest contributor to the total global atmospheric greenhouse
effect, accounting for approximately 20 percent of global emissions on a CO$_2$ equivalent basis.$^2$
Globally, CH$_4$ emissions in 2020 were estimated by the EPA to be 9,390 million metric tons of
CO$_2$ equivalent (MMTCO$_2$E).$^3$

Atmospheric CH$_4$ levels reached above 1850 ppb in 2018, over 2.5 times higher than the
estimated pre-industrial equilibrium value in 1750.$^4$ The large increase in the atmospheric
concentrations in that time frame can be mostly attributed to anthropogenic emissions.
Anthropogenic sources of CH$_4$ emissions include agriculture, energy industry, and waste from
homes and businesses, among other sources. In agriculture the production of CH$_4$ emissions is
largely tied to enteric fermentation by domestic livestock, which includes animals that produce
CH$_4$ as part of their digestive process. In the energy industry natural gas and petroleum are large
sources of CH$_4$ emissions. Methane is the primary constituent of natural gas and is emitted
during many phases of natural gas production processes. Oil and coal production are also
industry sectors that produce large quantities of CH$_4$ emissions. With regards to waste from
homes and businesses, CH$_4$ is generated as waste decomposes in landfills and wastewater
treatment, as well as in composting. Anthropogenic CH$_4$ emissions for the decade 2008-2017
were estimated to be 366 Tg CH$_4$ yr$^{-1}$, an increase of nearly 10% from the previous decade. The major anthropogenic sources of CH$_4$ emissions are projected to continue to increase and are estimated to contribute over 10,000 MMTCO$_2$E by 2030 (Figure 1).

![Figure 1 - Estimated and projected global anthropogenic methane emissions by source, 2020 and 2030. From the Global Methane Initiative, see www.globalmethane.org.](image)

Atmospheric CH$_4$ emissions have a relatively short lifetime, lasting approximately 9 years in the atmosphere before being oxidized by free radicals. However, CH$_4$ emissions have a large global warming potential relative to CO$_2$ and have made a large contribution to the total radiative forcing accumulation in the atmosphere since the Industrial Revolution. Specifically, the radiative forcing increase attributable to methane is approximately 0.62 W m$^{-2}$ since 1750. This represents approximately 23% of the total radiative forcing accumulated in the atmosphere in that time period. Furthermore, the total radiative forcing attributable to anthropogenic sources of CH$_4$ emissions is estimated to be approximately 1 W m$^{-2}$. The large impact of methane emissions relative to their short atmospheric lifetime is compounded by low oceanic contribution to the global GHG budget as a CH$_4$ sink compared to as a CO$_2$ sink, which is in turn
related to the large discrepancy of Henry’s law constant for CO₂ and CH₄. This is significant from a mitigation perspective. While new CH₄ emissions are especially detrimental because of their high heat trapping efficiency and smaller sinks, efforts to capture, utilize or reduce these emissions become especially fruitful because of their shorter lifetime.⁴,⁷

Economic constraints for research and technology development of climate change mitigation techniques are such that the development of such techniques should be specifically selected to maximize efficacy and efficiency of climate benefits. While it should be noted that CO₂ emission treatment and reduction measures represent a more obvious solution for long term climate change effects, methane mitigation techniques represent an extremely effective option for rapid climate changes. Furthermore, unexpected growth in global CH₄ emissions have already considerably negated the expected impact of progress in managing CO₂ emissions.⁷,⁸ Significant benefits of mitigating CH₄ emissions have been modeled and predicted, including a 0.28 ºC avoided warming by 2050 and an estimated economic value of roughly $3500 per metric ton CH₄ treated.⁹ These benefits are expected to far outweigh abatement costs, even economically.⁴

1.1.2. Mitigation Strategies

As previously mentioned, not all GHG mitigation strategies are created equal, and some should be prioritized over others. For methane mitigation, some strategies are relatively inexpensive, while others may require a much higher investment or tax incentive.⁷ Methane mitigation techniques that target certain gas emission streams from the energy industry, waste, and agriculture may be seen as the most likely to make impactful changes in the global CH₄ budget.
Agricultural CH₄ emissions are sourced primarily from enteric fermentation in ruminants and from manure management. CH₄ emissions from manure can be minimized by switching from liquid to dry management systems, or through lower stacking, dilution, or an anaerobic digestion preprocess. CH₄ from enteric fermentation has been reported to be decreased through implementation of various nutritional techniques and feed strategies. However, most methane-climate models agree that enteric fermentation can by far be considered the largest mitigation bottleneck in a strong climate policy case.

The energy industry includes the oil and gas industry, where CH₄ emissions were estimated at over 2200 MMTCO₂E globally in 2020, and the coal industry, where CH₄ emissions were estimated at approximately 800 MMTCO₂E globally in 2020. Methane is emitted in this sector during normal operation, but can be exacerbated by system disruptions, maintenance, and system failures. Equipment upgrades and implementation of new technologies can significantly reduce quantity of emissions from the energy industry. Such improvements may be driven by regulatory change, penalty fines, tax incentives or a mixture of these with a relatively small public economic cost. Recommendations for CH₄ mitigation in the gas and oil industry include use of vapor recovery units, flaring, catalytic converters, replacing wet seals with dry seals, direct use, and reinjection of gas into oil fields for enhanced oil recovery. In the coal industry, proposed CH₄ mitigation techniques include degasification and pipeline injection and oxidation of ventilation air methane.

The waste industry includes the solid waste industry, which was estimated to contribute over 1000 MMTCO₂E globally in 2020, and the wastewater industry, estimated to contribute over 650 MMTCO₂E. Mitigation of CH₄ emissions in these sectors has been proposed via a range of options, from waste diversion including recycling and reuse, to gas extraction using
collection wells and vacuum systems in landfills, and inclusion of anaerobic sludge digestions and biogas capture systems in wastewater treatment facilities.\textsuperscript{2, 12} Furthermore, landfill gas can be treated in various stages with more intensive treatment yielding more purified products that can be used in a variety of applications (Figure 2).

![Figure 2- Stages of landfill gas treatment. Primary treatment removes moisture, secondary treatment and advanced treatments remove impurities, purify and compress the gas into a high-energy gas for use as vehicle fuel or pipeline injection. From the EPA Landfill Methane Outreach Program (www.epa.gov/lmop).](image)

A large portion of the previously provided suggestions include reducing atmospheric CH\textsubscript{4} concentrations by avoiding escape of gaseous CH\textsubscript{4} emissions either by updating processes to prevent leaks or by sequestering the gas \textit{in situ}. Other suggestions require chemical processing, purifying, concentrating, and compressing dilute CH\textsubscript{4} into a more easily useable product, which is eventually oxidized and used for energy or fuel. While both approaches are required to reduce atmospheric methane, the combination of the two approaches – consumption or utilization of dilute CH\textsubscript{4} \textit{in situ} is an attractive future option of CH\textsubscript{4} removal. Such CH\textsubscript{4} treatment can be achieved using biological options. Many anthropogenic CH\textsubscript{4} emissions, especially in the agriculture and waste industries, are caused indirectly though creation of an environment
favorable to methanogens i.e., methane generating microorganisms. However, another class of microorganism may be used to counteract these emissions, namely methanotrophs – methane metabolizing microorganisms.

1.1.3. Methanotrophs: The Biological Solution

Methylotrophs are a group of microbes capable of using reduced single carbon compounds as a carbon source and electron donor. Methanotrophs are a subset of methylotrophs that assimilate methane as their carbon source. Methanotrophs can be further classified as Type I, Type II, or Type X depending primarily on the metabolic pathway used for formaldehyde assimilation and associated characteristics including membrane composition and cell morphology. Type I methanotrophs are typically classified as γ-proteobacteria, that assimilate formaldehyde via the ribulose monophosphate pathway (RuMP), while Type II methanotrophs are α-proteobacteria, that assimilate formaldehyde via the serine pathway. Type X methanotrophs have the ability to assimilate formaldehyde via the RuMP similar to Type I methanotrophs, but are distinct in that they also have low levels of the enzymes required for the serine pathway, present in the Calvin-Benson-Bassham cycle (Figure 3).

In general, methanotrophs produce unique enzymes, including methane monooxygenase (MMO), methanol dehydrogenase (MDH) and formate dehydrogenase (FDH), which allow them to utilize methane as a carbon and energy source. In the case of aerobic methane oxidation, the MMO enzyme is used to oxidize methane to methanol, which is in turn oxidized to formaldehyde. While MMO genes have been cloned into faster growing bacterial strains, a heterologous expression system remains difficult. Thus, fermentation based methane oxidation systems may be most successful through utilization of methanotrophic bacteria.
The operational costs of chemical catalytical technology for methane oxidation are significant and leave room for improvement via implementation of biological processes. Conventional chemical CH$_4$ conversion technologies generally require large scale production facilities. Inversely, biological methane conversion processes are relatively simple and can be operated at or close to ambient temperature and pressure with reduced capital investment and operating expenditures. Additionally, perhaps foremost among the advantages of using methanotrophs for bioremediation of methane emissions is the ability to oxidize dilute methane streams. This is important, as methane emissions in low concentrations cannot be treated by thermal oxidation. Finally, the carbon conversion efficiency of biological CH$_4$ treatments is much higher than that of chemical CH$_4$ treatment processes. Use of methanotrophs for methane bioremediation is generally performed through use of various bioreactor configurations that include biofilters, biotrickling filters, airlifts, and stirred tanks.
While bioremediation of methane emissions via methanotrophs is known to be possible, for widespread implementation it is important that the process be economically feasible. Sustainable waste CH$_4$ streams that are unable to be valorized by tradition methods, such as natural gas production, can be converted into bioproducts that offer an economic incentive. Products that are created using methanotrophs include Single-cell Protein (SCP); biopolymers including bioplastics such as poly(3-hydroxybutyrate), or PHB; extracellular polysaccharide biopolymers that are used in the food industry; osmo-protectants such as ectoine; lipids for use in health supplements or biodiesel applications; vitamins; and soluble metabolic intermediates such as methanol, formaldehyde and organic acids.$^{13}$

1.1.4. *Methylomicrobium alcaliphilum*

*Methylomicrobium alcaliphilum* is a Gram-negative, halotolerant, obligate methanotroph, isolated from surface sediment of the soda lake Shara-Nur in Tuva, Russia (which is notable for its dynamic seasonal changes).$^{20-22}$ *M. alcaliphilum* is a Type I methanotroph, utilizing the pMMO and MDH enzymes in conjunction with the RuMP pathway to oxidize CH$_4$ (Figure 3). The bacterium has an optimal pH range of 7.2-9.5 and tolerates salt concentrations as high as 1.5 M NaCl.$^{21}$ In general, cultures of *M. alcaliphilum* stay active over a wide range of physicochemical parameters including pH, temperature, and salinity. Moreover, the species quickly adapts to environmental perturbations, making it ideal for environmental bioprocess and methanotrophy experiments.$^{22}$

Importantly, *M. alcaliphilum* is capable of ectoine biosynthesis, while utilizing CH$_4$ as the sole carbon and energy source.$^{23-26}$ Ectoine represents one of the most valuable bioproducts naturally produced by microorganisms.
1.2. The Significance of Ectoine

1.2.1. Ectoine as a Compatible Solute

Selection pressures on organisms are caused by unfavorable environmental stresses such as temperature, pH, or toxic levels of various compounds. In response to these selection pressures, bacterial populations mutate and adapt in order to provide mechanisms to survive. Halophilic bacteria found in highly saline environments have developed strategies to cope with hyperosmotic stress, perhaps most notably including the accumulation of compatible solutes. Compatible solutes are generally compounds that are highly soluble, of low molecular weight, and are often either uncharged or zwitterionic organic molecules. These molecules are produced and collected in the cell and unlike most inorganic salts, are biocompatible at high concentrations. While inorganic salts can become toxic when accumulated in excess, compatible solutes are capable of providing osmotic balance without interrupting cell metabolism, lowering enzyme efficiency, or necessitating specially adapted proteins. Furthermore, compatible solutes allow for prompt adaptation to environmental salinity perturbations, as they can be rapidly released from the cell via efflux pumps in response to a hypoosmotic shock. Some commonly known compatible solutes include structures from various chemical classes, including some sugars (e.g., sucrose and trehalose), polyols (e.g., sorbitol and glycerol), betaines (e.g., betaine and glycine), as well as some amino acids and derivatives.

Although somewhat lesser known, another significant compatible solute is ectoine (1,4,5,6-tetrahydroydro-2-methyl-4-pyrimidinecarboxylic acid). Ectoine is named for its discovery in Ectothiorhodospira halochloris (now known as Halorhodospira halochloris). The ability to synthesize the compound has since been identified in a wide range of halophilic organisms from several different taxonomic classes. Among the many organisms naturally capable of ectoine
synthesis, *Halomonas elongata* has received considerable attention and industrial scale production processes have been developed to use the organism as a host for ectoine production.\(^{32}\)

### 1.2.2. Physicochemical Properties of Ectoine
Ectoine (PubChem CID: 126041) has a chemical formula of \(\text{C}_6\text{H}_{10}\text{N}_2\text{O}_2\), with a molecular weight of 142.16 g/mol. The zwitterionic compound has a melting point of approximately 280 °C. Ectoine has a high solubility in water of approximately 4 mol/L at 20 °C.\(^{33}\) Solubilities of ectoine at 25 °C have also been reported in water, methanol, and ethanol as approximately 550 g/L, 36 g/L, and 5 g/L, respectively.\(^{34}\) Additionally, ectoine is stable within a wide pH range (1-9) and at high temperature. A summary of physicochemical properties of ectoine is provided in Table 1.

<table>
<thead>
<tr>
<th>Molecular Weight</th>
<th>Density</th>
<th>Melting Point</th>
<th>Solubility</th>
<th>pH Stable range</th>
<th>pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>142.16 g/mol</td>
<td>1570 kg/m(^3)</td>
<td>280 °C</td>
<td>(550 \text{ g/L (Water)}^{33,34})</td>
<td>1-9</td>
<td>2.44(^{34})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(36 \text{ g/L (MeOH)}^{34})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(5 \text{ g/L (EtOH)}^{34})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 1.2.3. Ectoine as a Bioproduct
Industrial scale ectoine production began as a result of considerable biotechnological attention given to the compound due to its desirable applications.\(^{35}\) One of these applications is use of ectoine as a cellular protectant. It has been proposed that ectoine can increase cell membrane fluidity to cope with extreme conditions of not only high osmotic pressure but also temperature.\(^{30}\) Interestingly, extremes in either high or low growth temperatures have been shown to trigger enhanced ectoine production in some organisms, and other organisms have been observed to accumulate ectoine from environmental sources via cellular transport processes.\(^{36}\) Because ectoine can act as a chemical chaperone, this intracellular ectoine accumulation under
extreme temperature conditions may be a cellular response to increase protein stability.\textsuperscript{35} Thus, ectoine is useful for its properties as both an osmolyte and a thermolyte.

Besides valuable thermal and osmotic pressure protection, ectoine is also notable for its UV protectant properties.\textsuperscript{13} Although the exact mechanisms by which ectoine provides UV protection are not completely understood, it is thought that “ectoine acts as a radioprotector in terms of its electron as well as radical scavenging properties”.\textsuperscript{37} Ectoine has been also shown to inhibit early UV-A radiation induced ceramide signaling, as well as decrease UV-induced DNA single strand breaks.\textsuperscript{29} Ectoine is known to absorb UV radiation and protect DNA in various cell types. Notably, these UV protection properties have led to use of ectoine as an ingredient in skin care, anti-aging and sun protection products.\textsuperscript{29, 38}

Additional functions of ectoine have been found including use for protein and enzyme stabilization, cryoprotection, as well as inflammation alleviation and protection against neurodegenerative diseases.\textsuperscript{27, 30} As a result, ectoine can be found as an active ingredient in many human and animal health products. For example, Bitop AG, a Germany based company that specializes in ectoine production and ectoine based treatments, includes ectoine as an active ingredient in products ranging from allergy and cold nasal spray, inhalation solution, dermatitis and psoriasis creams, moisturizers, and eye drops. Bitop has even claimed that ectoine has potential for use in inhibition of the binding of SARS-CoV-2 spike protein.\textsuperscript{39}

The previously described properties of ectoine have garnered much interest in the compound in recent years. Ectoine has an estimated sales price of over USD 1,000 kg\textsuperscript{-1} and has a global demand of approximately 15,000 metric tons per year.\textsuperscript{13, 36, 40} However, at least one study has listed prices for ectoine at well over USD 10,000 kg\textsuperscript{-1}.\textsuperscript{41} At the time of writing, HPLC grade ectoine was found to have a cost of USD 1,330 for a 100 g sample offered by Sigma-Aldrich.
1.2.4. Ectoine Production

On an industrial scale, ectoine production most commonly includes a ‘bio-milking’
process of a halophilic organism, usually the previously mentioned *H. elongata* as performed by
the previously mentioned Bitop. In this bio-milking bioprocess, bacteria are cultured in
highly saline environment, then biomass is concentrated by microfiltration, and the concentrate is
exposed to a hypoosmotic shock by dilution with water. This forces organisms to release the
intracellular ectoine, which is then separated from the biomass via a second round of filtration.

There are a number of drawbacks associated with this process, including the relatively high
cost of using a glucose feedstock for *H. elongata* and potential negative effect on equipment due
to the highly saline environment.

1.2.5. Ectoine Biosynthesis Pathway

The enzymes responsible for the biosynthesis of ectoine are L-2,4-diaminobutyrate
transaminase (EctB), L-2,4-diaminobutyrate acetyltransferase (EctA), and ectoine synthase
(EctC). These are typically organized in a single operon, and usually the transcription of the
*ectABC* gene cluster is osmotically inducible. It should also be noted that 5-hydroxyectoine, an
ectoine derivative, is formed through hydroxylation of ectoine via the ectoine hydroxylase
(EctD) enzyme. Prior to reactions performed by the EctABC proteins, ectoine synthesis requires
phosphorylation of L-aspartate to form β-aspartylphosphate, which is subsequently reduced via
the aspartate semialdehyde dehydrogenase (ASD) enzyme to form L-aspartate-β semialdehyde
(Figure 4).
2. Project Description
2.1. Project Purpose
The goal of this work was to utilize biological engineering methods of mitigating harmful methane emissions. Specifically, insight was desired regarding the feasibility of using \textit{M. alcaliphilum} in a bioreactor for simultaneous waste methane treatment and ectoine production. Two specific objectives of this project were: 1) determine biokinetic constants for \textit{M. alcaliphilum}; and 2) design a methane emissions treatment process for a landfill using the biokinetic constants.

2.2. Engineering Significance
Use of \textit{M. alcaliphilum} for bioremediation of CH$_4$ looks to be one of the most promising methods of reducing the negative environmental impacts of CH$_4$ emissions while simultaneously providing an economic incentive to do so. However, the discovery of the bacterium is relatively recent, and efforts to accomplish fermenter design utilizing the bacterium remain in the nascent stage. Fermenter or bioreactor design requires biokinetic constants and microbial growth information. Prior to widespread implementation of \textit{M. alcaliphilum} based reactors for CH$_4$
treatment, information including growth rate ($\mu$), biomass yield ($Y_{x/s}$), specific consumption rate ($q_{\text{CH}_4}$), product yield ($Y_{p/x}$) is needed.

3. Methods

3.1. Growth Medium

*M. alcaliphilum* cultures were grown in modified “P” media similar to that used by Akberdin et al., Ojala et al., and reported for *M. alcaliphilum* cultures by the Kalyuzhnaya Laboratory at San Diego State University.\textsuperscript{22,42} This growth medium consists of KNO$_3$ (1 g/L), MgSO$_4$ x 7H$_2$O (0.2 g/L), CaCl$_2$ (0.02 g/L), NaCl (30 g/L), trace solution\textsuperscript{22} (1 mL/L), phosphate buffer (20 mL/L), and carbonate buffer (40 mL/L).

Cultures were grown in 250 mL glass serum vials, sealed with butyl rubber stoppers (Chemglass, CLS-4209-14) and aluminum crimp caps. Aliquots of 53 mL (50 mL base medium + 3 mL buffer) were added to the vessels, resulting in a headspace to medium ratio of approximately 4:1 (v/v). Prior to inoculation, 20% (volumetrically) of the headspace was removed and replaced with CH$_4$ (99.9% purity) to give an initial headspace concentration of 80% to 20% v/v air/CH$_4$. Headspace composition was selected to avoid O$_2$ limited growth. Methanotrophs have generally been observed to have a maximum oxidation rate at O$_2$ concentrations up to 10.5%.\textsuperscript{19}

3.2. Microorganism and culture conditions

*M. alcaliphilum* 20Z was obtained from DSMZ (Leibniz-Institut, Germany). Cryogenic stocks were stored at -72 °C and prepared by adding 30% glycerol to aliquots of culture broth in a 1:1 ratio. Cultures were grown in a shaking incubator maintained at 28 °C and agitated at 200 rpm. The inoculant used for each test was incubated to a biomass concentration of at least 0.1 g/L prior to use. Unless otherwise stated, 5 mL of inoculum were used to inoculate culture vessels in the tests performed for this project.
3.3. Growth Curve and Dry Cell Weight
A growth curve was generated by indirect measurement of cell growth via optical density (OD). Cultures of *M. alcaliphilum* were grown in triplicate with OD measurements (\( \lambda = 600 \) nm) for each vessel being recorded every 2-4 hours until cell growth entered the late stationary phase. The data were plotting using semi-logarithmic transformation applied to linearize the data corresponding to the exponential growth phase. Analysis of covariance (ANCOVA) was performed on the linearized data using SAS® Studio to determine the growth rate of the bacteria.

Dry cell weight of the liquid cultures was determined by drying the cell pellet acquired following centrifugation of cultures at various culture absorbance levels. Centrifugation was performed at 3500 g for 25 minutes at 4 °C. After decanting the supernatant, the cell pellet was resuspended in 5 mL DI water, and dried overnight at 105 °C. Pellet mass was determined following cooling in a glass desiccator.

3.4 Methane Consumption
Two treatment conditions were used while observing the methane consumption rate of *M. alcaliphilum*. The first treatment used an initial headspace concentration of 80% to 20%, air to synthesized landfill gas (SLFG), where the SLFG was comprised of 50% CH\(_4\) and 50% CO\(_2\).\(^{43}\) This treatment will be referred to as DSLFG (Diluted SLFG). The second treatment used an initial headspace concentration of 80% to 20%, air to pure CH\(_4\). This treatment will be referred to as DPM (Diluted Pure Methane). Both treatments were tested in triplicate, with the headspace being flushed and reestablished at the initial concentration each day. The gas composition of the headspace for each culture vessel was monitored by taking 2 measurements daily, one immediately before the headspace was reestablished and one immediately after. Culture absorbance measurements were acquired daily immediately after gas chromatography (GC) measurements of gas concentrations.
Gas concentrations were determined in an Agilent 7890B GC-TCD with a GS-GasPro GC Column (60 m x 0.32 mm x 0.0 μm, Agilent Part Number: 113-4362). The inlet temperature was set at 120 °C, with pressure set to 20 psi and purge flow set at 3mL/min. Helium was used as the carrier gas, with the column flow set to 3.06 mL/min and a pressure of 20 psi. Oven temperature was set at 25 °C, and the detector temperature was set to 250 °C.

Culture absorbance measurements were acquired at 600 nm using a HACH DR5000 spectrophotometer. The DR5000 became inoperable during the test, after which a HACH DR1900 spectrophotometer was used with wavelength set to 600 nm.

The daily CH$_4$ consumption was calculated by subtracting the mass of CH$_4$ remaining from the initial mass of CH$_4$ present. This cycle was repeated daily. The CH$_4$ consumption was divided by time and dry weight of biomass to determine a specific CH$_4$ consumption rate. The dry weight of biomass used to perform this calculation was an approximated value based on the dry weight at the start and end of the measurement period.

3.5 Ectoine Analysis

The DSLFG and DPM treatments were used as described in the previous section. Likewise, the headspace for each sample vessel was reestablished to the initial concentration daily. OD measurements were acquired daily at the time of harvesting samples for ectoine analysis.

Intracellular ectoine was separated following a method similar to Cantera et al., by drawing a 2mL sample of cultivation broth and placing it in a 2 mL screw cap vial. Samples were centrifuged at 9000g and 4 °C for 15 minutes, and the supernatant was discarded. Next, 0.1 mm diameter zirconia beads (Biospec, Catalog Number: 11079101z) were added to cover the pellet, and 2mL 80% ethanol added. The pellet was disrupted in a Mini Beadbeater (Biospec) at
4200 oscillations/min for 90 seconds. The supernatant of this suspension was then filtered with a 0.2 μm syringe filter. Extracellular ectoine was separated by directly filtering a 1 mL sample of cultivation broth.

Prior to analysis, the filtrate of the intracellular and extracellular ectoine samples was centrifuged at 21000 g for 10 minutes. Attempts to analyze the ectoine concentration of the samples were performed via high performance liquid chromatography (HPLC) using an Agilent 1100 Series Capillary HPLC, with a Polaris NH2 column (150 mm x 4.6 mm, Part Number: A2014150X46) and Polaris NH2 MetaGuard (3 μm, 4.6 mm, Part Number: A2014MG). The column was shipped in heptane. To switch the column from normal phase to hydrophilic interaction chromatography (HILIC) phase separation it was flushed with HPLC grade IPA for 5 hours at 0.5 mL/min, after which the column was equilibrated with mobile phase composition to be used during the analysis method. For analysis, the flow rate was set at 1.0 mL/min with an eluent composition of 75% acetonitrile and 25% ddH2O, and an injection volume of 10 μL. The temperature was set to 30 °C, and UV detection at 210 nm.

4. Results and Discussion
4.1. Growth Curve
One of the most important pieces of biological information required for bioreactor design is the growth rate (μ, in terms of time⁻¹). To determine the growth rate of *M. alcaliphilum*, first the growth curve was generated by plotting culture OD against time (Figure 5).
The growth rate is calculated by performing a semi-logarithmic transform of the plot and determining the slope of the resultant linearized section of data. The natural logarithm transform of OD was plotted against time to generate Figure 6. Using ANCOVA with the data points for each culture vessel in the time period between 2 h and 17 h, the growth rate was calculated as $\mu = 0.132 \text{ h}^{-1}$ (standard error = 0.0025, 95% confidence interval (CI) [0.127, 0.137]).
The semi log transform of the growth curve. The plot is approximately linear from $t = 2$ h to $t = 17$ h, where the slope is representative of the growth rate. Error bars represent the standard deviation to provide a measure of dispersion of data.

4.2. Dry Weight

The dry cell weight (DCW) of the liquid cultures was experimentally determined to be 0.330 gDCW/L-OD (standard error = 0.007, 95% CI [0.315, 0.343]). Per OD unit the DCW appeared to be slightly lower for cultures at a higher OD compared to cultures at a lower OD. This observation would require many more sample replicates to substantiate statistically.

4.3. Methane Utilization

A plot of the headspace methane content for each sample replicate over time allows for observation of the behavior of the cultures (Figure 7). Ambient conditions in the location of testing were an approximate atmospheric pressure of 86 kPa and temperature of approximately 22 °C. Using the ideal gas law with these ambient conditions, a methane content of 20% is expected to correspond to a mass of approximately 112 mg/L headspace. The daily peaks (local maxima) in the plot show the reestablishment of the headspace composition to be precise and accurate. The daily methane consumption of the $M.$
*alcaliphilum* cultures in each vessel is shown by the downward trends. Note that vessels 1-3 correspond to the DSLFG treatment and vessels 4-6 correspond to the DPM treatment.

![Headspace Methane Concentration](image)

*Figure 7 - Daily methane concentration fluctuations among all culture vessels. Vessels 1-3 correspond to DSLFG, while vessels 4-6 correspond to DPM. Individual data points are shown.*

The headspace methane concentration over time for treatment averages of DSLFG and DPM shows overall trends for the treatments, see Figure 8. Results show the daily reduction of methane is less for DSLFG than for DPM, especially at the beginning of the experiment. This can be linked to at least two factors: 1) the higher initial concentration of methane available for vessels exposed to DPM, and the subsequent increased amount of methane dissolved in the culture broth related to Henry’s Law; and 2) high variability of growth among culture vessels exposed to DSLFG. Within the DSLFG group, one of the culture vessels showed the fastest initial growth among all vessels and complete methane depletion each day after the first day, while the remaining two cultures showed the slowest initial growth among all vessels, with a related relatively small daily decrease in methane concentration.
Figure 8 - Daily methane fluctuations among vessels, averaged by treatment condition. Error bars represent the standard error in order to provide a measure of the precision of the means.

One of the reasons for monitoring the methane consumption by *M. alcaliphilum* cultures over time is to provide evidence that methane is being oxidized under both treatment conditions. The results in Figure 7 and Figure 8 show methane oxidation via *M. alcaliphilum* growth using DSLFG occurs despite a relatively high initial CO₂ concentration. Thus, treatment of CH₄ emissions from landfill sources via *M. alcaliphilum* is feasible. Another primary reason for monitoring the methane consumption of cultures over time is to provide biological constants needed for reactor design, namely yield \((Y_{x/s})\), specific methane consumption rate \((q_{CH_4})\), and maintenance uptake rate of methane \((m_{CH_4})\). The relation between \(q_{CH_4}\), \(\mu\), \(m_{CH_4}\), and \(Y_{x/s}\) can be explained by Equation 1.⁴²

\[
q_{CH_4} = m_{CH_4} + \frac{\mu}{Y_{x/s}}
\]

Direct calculations using experimental data were made to provide an approximation for \(q_{CH_4}\) for each culture for each day by dividing the mass of methane depleted in the culture vessel by both the approximated average biomass in the culture vessel for the depletion period and the
duration of the depletion period. Equation 1 takes the form of a line, where \( q_{CH_4} \) can be expressed as the dependent variable, and \( \mu \) can be expressed as the independent variable. Thus, by plotting \( q_{CH_4} \) against \( \mu \), it is possible to extract \( m_{CH_4} \) and \( Y_{x/s} \) from the plot as the y-intercept and the inverse of the slope, respectively. A linear regression model was used to estimate the slope of this plot (Figure 9).

![Specific Methane Consumption Rate](image)

**Figure 9** - The growth rate plotted against the specific methane consumption rate. The linear regression model and least squares regression fit with 95% CI bounds shows predicted values and anticipated boundaries.

Based on the model, \( Y_{x/s} = 1.19 \) gDCW/gCH\(_4\) (standard error = 0.02) and \( m_{CH_4} = 16.9 \) mgCH\(_4\)/gDCW-h (standard error = 2.4). The coefficient of determination value, \( r^2 = 0.56 \), is not unusual for biological systems, compared with physical and chemical systems.

Additionally, the model gives higher weight to the few points at high growth rate compared to those at a lower growth rate, which leaves estimates prone to high variability upon reproducing the experiment.

An alternative method of determining \( Y_{x/s} \) is by directly calculating yield as an observed increase in biomass divided by an observed decrease in mass of methane over the
same period. This calculation was performed for each culture vessel and sampling period. Based on this method of calculation, the apparent yield, \( Y_{x/s}^A = 0.49 \) gDCW/gCH\(_4\) (standard error = 0.03). The value for the direct calculation of yield is approximately 41% of the value calculated based on the model. The discrepancy can be attributed to at least two factors: 1) the direct calculation for \( Y_{x/s}^A \) does not account for \( m_{CH_4} \) of the cultures; and 2) the previously mentioned variability in the model prediction, given the coefficient of determination and heavier weight given to points corresponding to a high growth rate. From their work with \( M. \) alcaliphilum, Akberdin et al. reports a maximum yield, \( Y_{x/s}^M \) of 0.6 gDCW/gCH\(_4\).\(^{42}\) This falls between the two values reported herein of \( Y_{x/s}^A = 0.49 \) gDCW/gCH\(_4\) and \( Y_{x/s} = 1.19 \) gDCW/gCH\(_4\). Thus, with estimates from this study, assuming a peak growth rate of approximately 0.13 h\(^{-1}\) and \( Y_{x/s}^A \) of approximately 0.5 gDCW/gCH\(_4\) (which incorporates \( m_{CH_4} \)), \( q_{CH_4} \) during growth can be estimated at approximately 0.26 gCH\(_4\)/gDCW-hr. Using the same growth rate with \( Y_{x/s} = 1.19 \) gDCW/gCH\(_4\) and \( m_{CH_4} = 17 \) mgCH\(_4\)/gDCW-h, \( q_{CH_4} \) is approximately 0.13 gCH\(_4\)/gDCW-hr.

Additional information acquired during this test includes biomass concentration over time, as well as the approximate growth rate over time. The growth rate approximation provided in Figure 10 is based on the formula \( \mu = \frac{dX}{dt} \), and its subsequent solution for growth rate (Equation 2).

\[
(Equation 2) \quad \mu = \frac{\ln(X_2/X_1)}{t_2-t_1}
\]

It should be noted that because biomass measurements were performed only once daily, the reported growth rate in this section is an approximate growth rate over the entire day-long period. More frequent measurements would be required for a better approximation
but would be impractical based on the small reactor size used in this study. The approximate growth rate rapidly decreased for all culture vessels through the first 50 – 100 hours. While the growth rate of Vessel 2 and Vessel 3 is low relative to the other vessels at the beginning of the test, both Vessel 2 and Vessel 3 eventually began a phase of faster growth. Eventually, the biomass concentration of these vessels began to increase at a similar rate to all other vessels, as observed upon comparing Figure 10 and Figure 11.

The biomass concentration over time for all culture vessels is provided in Figure 11. While Vessel 2, Vessel 3, and Vessel 5 lagged in initial biomass concentration, it is interesting to note that the rate of increase of biomass concentration, as shown by the slope, is nearly identical for all Vessels by the last 3 days of the experiment. The rate of increase of biomass concentration across all culture vessels over this period was calculated to be 0.00525 g/L-h (standard error = 8.85 x 10^-5). It is likely that the slower initial growth of some cultures compared to others is due a longer lag or acclimation phase of growth related
to the inherent variability of biological systems. Comparison of Figure 7 and Figure 10 shows methane concentrations in slower growing culture vessels to have a smaller decline than faster growing culture vessels. This observation is to be expected as total methane consumption is related to both biomass concentration and growth rate.

![Biomass Concentration](image)

*Figure 11 - Biomass Concentration over time for all culture vessels. Vessels 1-3 correspond to DSLFG and Vessels 4-6 correspond to DPM.*

Finally, information regarding the CO$_2$ generation with its comparison to CH$_4$ oxidation is reported in Figure 12. The relationship between CO$_2$ production and CH$_4$ consumption was modeled by creating a linear regression model, with CH$_4$ consumption as the independent variable and CO$_2$ production as the dependent variable. The slope of the linear regression model was calculated to be 1.45 gCO$_2$/gCH$_4$, and coefficient of determination was calculated to be $r^2 = 0.744$. While more CO$_2$ was produced than CH$_4$ consumed on a mass basis, the environmental benefit of oxidizing methane with relation to its higher heat trapping efficiency compared to CO$_2$ remains.
Converted to a molar ratio, the slope of regression model in Figure 12 is equivalent to 0.53 mol CO₂ / mol CH₄. Thus, under aerobic conditions, approximately half of the carbon consumed by the bacterium is converted into CO₂, with the remaining fraction being used in biosynthesis pathway such as ectoine synthesis and biomass generation. This is consistent with expectations for aerobic transformation of a carbon source.

![Graph showing CH₄ consumption vs CO₂ production](image)

*Figure 12 - Linear regression model of the relationship between CH₄ consumed and CO₂ produced by M. alcaliphilum cultures.*

Table 2 provides a summary of biological constants and kinetic information calculated in this report along with comparisons to values found in the literature. Growth rate (μ) and dry cell weight values determined for *M. alcaliphilum* cultures in this work were similar to values reported in the literature. Additionally, values for specific CH₄ degradation rate ($q_{CH₄}$) and biomass yield on methane ($Y_{X/S}$) were comparable to values reported in the literature.
Table 2 - Biological constants and kinetic information in this study, with comparison to values found during literature review.

<table>
<thead>
<tr>
<th></th>
<th>Growth Rate (μ) [h⁻¹]</th>
<th>Dry Cell Weight [g/L-OD]</th>
<th>Specific CH₄ Degradation [gCH₄/gDCW-h⁻¹]</th>
<th>Maintenance Methane Uptake [mgCH₄/gDCW-h⁻¹]</th>
<th>Biomass Yield on Substrate [gDCW/gCH₄]</th>
<th>Extracellular Product Concentration [mg/L]</th>
<th>Intracellular Product Concentration [mg Ectoine gDCW⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td>0.132 ± 0.0025</td>
<td>0.330 ± 0.007</td>
<td>0.13-0.26</td>
<td>16.9 ± 2.4</td>
<td>0.49 ± 0.03 (Yₐ/sₐ)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Akberdin et al. 42</td>
<td>0.14 ± 0.02</td>
<td>0.336 ± 0.025</td>
<td>0.30 ± 0.02</td>
<td>58</td>
<td>0.6 (Yₐ/sₐ)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(Batch)</td>
<td>(Batch)</td>
<td>(Continuous)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cantera et al. 23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>253.4 ±55 mg/L after shock</td>
<td>70.4 ± 14.3</td>
<td></td>
</tr>
<tr>
<td>Cantera el al. 44</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>94.2 ± 10.1</td>
<td></td>
</tr>
<tr>
<td>Khmelelena et al. 45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.15</td>
<td></td>
</tr>
<tr>
<td>Cantera et al. 24</td>
<td>-</td>
<td>0.300</td>
<td>0.22 – 2.03</td>
<td>-</td>
<td>1.2 – 4.7</td>
<td>12.4 – 66.9</td>
<td></td>
</tr>
<tr>
<td>Cho et al. 46</td>
<td>-</td>
<td>0.198 ± 0.031</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nguyen et al. 47</td>
<td>0.089 – 0.121</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nariya et al. 48</td>
<td>0.15-0.16</td>
<td>0.345</td>
<td>0.4-0.6</td>
<td>-</td>
<td>0.6 – 1.5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

4.4. Ectoine Production

4.4.1. Indeterminate Tests

Unfortunately, the ectoine production of *M. alcaliphilum* was unable to be quantified in this work. Difficulties with the HPLC analysis resulted in a failure to be able to accurately determine ectoine concentration of the produced samples. Two example chromatograms are provided to show the issues encountered during HPLC analysis for determining ectoine content of samples. In general, the retention time was extremely poor prior to troubleshooting, lasting approximately as long as the column void time, indicating no interaction of the sample with the column (Figure 13).
While washing the column did improve retention time slightly, the peak shape worsened, with the peak splitting and becoming wider (Figure 14). The resulting poor peak shape is problematic, as split peaks and peak asymmetry may result in unreliable or inaccurate interpretation of results.
4.4.2. Pitfalls and Obstacles

A number of factors may have contributed to the inability to accurately quantify ectoine in the samples tested via HPLC. During the first attempt to use Polaris NH$_2$ column, 0.1% Trifluoracetic Acid (TFA) was mistakenly added both to the acetonitrile and H$_2$O that were used as the mobile phase. TFA is an extremely electronegative compound, and it is likely that upon entry into the column, the TFA strongly interacts with and binds the amine groups within the column. This resulted in a retention time of the compounds through the column equal to the void time, or in other words no interaction occurred between the column and the sample.

In an attempt to remove TFA bound inside the column, the column was flushed with pure H$_2$O and pure acetonitrile in 50/50, and later 75/25, and 90/10 mixtures (H$_2$O/Acetonitrile) at various flow rates per recommendations given by Agilent applications engineers. This wash cycle was repeated several times. The flushing resulted in increased retention times, but peak shape quality of injected ectoine standards suffered considerably. The peak width increased dramatically, and eventually split peaks were observed in the analyzed ectoine standards. The guard column was also replaced, but issues persisted. These issues could be due to creation of a void space in the column. It is possible that the column stationary phase was deteriorated or dissolved.

Upon replacing the column, the method was attempted again, this time using pure acetonitrile and H$_2$O for the mobile phase, with no TFA added. Additionally, care was taken to purge the HPLC system before attaching the column and guard. Unfortunately, retention time of the ectoine standard shifted rapidly over the first few sample injections until once again it became approximately equal to the void time. Again, flushing the column with a mobile phase at a high water to organic solvent ratio helped increase the retention time, but at the cost of poor
peak shape. After over 10 cycles of flushing, equilibrating, and sampling, the method was abandoned.

4.4.3. Recommendation for Future Analyses
When using the Polaris NH₂ column used in this project, or a similar column, it would be recommended, if possible, to avoid use of an HPLC that is being used or has recently been used for separation techniques which utilize an incompatible compound, such as a highly electronegative chemical. Additionally, avoiding use of HPLC systems that are being used or have recently been used with reverse phase separation techniques may be advisable. It is possible that the HPLC system used in this project was not purged sufficiently prior to use with the Polaris NH₂ column, which may have resulted in introduction of an incompatible residue from the system into the column. Thus, if other HPLC separations are being performed on the system, care should be taken to purge the system thoroughly.

While HILIC phase separation techniques often yield methods that have high analyte sensitivity and limit of detection, the associated columns are often more difficult to use than more common reverse phase C18 columns. Additionally, NH₂ columns may be more susceptible to rapid stationary deterioration, which can affect retention time, peak symmetry and reproducibility. Because of these factors, it may be advisable to perform reverse phase chromatography with a C18 column (similar to Cantera et al.²⁴) or TSK-GEL column (similar to Ling-hua et al.⁴⁹) for ectoine separation and analysis, unless the method is not found to have a sufficiently high limit of detection or sensitivity.

4.5. Additional Recommendations
In future work, it may be beneficial to determine the kinetic constants discussed in this report using a larger reactor size. The relatively large ratio of sample volume to reactor
growth medium volume used in most of the tests described in this report may be a source of variability in culture behavior. By using a larger reactor that contains a larger volume of growth medium, sampling the culture broth for analysis of OD, DCW, and ectoine content may have a smaller effect on future growth characteristics and behavior compared to drawing samples of an equal volume when using a smaller reactor. The corresponding increase in headspace volume gained by using a larger reactor may yield similar benefits when dealing with gas content samples used for methane utilization analyses. Thus, increasing the reactor sized used when determining kinetic constants may also increase accuracy and precision of the determined values.

While lab scale attempts to harvest ectoine from *M. alcaliphilum* may be accomplished using bead-beater based homogenization methods, large scale ectoine production using the organism would require alternate methods of product separation such as chemical disruption of the cell or osmotic shock. Ectoine production by *H. elongata* is done using the bio-milking approach, described in Section 1.2.4. A similar approach may be used in ectoine production by *M. alcaliphilum*.

### 4.6. Landfill Emission Treatment – Preliminary Design

The biokinetic constants determined in this work were used to generate a preliminary design for treating landfill emissions, as visualized in Figure 15.
The assumed and calculated input values for this design are summarized in Table 3.

**Table 3 - Assumptions used and calculated values for a preliminary design for treatment of methane emission from North Valley Landfill in Cache County, Utah.**

<table>
<thead>
<tr>
<th>Assumptions used</th>
<th>Calculated Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste in place</td>
<td>Methane Production</td>
</tr>
<tr>
<td></td>
<td>277856 Mg</td>
</tr>
<tr>
<td>Methane emission volume</td>
<td>Biomass Requirement</td>
</tr>
<tr>
<td></td>
<td>808700 m³</td>
</tr>
<tr>
<td>Atmospheric pressure</td>
<td>Total growth medium volume</td>
</tr>
<tr>
<td></td>
<td>86 kPa</td>
</tr>
<tr>
<td>Ambient temperature</td>
<td>50000 L</td>
</tr>
<tr>
<td>22 °C (295 K)</td>
<td></td>
</tr>
<tr>
<td>Specific methane degradation rate</td>
<td></td>
</tr>
<tr>
<td>0.26 g CH₄/gDCW-h</td>
<td></td>
</tr>
<tr>
<td>Growth rate (Dilution rate)</td>
<td></td>
</tr>
<tr>
<td>0.13/h</td>
<td></td>
</tr>
<tr>
<td>Reactor Type</td>
<td></td>
</tr>
<tr>
<td>CSTR</td>
<td></td>
</tr>
<tr>
<td>Liquid Phase Reactor Volume</td>
<td></td>
</tr>
<tr>
<td>50000 L</td>
<td></td>
</tr>
</tbody>
</table>

A landfill gas emission model (LandGEM) created in 2019 for the new North Valley Landfill, in Cache County, Utah was used to estimate methane emission quantity. The LandGEM model is based on a spreadsheet provided by the EPA. For the year 2022, the model uses a projected 277856 Mg of waste in place, resulting in an estimated annual methane emission volume of 808700 m³. Using atmospheric pressure of 86kPa and temperature of 22 °C, this is equivalent to approximately 510.8 Mg CH₄/yr., or 58.3 kg CH₄/h.

Bioremediation of this methane could be accomplished using a *M. alcaliphilum* based fermenter system, consisting of a pretreatment holding vessel, and a treatment fermenter. In such a system, the pretreatment holding vessel would provide an environment in which growth medium is agitated and sparged with the captured landfill gas, and either air or pure O₂. This purpose of the pretreatment step would be to solubilize the landfill gas into the growth medium, allowing for the influent of the treatment fermenter to be a pre-
saturated growth medium. Design of this pretreatment step would likely require testing beyond the scope of this project to evaluate solubility, mass transfer limitations, and mixing requirements.

The preliminary design for the treatment fermenter can be initiated using biokinetic constants evaluated in this study, in conjunction with estimates for landfill gas production. Using the previously calculated $q_{\text{CH}_4} = 0.26 \text{ gCH}_4/\text{gDCW-h}$, bioremediation of the methane production estimate using the LandGEM model would require approximately 225 kg DCW of biomass in growth phase ($\mu=0.13 \text{ h}^{-1}$). As such, using continuous stirred tank reactor (CSTR) treatment fermenters, a total liquid medium volume of approximately 50000 L at an approximate biomass concentration of 4.5 g/L would be required for treating all methane produced by the landfill (additional sizing may be required to provide a gas headspace). With dilution rate of the fermenters, $D$, set to 0.13 h$^{-1}$, the total resultant flow rate of growth medium would be 6500 L/h. It is possible that the large reactor volume requirement could be significantly reduced through reactor designs that allow for increased biomass density or increased solubility of gases (through various sparging techniques).

5. Conclusion

Methane emissions, especially dilute methane emissions from anthropogenic sources are important to remediate. Biological remediation of these emissions is possible through fermentation using the bacterium *M. alcaliphilum*. Ectoine production tied to this process provides an economic incentive for large scale methane oxidation.

Biological constants, including growth rate ($\mu$), biomass yield ($Y_{x/s}$), specific consumption rate ($q_{\text{CH}_4}$), and a correlation between methane oxidation and carbon dioxide production for *M. alcaliphilum* are reported. Treatment of a synthetic landfill gas using *M.
*alcaliphilum* was shown, indicating the feasibility of using the bacterium for treating landfill gas, as well as other methane sources such as wastewater treatment processes.

**Acknowledgement**

I would like to thank Judith Sims and Dr. Ronald Sims for giving me the opportunity to develop this project and for providing the necessary funding to make the project possible. I appreciate their input, direction, and flexibility in helping to form the vision for this work. I also want to thank Dr. Charles Miller and Dr. Jixun Zhan for providing access to laboratory workspace and instrumentation. A special thanks is given to Dr. Sara Cantera for subject matter expertise and advice regarding *M. alcaliphilum*.

Finally, thanks to family and friends for their encouragement and support of my academic and life endeavors.

**References**

2. Global Methane Initiative, Global methane emissions and mitigation opportunities. 2015.


