## ABSTRACT

SCHUPP, SIERRA LAUREN. Evaluation of the Temperature Range for Biological Activity in Landfills Experiencing Elevated Temperatures. (Under the direction of Dr. Morton A. Barlaz).

A limited number of landfills in the U.S. have exhibited temperatures of 80 – 100 °C. These landfills, referred to as elevated temperature landfills (ETLFs), require intensive management as the elevated temperatures are a threat to a landfill's gas and leachate collection systems, liner integrity, and geotechnical stability. In addition to increasing temperature trends in gas wells, key indicators of elevated temperatures are changes in gas composition and quantity, specifically a decrease in the methane concentration. The objectives of this research were to (1) determine an expected upper temperature limit for methanogenesis and fermentation in landfills and (2) determine qualitatively if microbial populations can adapt or acclimate when perturbed to either lower or higher temperature conditions. Sixteen samples excavated from two Southeastern landfills exhibiting elevated temperatures were utilized as inocula for small-scale (30 – 160 mL) and reactor-scale (2-L) perturbation experiments. A metagenomic analysis was conducted on the samples before and after incubation to identify community shifts in the archaeal population. Results showed that methane production is possible in landfilled waste up to 62.5 to 67.5 °C, albeit with significantly reduced yields. It appears that a smaller population of methanogens capable of activity at elevated temperatures may be responsible for reduced methane generation beyond the observed optimal range of 47.5 to 57.5 °C. The results will improve the predictability of methane generation in ETLFs and also support discussions with regulatory agencies on appropriate upper limits for gas wells. In addition, 16S rRNA sequencing revealed that *Methanothermobacter* likely plays a key role in facilitating methane production in landfills experiencing elevated temperatures. Finally, there was evidence that fermentation is the dominant biological process between 72.5 to 77.5 °C. The implications of the higher temperature

limit for fermentation are that intermediates can accumulate and act as early indicators of an ETLF, and that fermentation can contribute to heat generation at temperatures above the range for methanogenesis. An existing batch reactor model estimated that fermentation above the upper limit of methanogenesis could add an additional 3 to 10 °C to landfill temperatures.

# Evaluation of the Temperature Range for Biological Activity in Landfills Experiencing Elevated Temperatures

by
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## DEDICATION

I would like to dedicate my thesis to my parents, Nicole and Ken, and to my sister, Sydney.

Thank you for your unconditional love and support.

## BIOGRAPHY

Sierra was born and raised in Raleigh, NC. She received a Bachelor of Science degree from North Carolina State University in Environmental Engineering in 2018.

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# CHAPTER 1 - INTRODUCTION

In the United States, a limited number of municipal solid waste (MSW) landfills have reported waste and gas wellhead temperatures in excess of 80 – 100 °C (Stark et al., 2011; Martin et al., 2012; Benson, 2017; Jafari et al., 2017a). This range is above the temperature of waste excavated from typical landfills which has been reported as 20 to 65 °C (Hanson et al., 2010; Yeşiller et al., 2005, 2015). Landfills experiencing heat accumulation over a broad area are often referred to as elevated temperature landfills (ETLFs). Circumstances surrounding the initial cause of increasing temperatures in landfills as well as the effects of elevated temperatures on landfill performance, specifically in regard to methane production, are understudied. Landfill operational challenges associated with elevated temperatures have been reported to include some or all of increased leachate strength and quantity, accelerated settlement, damage to leachate and gas collection systems, increased temperature on the geomembrane liner, and malodors (Stark et al., 2011; Jafari et al., 2017a).

Methane ($CH_4$) and carbon dioxide ($CO_2$) are the final products in the anaerobic biodegradation of waste and typically comprise 50 – 60 % and 40 – 60 % of landfill gas, respectively. As temperatures increase, landfills have reported decreases in the $CH_4/CO_2$ ratio to values that approach 0, indicating the cessation of methane generation (Stark et al., 2011; Martin et al., 2012; Benson et al., 2017; Jafari et al., 2017a,b). However, details of the extent of inhibition as a function of temperature are lacking and useful for at least two reasons. Recently, Hao et al (2017, 2020 submitted) published a model of heat generation and accumulation in a landfill. This model is dependent in part on the rate of heat generation from anaerobic biological waste decomposition. Thus, information on the impact of temperature on methane generation is needed to better parameterize landfill heat generation models. Second, under the New Source

Performance Standards (NSPS) [40 CFR § 60.753(c) and § 60.755(a)(5)], landfills with gas wells that operate above 55 °C must request a variance. This temperature was originally selected based on the assumption that higher temperatures are indicative excessive vacuum on the gas well that results in air intrusion and the risk of initiating a landfill fire. More recently, it has become apparent that landfills can operate at gas well temperatures above 55 °C without air intrusion or a fire (Sadri et al., 2008). However, better data are needed to evaluate appropriate upper temperature limits for landfill gas collection wells.

While there have been many studies on the effect of temperature on methane generation in anaerobic digestors, there has been little study of temperature impacts under landfill-relevant conditions (Pfeffer, 1974; Cecchi et al., 1993; Tchobanoglous et al., 1993). Hartz et al. (1982) showed that the optimal temperature for refuse methanogenesis was 41 °C but did not evaluate temperatures above 48 °C. As such, predictive models for heat accumulation lack data on temperature limits of anaerobic reactions, particularly in the thermophilic range. Furthermore, there are a limited number of studies that have evaluated traditional landfill microbiology (Staley et al., 2018) and there have been no studies to date on microbial communities in landfills experiencing elevated temperatures.

The objectives for this research were to 1) determine an expected upper temperature limit for methanogenesis and fermentation in landfills and 2) determine qualitatively if microbial populations can adapt or acclimate when perturbed to either lower or higher temperature conditions.

# CHAPTER 2 - LITERATURE REVIEW

This review will summarize literature available on elevated temperature landfills, provide an overview of anaerobic biodegradation, discuss relevant microbiology, and finally summarize research on the impact of temperature on anaerobic biological activity in landfills.

## Elevated Temperature Landfills

This section will review four published case studies of landfills that have experienced elevated temperatures (Sites 'A' – 'D'), with a focus on the reported site-specific impacts of temperature on biological activity.

Site A (Stark et al., 2011; Martin et al., 2012)

Site A is a Subtitle D landfill that accepted approximately 5440 metric tons of municipal and industrial waste per day from 1991 through 2006. Aluminum production waste (APW) comprised about 2.6 % of the total waste stream, resulting in the disposal of ~0.54 - 1.03 million metric tons over time. Initial signs of elevated temperatures, including odors, gas and leachate outbreaks, decreases in the $CH_4$ content of the gas, and waste temperatures exceeding 150 °C, began to appear in 2005 after an excessively high vacuum was applied to the horizontal gas main.

Monitored landfill gas temperatures were originally slightly below 55 °C, excluding an instance of air intrusion that temporarily dropped temperatures in the gas wells to about 20 °C in 2005. The impact of the observed air intrusion is unclear as the primary gas ratio of $CH_4/CO_2$ returned to normal levels (~ 1.4) following this event, however, both gas flow rates and the methane composition substantially decreased later. Beginning in 2007, gas well head temperatures rapidly increased from about 55 °C to 99 °C. Ultimately, gas and waste temperatures were

measured up to 110 °C and 149 °C, respectively. Elevated temperatures in the gas wells were observed over 6 years.

Methane concentrations began to gradually decrease to about 30 % before gas temperatures were measured in excess of 55 °C. The authors attributed this as likely due to temperatures increasing in the waste mass that were not captured immediately by surrounding wells. This could also be attributed to a decrease in gas production relative to the area of influence. As waste temperatures increased to approximately 93 °C, carbon monoxide (CO) levels typically ranged from 2000 – 5000 ppmv and hydrogen concentrations of 20 – 40 % were reported. In gas wells with temperatures greater than 88 °C, an increase of $CO_2$ content to 60 – 80 % was observed. The $CH_4/CO_2$ ratio dropped sharply to zero, while overall gas production and pressure increased. No significant $O_2$ levels, above 5 % v/v, were detected during the period of elevated temperatures.

Changes in landfill gas composition were attributed to the progression of an aluminum waste reaction and subsequent subsurface combustion reaction. Hydrogen can be produced from an amphoteric reaction between aluminum and water under alkaline conditions. This reaction, likely exacerbated by leachate recirculation, was identified as the most probable cause of temperature increases and hydrogen accumulation in this landfill. The heat from the exothermic aluminum waste reaction was thought to initiate smoldering combustion in the surrounding waste mass that propagated and was apparently sustained by the presence of low oxygen at subsurface depths. The high CO levels were considered to be a by-product of incomplete combustion, while the $CO_2$ increase was attributed to an increase in waste combustion. The atypical production of these gases was exclusively explained by the aluminum corrosion and smoldering combustion reactions because the gas concentrations were not considered to be consistent with biological

degradation. Accordingly, the methane decrease was likely due to inhibition of methanogens above temperatures of 80 °C.

Site B (Jafari et al., 2017b; Jafari et al., 2018)

Site B was a former limestone quarry. 13 million $m^3$ of municipal, commercial, and industrial waste was accepted between 1974 and 2005. The landfill is unlined and the waste thickness is approximately 100 m. Post-closure care began in 2005 and elevated gas temperatures were first reported in 2010. Operators noted a decrease in methane content from 50 % to 1 %. in two gas wells in which the temperature increased to 90 °C. The site experienced associated leachate outbreaks and settlement.

Jafari et al. (2017b) began to monitor trends associated with changing temperatures in 2012. The authors used four downhole thermocouple arrays near two gas wells located ~200 m from the location where elevated temperatures were observed. Initially, the center of the waste mass had a temperature of 70 °C, while the gas temperatures were recorded as 55 °C. However, after the approach of what the authors described as the 'temperature front', the nearby gas well temperature reportedly reached the waste temperature. A maximum waste temperature of 135 °C (gas well = 105 °C) was recorded and 40 % of the waste depth in this location experienced temperatures equal to or in excess of 80 °C.

CO concentrations were reported above 1500 ppmv in one of the wellheads throughout the monitoring period. It is unclear if air intrusion was detected in this well. In the second gas well, nitrogen and oxygen concentrations were noted at one time point to reach 30% and 2.3%, respectively. This could indicate air intrusion via overapplication of gas well vacuum. It is unclear if CO was detected in this well. The authors did not report methane, hydrogen, or carbon dioxide levels in the studied gas wells.

Jafari et al. (2017b) reason that air intrusion potentially introduced oxygen in the subsurface and triggered smoldering combustion that rapidly propagated the temperature front. The authors suggest that "thermal degradation via smoldering combustion and/or pyrolysis are the dominant processes inside the landfill." This was understood to be corroborated by CO levels and the high extent of settlement.

Site C (Jafari et al., 2017a,b; Benson, 2017)

Site C is a Subtitle D MSW landfill that received approximately 9,000 tons of waste per day beginning in 1997. The cell of initial concern was closed in 2005 and temperatures ranging from 68 – 95 °C in five gas wells were reported in 2009. Routine monitoring also revealed CO concentrations above 500 ppmv (up to 10,200 ppmv) in wells with temperatures greater than 55 °C. These observations led to a temporary shut-down of the gas system to prevent air intrusion. However, when operations were resumed it was observed that an exothermic reaction had spread and caused temperature increases of 20 – 45 °C and substantial decreases in the primary gas ratio of several wells. The elevated temperatures propagated over 4 years to neighboring cells and the landfill experienced extensive settlement. Waste thickness ranged from 50 to 80 m and a majority of the waste was saturated or below the water table.

Jafari et al. (2017a,b) reported temperature measurements at gas wellheads, in leachate sumps, and in downhole waste samples when possible. Landfill gas temperatures were consistently 20 °C lower than waste temperatures. In the triggered cell, one gas well temperature rose from 40° C to 90 °C within a year. After 2 years of stable elevated temperatures, this well dropped to 20 °C due to air intrusion, confirmed by oxygen levels of 15 %, before again rising to 90 °C. While the landfill gas temperature also dropped in another well, no $O_2$ data was provided and the sump data

did not register a temperature drop. This would imply that air intrusion into gas wells likely did not affect the entire waste mass.

Jafari et al. (2017a,b) demonstrated that changes in gas composition occurred gradually and about 1 – 2 months prior to an observed increase in gas well head temperatures to 55 °C in several wells. The $CH_4/CO_2$ ratio typically dropped from 1.4 to 0.2 as temperatures increased to ~75 °C. Elevated temperatures also corresponded with $H_2$ concentrations of 20% and steady CO levels of 2000 – 3000 ppm. Elevated oxygen in gas wells, greater than 5% v/v, was linked with temperature drops to approximately 20 °C but was also sporadically detected in regions of elevated temperatures. Air intrusion was likely from compromised gas wells. Although it did not have any apparent impact on the primary gas ratio, both $CH_4$ and $CO_2$ decreased in the system.

As for Site C, Jafari et al. (2017a,b) proposed that heat and CO accumulation could be explained by smoldering combustion and/or pyrolysis. Hydrogen production at this site was attributed to either acetogenesis becoming the dominant biological process at elevated temperatures, a water-gas shift reaction, or pyrolysis of cellulosic materials. The authors also suggested that the primary gas ratio is a better measure of identifying an elevated temperature event than lagging changes in gas well temperatures.

In an independent study of Site C, Benson (2017) observed an abrupt and substantial temperature increase in 85 % of monitored gas wells following the re-start of the system over a 5-year period. While the temperature in some gas wells increased at faster rates than others, overall the final temperature increases were not dependent on the original landfill gas temperatures. Thermocouples recorded maximum temperatures at depths of 20 m where the waste was fully saturated.

Gas well data from Benson (2017) showed that temperatures were originally close to 55 °C and had a primary gas ratio greater than 1.0, which indicated stable methanogenic conditions. As the wells experienced a sharp 20 °C increase following the system re-start, the $CO_2$ content of the gas also sharply increased to 60 – 80 %, while the methane content decreased to less than 30 %. Overall it was observed that the primary gas ratio dropped to 0 – 0.5 when gas well head temperatures reached approximately 70 °C. No significant oxygen was reported and no CO or $H_2$ data was presented.

Benson (2017) cited an undefined exothermic reaction as the cause for heat accumulation and clarified that combustion is unlikely due to waste saturation. Later Comments by Barlaz et al. (2018) also point out that the lack of a continuous oxygen source, in addition to the fact that the waste was fully saturated, suggesting that combustion was unlikely the cause of initiation and propagation of heat at this site. The authors instead suggested the consideration of exothermic pyrolysis.

Site D (Sadri et al., 2008)

Site D is a Subtitle D landfill. In addition to a waste stream of 26% MSW, the site accepted incinerator ash from a nearby waste-to-energy facility (31%), bulky waste (26%), biosolids (6%), automobile shredder residue (7%), and miscellaneous wastes (4%). Both temperature and gas composition in this landfill were found to be atypical of MSW landfills. Elevated waste mass temperatures were first noted in 2005 during the installation of new gas wells.

Monitoring of both waste and gas temperatures from 2005 to 2008 confirmed the landfill was experiencing elevated temperatures. Subsurface waste temperatures of up to 84 °C were recorded. Accumulation of both $H_2$, up to 26%, and CO, up to 170 ppmv, were detected in some gas wells. The accumulation of these gases corresponded with a reduction in methane production.

Based on not only the field data, but also laboratory reactor data using samples from the site that successfully replicated the observed field conditions, the authors determined that the accumulation of CO and $H_2$ were end-products of biological and chemical processes rather than a subsurface oxidation or smoldering event.

Summary

The case studies have both similarities and differences related to the initiation of the elevated temperatures, manner of heat propagation, and associated symptoms. Several of the indicators for the progression of the aluminum waste reaction that took place in Site A, notably $H_2$ accumulation, were also observed at Sites C and D. One measurable difference offered by Martin et al. (2012), was that temperatures can reach up to 260 °C for landfills experiencing combustion, whereas the maximum appears to be approximately 93 °C for APW reactions only. However, given that Site A reported temperatures comparable to the other studied sites without APW and with suggested combustion, the data do not support this claim. There are a number of factors known to influence heat accumulation in landfills that were not considered, such as non-MSW waste placement and depth, makes it unwise to attribute temperature changes to a single reaction (Hao et al., submitted for publication).

In addition, there are limitations to the explanation that combustion was responsible for heat accumulation as suggested for Sites A, B, and C. Much of the data presented for oxygen levels in the subsurface waste mass are insufficient as well as inconsistent with the temperature trends observed. Therefore, as suggested by Barlaz et al. (2018), future studies should focus on the influence of exothermic pyrolysis as opposed to combustion as a possible source of heat, CO, and $H_2$ accumulation. Furthermore, Sadri et al. (2008) was the first to propose anaerobic biological

activity, specifically fermentation, as the main plausible source of $H_2$ and $CO_2$ accumulation at Site D.

Overall, the underlying chemical and/or biological mechanisms that can explain the observed accumulation of heat and atypical gases in landfills experiencing elevated temperatures still remain somewhat uncertain and are likely site-specific. Therefore, this review aims not to present a universal insight of elevated temperature landfills, but to use these publications to understand more about the impact on biological activity.

## Overview of Anaerobic Biodegradation

The anaerobic biodegradation process must first be considered to understand what specifically is happening in elevated temperature landfill systems. During anaerobic biodegradation, a microbial consortium is responsible for the conversion of organic waste to $CH_4$ and $CO_2$ (Figure 1).

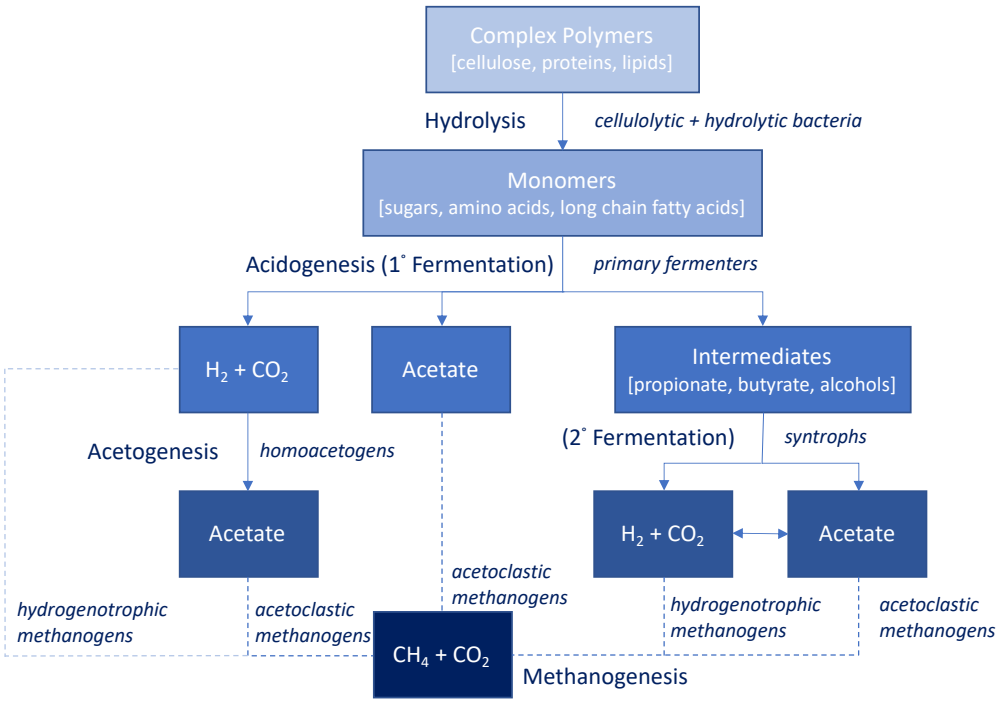


**Figure 1.** An overview of the complex processes and components that constitute the anaerobic biodegradation of organic materials such as solid waste (adapted from Angelidaki et al., 2011 & Madigan et al., 2017)

Complex polymers are hydrolyzed to monomers in the first step of the anaerobic biodegradation of solid waste. Carbohydrates, namely cellulose and hemicellulose, are the major biodegradable components of refuse but proteins and lipids are biodegraded as well. These substrates are converted to sugars, amino acids, and long-chain carboxylic acids by cellulolytic and hydrolytic bacteria. The hydrolysis products (monomers) are then converted to $H_2$, $CO_2$, acetate, short-chain volatile fatty acids (VFAs), alcohols, and ammonia through a process known as fermentation. Short-chain VFAs, such as propionate and butyrate, and alcohols are intermediates that can be further oxidized to $H_2$, $CO_2$, and acetate by syntrophic microorganisms, namely acetogens. Finally, acetate and $H_2$ plus $CO_2$ are converted to $CH_4$ plus $CO_2$ by acetoclastic and hydrogenotrophic methanogens, respectively. The ratio of $CH_4/CO_2$ in biogas should theoretically be greater than or equal to 50/50 (1) in stable systems in which carbohydrates are the major substrate.

If any particular microbial group involved in this process becomes inhibited, the entire process will become imbalanced, resulting in a build-up of intermediates rather than the desired methane end-product. A main indicator of a problem with any system involving anaerobic biodegradation is low methane content in biogas. Since fermentation precedes methanogenesis, accumulation of fermentation intermediates that are typically consumed by methanogens, can be helpful indicators of underlying biological mechanisms. Mainly, if methanogenesis becomes inhibited while fermentative activity continues, the system will become imbalanced and accumulate $H_2$, $CO_2$, and VFAs (Valdez-Vazquez et al., 2005; Aghdam et al., 2017).

However, Figure 1 shows that fermentation is not a straightforward process and the major intermediates are both produced and consumed via several biological pathways. Acetogenesis is the production of acetate, propionate and butyrate. Homoacetogens, better defined as hydrogen-

utilizing acetogens, use $H_2$ plus $CO_2$ to form acetate via the Wood-Ljungdahl pathway (Drake, 1994; Angelidaki et al., 2011). In contrast, short-chain fatty acids and alcohols are oxidized to acetate by hydrogen-producing acetogens, which can be defined as syntrophic acetate oxidation (Angelidaki et al., 2011). Interspecies hydrogen transfer in this process is crucial as syntrophs are obligately dependent on methanogens, homoacetogens, or other hydrogen-utilizing microorganisms that may be present (Stams & Plugge, 2009; Madigan et al., 2018). From an energetic standpoint, this means that the conversion of fermentation intermediates (propionate, butyrate) require the partial pressure of $H_2$ to be maintained below a certain level as demonstrated in Table 1.

**Table 1. Major reactions in anaerobic biodegradation of organic materials such as solid waste[a]**

| Reaction Type | Reaction | (kJ/reaction)$^0$ | | | |
|---|---|---|---|---|---|
| | | $\Delta G^b$ | $\Delta G^c$ | $\Delta G^d$ | $\Delta G^e$ |
| **Glucose fermentation** to acetate, $H_2$, and $CO_2$ | $Glucose + 4H_2O \rightarrow 2\ acetate^- + 2HCO_3^- + 4H^+ + 4H_2$ | -207 | -319 | -329 | -339 |
| **Glucose fermentation** to butyrate, $H_2$, and $CO_2$ | $Glucose + 2H_2O \rightarrow butyrate^- + 2HCO_3^- + 3H^+ + 2H_2$ | -135 | -200 | -205 | -211 |
| **Butyrate fermentation** to acetate and $H_2$ | $Butyrate^- + 2H_2O \rightarrow 2\ acetate^- + H^+ + 2H_2$ | +48 | -14 | -19 | -24 |
| **Propionate fermentation** to acetate, $H_2$, and $CO_2$ | $Propionate^- + 3H_2O \rightarrow acetate^- + HCO_3^- + H^+ + H_2$ | +76 | -5 | -7 | -10 |
| **Acetogenesis** from $H_2$ + $CO_2$ | $4H_2 + 2HCO_3^- + H^+ \rightarrow acetate^- + 4H_2O$ | -105 | -12 | -4 | +4 |
| **Methanogenesis** from $H_2$ + $CO_2$ | $4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ | -136 | -37 | -28 | -20 |
| **Methanogenesis** from acetate | $Acetate^- + H_2O \rightarrow HCO_3^- + CH_4$ | -31 | -25 | -24 | -24 |

[a] Data adapted from Zinder & Koch, 1984; Angelidaki et al., 2011; Madigan et al., 2018
[b] free-energy change under standard conditions (solutes, 1 M; gases, 1 atm, 25 °C)
[c] free-energy change in typical anoxic freshwater ecosystems at 25 °C (fatty acids, 1 mM; $HCO_3$, 20 mM; glucose, 10 μM; $CH_4$, 0.6 atm; $H_2$, $10^{-4}$ atm)
[d] free-energy change in typical anoxic freshwater ecosystems at 50 °C
[e] free-energy change in typical anoxic freshwater ecosystems at 75 °C

Hydrogen as an Intermediate

Table 1 reveals that ecological factors play a role in what processes occur in-situ. For example, despite $H_2$ and $CO_2$ being more energetically favorable substrates for methane formation relative to acetate under standard conditions, approximately two-thirds of methane production in both mesophilic and thermophilic anaerobic digesters is carried out by acetoclastic methanogens (Zinder, 1990, 1993). This has been attributed to the high concentrations of acetate selecting for an acetoclastic methanogen population over time (McMahon et al., 2001; Montero et al., 2008). It was also determined that homoacetogens outcompete hydrogenotrophic methanogens for $H_2$ in lab-scale landfill studies (Chen et al., 2003), likely based on their substrate utilization efficiency under low $H_2$ partial pressures (Conrad, 1996). The predominance of any group of microorganisms will largely depend on the environmental conditions. Levels of different by- or end- products can provide insight for what is happening on a microbial scale. $CO_2$ is not useful indicator because it is an end-product of both anaerobic and aerobic biodegradation and has several biological and abiotic sources. Therefore, it will only be mentioned in association with intermediates such as hydrogen.

A pilot-scale anaerobic digester loaded with the organic fraction of MSW was successfully transitioned from mesophilic (37 °C) to thermophilic (55 °C) conditions in a matter of weeks, confirming the previous results of Rimkus et al. (1982) (Cecchi et al., 1993). Hydrogen was consistently present in the digester gas, up to 500 ppm, during both the transition period and steady state thermophilic conditions. Since the transition was gradual, the detection of hydrogen was attributed to fermentation briefly dominating over methanogenic activity with each rise in temperature. This logic is supported by the fact that methanogens typically grow more slowly than fermentative microorganisms (Zinder, 1993). The authors also speculated that the dominance of

acetoclastic methanogens over hydrogenotrophic is consistent with the presence of $H_2$. This study serves as an example of the sensitivity of functional microbial groups to temperature changes, evidence for adaptation, and the potential for biological hydrogen accumulation.

Sites A, C, and D exhibited accumulation of $H_2$ in gas well heads along with a $CH_4/CO_2$ ratio less than 1 and increased leachate strength, indicating VFA accumulation. $H_2$ and VFAs are fermentation intermediates consumed by methanogens. Potential non-biological sources of hydrogen are metal corrosion reactions and pyrolysis (Stark et al., 2011; Aghdam et al., 2017; Hao et al., 2017, Neves et al., 2011). The higher concentrations of $H_2$ observed in landfills may indicate the occurrence of both biological and chemical processes.

Acetate as an Intermediate

Although acetate formation is often linked to hydrogen formation, fermentation of glucose and homoacetogenesis are favorable regardless of hydrogen partial pressure (Table 1). Therefore, it is inferred that a build-up of acetate may serve as an alternative indicator of inhibition than hydrogen. However, given the complexity of the processes discussed, it is important to determine the temperature at which different steps in the anaerobic biodegradation process are inhibited to fully understand this process in an applied scenario.

As a simplified example, if methanogens are inhibited but homoacetogens are not, it could be reasoned that $H_2$ and $CO_2$ levels will remain low and only acetate accumulation will be measurable. Inter-microbial distances between acetogens and hydrogen-scavenging microorganisms have been shown to influence specific growth rates (Batstone et al., 2006). This may be important in field conditions depending on the rate of gas migration that could continue to keep $H_2$ partial pressure low within the waste mass. In addition, although the majority of ETLFs

have reported increased leachate strength, it may not be as practical to measure VFA production and track leachate migration in comparison to monitoring hydrogen in gas wells. At some point, an upper temperature limit of biological activity will be reached and anaerobic biodegradation of waste will cease along with the production of all VFAs.

Carbon Monoxide as an Intermediate

Although carbon monoxide (CO) was not included in the overview of anaerobic biodegradation, it is an important intermediate typically detected only in trace amounts. Certain methanogens, acetogens, and sulfate-reducers are capable of oxidizing CO, predominately via carbon monoxide dehydrogenase among other potential pathways (Conrad & Thauer, 1983; Rich & King, 1999) but the substrate serves a much less diverse consortium than $H_2$ (Conrad, 1996). These same microorganisms are also able to produce CO (Daniels et al. 1977; Enzmann et al., 2018), which implies they are the microorganisms responsible for regulating CO levels in-situ (Conrad, 1988). Biological accumulation of CO is typically limited for this reason.

Rich & King (1999) noted that CO levels would increase temporarily after organic loading and demonstrated that when methanogenesis was inhibited, the rate of CO consumption decreased by 30 % in peat samples. Generation of CO in peat samples was linked with hydrogen generation, likely from the incomplete oxidation of fatty acids. Similarly, Haarstad et al. (2006) showed an increase in CO from 100 to 400 ppm following intentional inhibition of methanogenesis in lab-scale solid waste reactors. Elevated CO in landfill gas is generally defined as greater than 1000 ppm (FEMA, 2002), as CO measured in landfill gas is about 100 ppm on average (Haarstad et al., 2006: Christensen & Manfredi, 2011). As previously discussed, caution should be taken before attributing elevated CO to combustion of waste given the limited conditions required for an actual

landfill ‘fire’ to occur. Chemical reactions, such as pyrolysis, are more practical potential chemical sources of CO in landfills (Ciuta et al., 2014, Hao et al., 2017; Barlaz et al., 2018).

Although it is known that CO is a byproduct with the potential to accumulate to some extent biologically and chemically, little research has been done to study this mechanism in landfills or to identify the dynamics of microorganisms potentially involved. It has been found that acetogens utilize CO most readily (Rich & King, 1999) and thus homoacetogens have been studied the most (Conrad, 1996). Ferry (2010) summarized the information available on the ambiguous role of CO in methanogenesis and acknowledged limitations to our understanding.

The microorganisms involved in each process and steps discussed for individual intermediates or end-products typically have different temperature ranges for activity and some are more adaptable to change than others. The role of a microorganism in various steps of the process can help clarify from a microbial perspective what biological activity, or lack thereof, may be expected at specific temperatures. These concepts will be addressed in the following sections.

**Microbial Diversity and Dynamics in Solid Waste Decomposition**

A microbial community analysis of landfills exhibiting elevated temperatures has never before been conducted, however, microbial populations and dynamics in conventional landfills have been studied. Communities were found to be spatially unique and distinctly influenced by waste composition, temperature, and moisture (Sawamura et al., 2009; Wang et al., 2017; Staley et al., 2018). Thermophilic and mesophilic anaerobic digestion systems each have distinguishable microbial profiles and it may be assumed that this will largely hold true between ‘mesophilic’ and ‘thermophilic’ landfills (Azizi et al., 2016). To identify potential overarching microbial

community structures in anaerobic biodegradation of solid waste, a synthesis of several studies is presented.

Methanogens belong to the *Archaea* domain but work closely with *Bacterial* communities to degrade organic matter (Bareither et al., 2013). Fresh refuse and cover soil contain a diverse population of microorganisms that are responsible for the anaerobic biodegradation of waste (Staley et al., 2012). Therefore, the populations present in-situ are crucial for determining the potential for microbial acclimation. *Clostridium* and *Thermotoga* were found in landfill field samples (Sawamura et al., 2010; Wang et al., 2017) and lab-scale landfill refuse studies (Staley et al., 2011b, 2018; Bareither et al., 2013). *Clostridia* are obligate anaerobes involved in cellulose hydrolysis to $H_2$ and some species prefer thermophilic conditions (Westlake et al., 1995; Holden & Topçuoglu, 2019). Likewise, *Thermotoga* is an obligate anaerobe and extreme thermophile capable of utilizing carbohydrates and major fermentation products (Counts et al., 2017). The presence of these *Bacteria* in mesophilic landfill environments demonstrates how it may be possible for a successful microbial shift to occur as temperatures rise. Bareither et al. (2013) linked *Clostridia* and *Thermotogae* phyla to hydrogenotrophic methane production. *Bacteroidetes*, a known acetogenic genera, were also found in landfill studies and was linked to acetoclastic methane production (Bareither et al., 2013; Wang et al., 2017). *Bacteroidetes* and *Thermotogae* were found to be most abundant during peak methane generation (Bareither et al., 2013; Staley et al., 2018).

If there exists a healthy *Bacterial* population in the ecosystem, methane production in landfills then hinges on the functional ability of methanogens. There are six orders of methanogens capable of carrying out hydrogenotrophic methanogenesis: *Methanococcales, Methanobacteriales, Methanosarcinales, Methanomicrobiales, Methanopyrales*, and

*Methanocellales* (Enzmann et al., 2018). Of these, only *Methanosarcinales* are capable of acetate-utilization. The *Methanosarcina* and *Methanosaeta* genera dominate in high- and low- acetate concentration environments, respectively (Karakashev et al. 2005; Montero et al., 2008; Staley et al., 2011b). *Methanosarcinales* are largely responsible for controlling methane production in anaerobic digesters (Shaw et al., 2017; Zinder, 1993).

The predominant methanogens found responsible for MSW degradation in lab-scale simulated bioreactor landfills were *Methanomicrobiales*, *Methanosarcinaceae*, and *Methanobacteriales* (Staley et al., 2012, 2018; Bareither et al., 2013). Bareither (2013) found that of these, *Methanomicrobiales* was dominant and the author inferred that the majority of methane was produced via the hydrogenotrophic pathway, in contrast to estimates of the acetoclastic and hydrogenotrophic pathways in anaerobic digestors. *Methanosarcina* were present throughout all stages and given these microorganisms can utilize both acetate and hydrogen, it is possible that hydrogenotrophic methane production is even more significant than is measurable. *Methanosarcina* have been found to grow optimally from 50 – 57 °C and are incapable of growth beyond 60 – 62 °C (Zinder, 1990). While the temperature limit for methane production from acetate conversion lies around 70 °C, hydrogen-utilization has been observed in methanogenic orders in excess of 80 °C (Zinder, 1990, 1993; Angelidaki et al., 2011; Holden & Topçuoglu, 2019). Staley et al. (2018) also found hydrogenotrophic methanogens were most abundant in solid waste biodegradation, specifically under low pH and high acetate conditions. Therefore, since hydrogenotrophic methanogens are appear predominant and capable of enduring higher temperature ranges, it may be expected that their role becomes even larger in landfills experiencing elevated temperatures as demonstrated in an anaerobic digestion study (Pap et al., 2015).

One limitation to the presented laboratory studies is the lack of consideration of waste age as a factor contributing to microbial population dynamics and abundance. All of the landfill case-studies discussed did not experience elevated temperatures until several decades after intial waste disposal. Liu et al. (2011) studied leachate sediments and determined that *Methanosaeta* dominated in aged landfills, which would align with the consumption of VFAs over time. Several thermophilic strains of *Methanosaeta* have been characterized and have been found to also dominate in digester studies (McMahon et al., 2004; Karakashev et al., 2005; Enzmann et al., 2018). In comparison, Tang et al. (2016) excavated eight samples from an MSW landfill and found that 92% of clones were related to the hydrogen-utilizing order *Methanomicrobiales*, namely *Methanoculleus*. This genus was previously only reported in studies conducted under mesophilic conditions until Weiss et al. (2009) found it to dominate in a thermophilic anaerobic digester study. Overall, it is clear that more than one species of methanogens will be necessary to successfully carrying out anaerobic biodegradation in any long-term system, especially one with varying temperature conditions.

Montero et al. (2008) looked at the microbial evolution in stabilization of lab-scale thermophilic-dry anaerobic digesters (55 °C) fed with the organic fraction of municipal solid waste. The microbial community was considered balanced when the $CH_4/CO_2$ ratio reached 50/50, at which point there was a 61/39 ratio of *Bacteria* to *Archaea*. Shaw et al. (2017) also found that the *Archaeal* population is much lower under thermophilic conditions compared to mesophilic in a lab-scale anaerobic digester study. In addition, syntrophic relationships with *Syntrophobacteraceae* under thermophilic conditions diminished significantly, likely due to the microorganism's preference for mesophilic conditions (Shaw et al., 2017). Bareither et al. (2013) found that the more organic substrate available initially, the greater methanogenic populations.

Accordingly, a higher number of methanogens equates to higher methane yields in the accelerated methane phase. Therefore, it may be expected that reduced organic waste fractions in aged landfills and a smaller population of methanogens capable of enduring thermophilic temperatures may limit the potential for methanogenesis in elevated temperature conditions.

The conclusion is that the success of a landfill in terms of methane production will be largely dependent on site-specific conditions and may not consistently implicate the same dominant microorganisms responsible for performance, especially given that landfills are more spatially and temporally heterogeneous than digester systems. Furthermore, considering the potential for different causes of elevated temperatures initially, it cannot be assumed that the presence of an active thermophilic population at one landfill ensures a presence at all. Nevertheless, a basic understanding of the underlying microbial ecology in landfills experiencing elevated temperatures would represent an important development in solid waste engineering. Just as physical and chemical properties may be utilized to identify and diagnose ETLFs, biological properties could also be used in this manner and further reinforces the need to explore microbial processes of landfills operating above the mesophilic range.

**Temperature Effects on Biological Activity in Landfills**

There is a lack of information available for the impacts of elevated temperatures in the context of landfill conditions. Papers have previously reported that methanogenesis completely ceases between 48 – 55 °C based on lab-scale waste studies (Kasali & Senior, 1989, Hartz et al. 1982). This was largely attributed to the idea that the mesophilic population of *Bacteria* and *Archaea* responsible for methane production became inhibited at this temperature. However, the

interpretation of their results is limited as the potential for a thermophilic population was not considered in these studies.

Kasali & Senior (1989) excavated waste from a landfill one month after placement and incubated the waste at four temperature regimes: ambient (18.7), 30, 40, and 55 °C. It was determined that methane generation completely ceased at 55 °C after 7 days. At Day 53, the pH was corrected from 5.7 to 7.2, however, after another 45 days of incubation no methane was produced. Interestingly, VFAs did not accumulate in this sample but solvents, ethanol and butanol, did. In addition, the 40 °C sample was raised to 55 °C and also experienced complete inhibition, although the time period of incubation in this case was not defined. The authors did point out that their results were unexpected as methanogenic digestion of MSW had been reported previously between 58 and 65 °C (Pfeffer, 1974, Cooney & Wise, 1975, Zinder et al., 1984). Kasali & Senior hypothesized their results were due to either a lack of thermophiles in their samples or solventogenesis.

Hartz et al. (1982) tested samples from two landfills and found that 41 °C was the optimum for methane generation during short-term incubations at temperatures ranging between 21 to 48 °C. In a review paper, Ward (1983) discusses the lack of acclimation period, low sample moisture content, and methane production not reported in terms of the organic fraction (VS) as being major conflicting issues with Hartz's claim that methane production ceases above 48 – 55 °C. Since these original publications, considerable research on thermophilic anaerobic digestion has been published and this may be able to clarify some of the discrepancies surrounding the results presented by Kasali & Senior (1989) and Hartz et al. (1982).

While there are several key differences between anaerobic digestors and landfills, the biological mechanisms governing activity in thermophilic anaerobic biodegradation are the same.

The optimum temperature range for methanogenesis in solid waste decomposition has been cited between 35 to 45 °C for mesophilic conditions (Hartz et al., 1982; Pfeffer, 1974; Rees 1980b; Cecchi et al., 1993; Tchobanoglous et al., 1993) and 50 to 60 °C for thermophilic conditions (Pfeffer, 1974; Cecchi et al., 1993; Tchobanoglous et al., 1993). The majority of hyperthermophiles and many thermophiles belong to the *Archaea* domain (Holden & Topçuoglu, 2019; Walther et al., 2011). For example, *Methanothermobacter*, a genus of the *Methanobacteriales* order, have been found to be abundant in thermophilic anaerobic digester studies (Illmer et al., 2014; Pap et al., 2015) and can grow at temperatures up to 75 °C (Zeikus & Wolfe, 1972; Enzmann et al., 2018). Optimal hyperthermophilic conditions are typically defined as 80 to 100 °C, but methanogenesis in the context of waste biodegradation has not been reported at these temperatures (Stetter et al. 2013; Walther et al., 2011). The theoretical upper limit of life for organisms is 120 °C (Counts et al., 2017).

Traditionally, mesophilic and thermophilic anaerobic digesters are operated at 37 and 55 °C, respectively. However, both research and practice differ on these values depending on the substrate type, retention times, solids content, etc. Several studies have been conducted on how to optimize this process by controlling digester conditions, such as temperature, operating reactors in series, and selecting for the microbial ecology to produce higher methane content via seeding. Although the controls that can be applied to a digester system are not viable options for landfills, their impacts can elucidate the relevant underlying biological mechanisms.

Lee et al. (2008) evaluated the feasibility of utilizing reactors in series to separate the phases of anaerobic degradation to optimize methane yields. A hyper-thermophilic acid fermenter reactor (70 – 80 °C) was followed by methanogenic reactors at temperatures ranging from 55 to 80 °C. The study was conducted using two 6-L lab-scale CSTRs run in series for 320 days and fed

with synthetic kitchen waste. Both reactors were seeded with thermophilic microorganisms. Results demonstrated that 85 % of potential methane conversion occurs at 55 °C, 62.5 % at 65 °C, 14.5% at 70 °C, and cessation at 73 °C. It was unclear whether the theoretical methane potential data was derived from COD data or otherwise from the manuscript. Microbial characterization results demonstrated that a methanogenic population was dominant below 65 °C and acidogens dominated over 73 °C. In addition, DNA concentrations in the methanogenic reactors decreased with increasing temperatures, indicating that the thermophilic microbial population is less dense as corroborated by additional studies (Karakashev et al., 2005; Shaw et al., 2017).

The knowledge that there are upper temperature limits to both fermentative and methanogenic activity in anaerobic digestion can be applied to landfills. Additionally, given that the reactors in the work of Lee et al. (2008) were seeded with thermophilic microorganisms and still had reduced populations as temperatures increased implies a naturally narrower range for thermophilic populations in landfills experiencing elevated temperatures. Recall the study done by Cecchi et al. (1993), in which an anaerobic digester was successfully transitioned from mesophilic (37 °C) to thermophilic (55 °C) conditions in a period of weeks without introducing a thermophilic inoculum. The authors found that thermophilic biogas production increased with time before reaching steady state and this was attributed to acclimation of the necessary thermophilic microorganisms. It is known that heat accumulation in landfills propagates over month- to year-long periods (Yeşiller et al., 2015; Martin et al., 2012). This lengthy transition period for temperature increases in landfills in comparison to digesters allows for the cautionary assumption that the microbial population may have adequate time to shift and acclimate.

It is generally concluded that thermophilic operation of anaerobic digesters is more efficient than mesophilic (Cooney & Wise, 1975; Rimkus et al., 1982; Cecchi et al., 1993;

Tchobanoglous et al., 1993; Lee et al., 2008). However, efficiency in these cases is often stated with respect to higher methane yields produced at a faster rate relative to mesophilic conditions. In landfills, where residence times are infinite, the landfill size is not dependent on biodegradation kinetics. Furthermore, disadvantages of thermophilic conditions include sensitive operational factors such as substrate loading rate, quality, and overall poor process stability (Cecchi et al., 1993; Lee et al., 2008). These factors are uncontrollable in landfills, in addition to the fact that landfills are heterogeneous batch systems. Therefore, it becomes difficult to translate the metabolic efficiency of anaerobic digestors to landfills.

Consideration of the impacts of temperature on thermodynamics of anaerobic biodegradation is another and perhaps simpler approach. The Gibbs free energy values for anaerobic biodegradation reactions at temperatures of 25, 50, and 75 °C under standard biological conditions are compiled in Table 1. In the fermentation reactions, the energetics become more favorable (ΔG decreases) with increasing temperatures. In comparison, increasing temperatures appears to have a negative effect on acetogenesis and hydrogenotrophic methanogenesis and no significant effect on acetoclastic methanogenesis. In all three cases, the available energy is relatively low. While the substrate conditions are estimates from literature assumed to be relevant to landfills and there are obvious limitations in considering energetics alone without kinetics, these calculations do show that the energetics of fermentation and methanogenesis move in opposite directions as the temperature increases. To overcome this dilemma, anaerobic digester studies, such as Lee et al. (2008), have utilized separate fermentation and methanogenic reactors. In the case that the processes cannot be decoupled, such as in landfills, Cecchi et al. (1993) reasoned that 55 °C was an 'appropriate' temperature to select based on the balance of hydrolytic, acetogenic, and methanogenic groups. If a portion of the microbial constituents capable of carrying out

hydrolysis and/or fermentation have temperatures for optimal growth greater than 60 °C, while the majority of methanogenic species are limited above 60 °C (Zeikus & Wolfe, 1972; Ferguson & Mah, 1983), then some sort of temperature agreement must be reached for all groups to be metabolically productive or there is likely to be a biological imbalance.

There is a gap in the literature outlining the impact of temperatures on methane generation once temperatures exceed the traditionally accepted mesophilic conditions in MSW landfills. This research aims to evaluate the impact of elevated temperatures on methanogenesis and fermentation in landfills by studying methane production and microbial populations. The objectives are to 1) determine an expected upper temperature limit for methanogenesis and fermentation in landfills and 2) determine qualitatively if microbial populations adapt or acclimate when perturbed to either lower or higher temperature conditions.

# CHAPTER 3 - MATERIALS AND METHODS

## Experimental Design

Solid waste samples from two southeastern landfills, 'LFA' and 'LFB', each reporting waste temperatures up to 90 °C, were collected to explore the impact of temperature on methanogenic activity. Two systems were used to maximize the number of samples that could be evaluated: (1) small-scale perturbation experiments and (2) reactor-scale perturbation experiments. Small-scale (30 - 160 mL) experiments were conducted to measure the impact of temperature on methane production at temperatures ranging from 47.5 to 72.5 °C. Tests were conducted in 5 °C temperature increments above and below the excavation temperature (Figure S1). Based on initial results from LFA, this design was later expanded for LFB to include temperatures up to 82.5 °C to evaluate the presence of fermentative activity above the temperature at which methanogenesis ceased (Figure S2). Duplicate tubes with added substrate were set up at each temperature.

Eight samples from each landfill were tested in the small-scale experiments using the fines fraction as an inoculum to represent the microbial population in a sample. A microbial analysis was conducted on the initial waste samples before and after incubation to identify microorganisms and population shifts associated with temperature. Samples from the small-scale perturbation experiment were chosen for analysis due to replication and compatible particle size.

Selected samples from each landfill were also tested in reactor (2-L) system such that the entire sample, as opposed to the fines fraction, could be tested. Reactors were incubated at 5 °C temperature increments between 52.5 and 72.5 °C (Figure S3). Reactors were initiated with samples excavated at 58 °C and 63 °C from LFA, to be referred to as samples 'A-58' and 'A-63', respectively, and with samples excavated at 61 °C and 71 °C from LFB ('B-61' and 'B-71'). Only two samples from each landfill could be tested due to space constraints. These samples were

chosen to compare the performance of the 61 °C sample to the 63 °C sample from separate landfills, to test performance at a temperature at which inhibition would likely be expected (71 °C), and to determine if the waste population could recover as the waste was incubated at lower temperatures.

**Sample Collection and Materials**

Samples were excavated using a ~1-m bucket auger and collected from two boreholes at each landfill. In each case, excavated waste was separated into ~3-m depth intervals and selected samples were collected based on their solid phase temperature so to obtain samples ranging from 50 °C to 80 °C (Table 1). Samples were placed in large plastic garbage bags, evacuated using a vacuum pump to remove residual air, and then placed in coolers in an attempt to maintain the waste temperature close to the original excavation temperature. Samples were processed within 24 – 72 hours of excavation after ground transport.

Each sample was manually mixed and quartered into representative subsamples for waste characterization, small-scale perturbation experiments, reactor-scale experiments, and microbial analysis. A portion of each sample was sieved through a 0.25-inch screen to obtain a fines fraction. This fraction was assumed to be representative of the sample's microbial population and allowed for use of a small-scale experiment to evaluate multiple (16) samples at multiple temperatures. A portion of the fines fraction for each sample was preserved for microbial analysis at –20 °C.

**Experimental Equipment and Operation**

For the small-scale experiments the fractionated samples were incubated as duplicates in either 30-mL pressure tubes or 160-mL serum bottles (Wheaton, Millville, NJ) and crimp-sealed with 20

mm butyl rubber stoppers (Bellco, Vineland, NJ). Sterilized anaerobic growth medium (Table S1), was added to each tube or bottle after flushing with $CO_2/N_2$ (20/80, v/v). Ground (<1 mm) synthetic-MSW was added as substrate (Table S2). Parallel treatments that did not contain added substrate were considered experimental 'blanks' and were also conducted in duplicate. The tubes/bottles containing media and substrate were autoclaved at 121 °C for sterilization prior to inoculation with fines. All further handling was done aseptically and anaerobically.

Sodium sulfide ($Na_2S$) solution was added as a reducing agent within 24 hours before inoculation. The tubes were inoculated with the sample fines and resealed inside an anaerobic chamber with a headspace of $H_2/N_2$ (5/95, v/v). 9 mL of media, 0.1 g substrate, 0.1 mL $Na_2S$, and 5 g of fines was added to the 30-mL tubes. To provide a larger volume of gas and liquid samples for $H_2$, CO, and VFA analyses, the LFB samples incubated between 72.5 – 82.5 °C were tested in 160-mL bottles. In this case, the bottles included 100 mL medium, 1 g of substrate, 1 mL $Na_2S$, and 50 g of fines.

Samples tested in the reactor-scale experiment were incubated in 2-L wide-mouth borosilicate glass bottles (Corning Inc., Corning, NY) and sealed with #13 rubber stoppers. 400 g of the non-fractionated solid waste sample and 100 g of shredded synthetic-MSW were well mixed and added to each reactor while flushing the system with $CO_2/N_2$ (20/80, v/v). 1-L of sterilized anaerobic growth medium was added to each reactor to saturate the waste and to ensure leachate generation for recirculation.

Reactors were modified with glass ports for leachate collection and distribution and for gas collection (Figure S4). Leachate was collected in intravenous bags (VWR, Randor, PA) to allow for recirculation and liquid sampling and gas was collected in FlexFilm gas bags (SKC Inc., Eighty Four, PA). The reactor system was connected by 0.25-inch (ID) Masterflex FEP-lined Tygon

transfer tubing (Cole-Parmer, Vernon Hills, IL). Assembled reactors were tested for leaks using a vacuum pump.

The tubes, bottles, and reactors were all incubated and maintained within +/- 1 °C of their respective temperatures in 28-L Isotemp General Purpose Water Baths (FisherBrand, Hampton, NH).

**Monitoring**

The excavated solids were characterized based on moisture content (MC), volatile solids (VS), and composition of cellulose, hemicellulose, and lignin (CHL) before testing. Given that one landfill reported acceptance of material from a system operated to remove hydrogen sulfide from the gas, total elemental sulfur (S) was analyzed. In addition, a modified anaerobic toxicity assay (ATA) was performed on the solids to determine if the conversion from cellulose to methane was inhibited in the presence of an increasing ratio of the mass of fines to growth medium. The ATA test has been described previously by Wang et al. (1997) and details are presented in the SI.

Gas volume and composition were measured weekly and bi-weekly for the reactor and small-scale experiments, respectively. $CO_2$, $O_2$, $N_2$, and $CH_4$ were monitored in both small- and reactor-scale experiments. CO, $H_2$, and VFAs were also monitored weekly and bi-weekly in the 2-L reactor and 160-mL bottle experiments, respectively. In the reactor experiment, 10 mL of liquid sample was removed for VFA analysis from the leachate bags and not replaced. In the bottle experiment, 5 mL of liquid slurry was removed and replaced with 5 mL of $N_2$ to maintain the same headspace volume. This step was done after gas composition and production was monitored. Reactor leachate pH was measured every 2-3 days initially until a stable neutral pH (6.8 – 7.5) was reached, after which pH was measured approximately weekly.

Small-scale treatments were destructively sampled for microbial analysis once methane production began to plateau for all samples at week 20. The reactors were monitored over the same 20-week time period.

**Analytical Methods & Data Analysis**

MC was measured by drying to constant weight at 75 °C. VS were determined by weight loss on ignition at 550 C for 2 h (APHA, 2017). CHL were analyzed as described previously (Lopez et al., 2016). Total S was determined by total combustion using a Perkin Elmer 2400 CHNS Analyzer at the EATS laboratory in the Soil Science Department. VFAs were measured with a GC-FID using standard methods (APHA, 2017).

Gas samples were taken from the tubes and bottles using a needle connected to an air-tight glass syringe to pierce the septum and measure overpressure volume. Gas samples were taken from the gas bags connected to the reactors using a luer lock and valve fitting. Gas volume was measured using an evacuated cylinder to empty the gas bags after composition analysis (Lopez et al., 2016). $H_2$ content was analyzed using an SRI gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) and 6 ft MolSieve 5A column. All other gases were analyzed using a Shimadzu GC-TCD equipped with a CTR-1 column.

All gas data were corrected to dry gas at 0 °C and 1 atm (STP). After initiating the experiment it was determined that methane generation data from the blanks (no substrate test) was not useful for evaluating methanogenic activity in the context of our objectives and therefore these data are not discussed but the results but are included in the SI for reference.

**Microbial Analysis**

Samples from the small-scale tests were prepared for analysis using two separate methods for the samples before (fines) and after testing (slurry) given the different physical forms. The fines fraction from the original excavated samples were prepared following the 'Direct Phosphate Buffer Method' described by Staley et al. (2011). In this method, the fines are blended with anaerobic phosphate buffer and centrifuged in 50 mL tubes at 3220 x g for 5 mins to form a representative pellet for DNA extraction. The post-incubation slurry samples were well-mixed, poured in four 1.7-mL tubes and centrifuged at 6000 x g for 10 mins form representative pellets for DNA extraction. All steps were done in a biological safety cabinet to maintain sterility and avoid cross-contamination of samples. The supernatant for all samples was decanted to avoid freeze/thaw lysing as the pellets were stored for a short period of time at -80 °C. Details are presented in the SI.

After sample preparation, DNA was extracted following the method for the Qiagen DNeasy PowerSoil Kit (Qiagen, Carlsbad, CA) with a few modifications added to minimize the high contamination levels of our samples as described in the SI. Following extraction, a next-generation sequencing (NGS) metagenomic analysis was conducted. Samples were analyzed at the Genomic Sciences Laboratory (GSL) using polymerase chain reaction (PCR) amplification and 16S rRNA sequencing. Amplifications were performed with modified 341F and 806R primers fused with Illumina adapters to target the V3 region of the bacterial and archaeal 16S rRNA gene prior to sequencing. Genomic DNA sequencing for 300 base pairs was performed using Illumina MiSeq. The results were analyzed using QIIME 2 and R packages.

# CHAPTER 4 - RESULTS & DISCUSSION

Waste characterization is presented in the first part of this section followed by results for the small-scale and then reactor-scale perturbation experiments. The final section will conclude the upper limit of biological activity and relevant application to elevated temperature landfills. The results of the microbial community analysis are presented and this is followed by modeling work to explore the implications of our results.

## Waste Characterization

The moisture and organic content of the samples are presented in Table 1. The relatively low VS of most samples indicates that the landfilled waste was likely diluted with cover soil and ash. The ratio of cellulose plus hemicellulose to lignin (CH/L) is an indicator of the extent of decomposition as lignin is recalcitrant under anaerobic conditions, and the ratio eliminates the impact of cover soil dilution on the measured organics concentrations. The average CH/L was 0.65 $\pm$ 0.41 and 1.19 $\pm$ 0.35 for samples excavated at temperatures below and above 65 °C, respectively ($p = 0.016$). 65 °C was chosen as a cut off as it is the mean temperature of the excavated samples. The higher CH/L for samples excavated above 65 °C suggests that in-situ anaerobic biodegradation may have been inhibited as there was more undegraded substrate remaining, although depth and waste age may also be confounding factors. The Total S for all samples was approximately 1% or less by dry weight (Table S3). As predicted in numerical simulations and reported for field data, the solids temperature generally increased with depth to the center of the waste mass (Hanson et al., 2013; Jafari et al., 2017b; Hao et al., 2020, in revision).

**Table 1.** Characterization of Excavated Solid Waste Samples and Methane Production

| LF | Temp (°C) | Depth (m) | $H_2O$ (%) | VS (%) | C (%)[a] | H (%)[a] | L (%)[a] | CH/L[b] | Incubation Temperature (°C)[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | **Max $CH_4$** | **≥ 75% of Max $CH_4$** | **≥ 10% of Max $CH_4$** |
| A | 55 | 6 | 23.6 | 24.5 | 18.1 | 6.9 | 23.3 | 1.1 | 47.5 | 52.5, 57.5 | 62.5, 67.5 |
| | 52 | 9 | 23.7 | 26.4 | 17.0 | 6.2 | 28.4 | 0.8 | 47.5 | 52.5 - 62.5 | 67.5, 72.5 |
| | 58 | 12 | 11.5 | 16.3 | 1.8 | 0.9 | 22.7 | 0.1 | 47.5 | 57.5, 62.5 | 52.5, 67.5 |
| | 58[d] | | | | | | | | 57.5 | 52.5 | 62.5 - 72.5 |
| | 63 | 15 | 13.5 | 5.5 | 8.0 | 2.9 | 30.8 | 0.4 | 47.5 | 52.5- 62.5 | 67.5 |
| | 63[d] | | | | | | | | 62.5 | 52.5 | 57.5, 67.5 |
| | 67 | 18 | 27.5 | 27.5 | 26.1 | 5.8 | 30.9 | 1.0 | 47.5 | 52.5, 57.5 | 62.5 - 72.5 |
| | 64 | 21 | 11.3 | 6.5 | 3.4 | 1.2 | 20.1 | 0.2 | 47.5 | 52.5 - 62.5 | 67.5 |
| | 70 | 21 | 21.6 | n.d. | n.d. | n.d. | n.d. | n.d. | 52.5 | 52.5 - 62.5 | 67.5, 72.5 |
| | 75 | 24 | 16.1 | 35.1 | 11.0 | 1.9 | 15.1 | 0.9 | 47.5 | 52.5 - 62.5 | 67.5, 72.5 |
| B | 52 | 9 | 28.3 | 19.9 | 10.4 | 3.5 | 26.6 | 0.5 | 52.5 | 52.5, 57.5 | 62.5, 77.5 |
| | 60 | 9 | 26.4 | 18.5 | 21.7 | 6.2 | 34.7 | 0.8 | 47.5 | 52.5, 57.5 | 62.5 - 77.5 |
| | 61 | 12 | 21.2 | 15.8 | 29.9 | 9.1 | 31.1 | 1.3 | 52.5 | 52.5, 57.5 | 62.5, 77.5 |
| | 61[d] | | | | | | | | 57.5 | 52.5, 62.5 | 67.5 |
| | 67 | 12 | 24.2 | 33.6 | 31.4 | 7.8 | 27.4 | 1.4 | 47.5 | - | 67.5 |
| | 70 | 15 | 25.6 | 26.1 | 18.8 | 4.2 | 26.0 | 0.9 | 52.5 | 47.5, 57.5 | 62.5 |
| | 71 | 15 | 28.2 | 60.7 | 30.8 | 7.1 | 24.1 | 1.6 | 47.5 | 52.5, 57.5 | 62.5 |
| | 71[d] | | | | | | | | 62.5 | 52.5, 57.5 | 67.5 |
| | 74 | 18 | 19.6 | 18.8 | 12.6 | 2.9 | 17.0 | 0.9 | 47.5 | 52.5 - 62.5 | 67.5 |
| | 79 | 21 | 25.4 | 45.5 | 28.9 | 5.4 | 20.7 | 1.7 | 47.5 | 52.5, 57.5 | 62.5 - 77.5 |

[a] Expressed as a % of volatile solids

[b] CH/L is the ratio of the major biodegradable to non-biodegradable components of solid waste.

[c] Cells contain incubation temperature (°C) at which the maximum, ≥ 75%, or ≥ 10% of the observed potential methane yield occurs within the range of temperatures tested. Maximum (100%) potential methane yield is normalized in this experiment to the results for each individual sample to account for the fact that each sample had different a methane potential based on the amount of C and H present.

[d] Results for Reactor-scale experiment with the same sample.

**Small-Scale Perturbation Experiment**

Samples are described by the landfill and the temperature of the excavated sample (e.g. the sample excavated at 52 °C from LFA is sample "A-52"). In general, methane generation gradually decreased as incubation temperature increased (Figures 1, S5, S6) and maximum methane generation occurred between 47.5 and 57.5 °C. In most samples, methane generation occurred up to 62.5 – 67.5 °C, though yields were reduced by ~ 50 %. There was more variability between samples and duplicates at incubation temperatures $\geq$ 62.5 °C and it is suspected that this is due to limited microbial populations at higher temperatures as discussed later in the microbial analysis section. There was measurable, but substantially reduced methane generation at 72.5 and 77.5 °C and no significant methane generation at 82.5 °C. These results were consistent across all samples tested.

These results demonstrate that methanogenic activity, or methane production capability, was not dependent on waste excavation temperature as samples excavated at a range of excavation temperatures exhibited similar methane production potential at the same incubation temperature. For example, sample B-74 did not perform better when incubated at temperatures above 72.5 °C than sample B-60, despite being excavated at 74 °C (Figure 1). The samples excavated at higher temperatures had the opportunity for in-situ microbial acclimation but did not produce more methane at higher incubation temperatures relative to lower. However, the microbial populations present in the samples excavated at higher temperatures were able to produce methane without a lag when incubated at lower temperatures, indicating recovery of methanogenic activity is possible (Figure S7a). After 16 weeks, the tubes inoculated with sample A-52 were spiked with additional substrate to further evaluate microbial acclimation potential (Figure S7b). As observed initially, substrate was consumed without a lag at incubation temperatures between 47.5 to 62.5 °C.

However, a second lag period and reduced methane generation was observed again in the 67.5 and 72.5 °C treatments, indicating that the microbial populations were required to re-acclimate and were slow to grow even in the high liquid to solids tube environment. These results also suggest that temperature impacts not only the ultimate methane yield but also the kinetics of the various reactions involved in anaerobic biodegradation.

As expected from the CH/L of the samples (Table 1), the average of the maximum methane yields for the samples excavated above 65 °C, 85 ± 50 mL $CH_4$, was significantly higher than the average of 30 ± 8 mL $CH_4$ produced for samples excavated below 65 °C ($p = 0.009$). This analysis was done based on methane production data in the 47.5 and 52.5 °C incubations where yields were generally highest. The amount of organic matter carried over in the hotter excavation samples implies that for landfill conditions at or above 65 °C, temperature may have limited the microorganisms ability to acclimate and inhibited waste degradation. This hypothesis is supported by our laboratory results, which demonstrate reduction in methanogenic activity at incubation temperatures at or above 62.5 – 67.5 °C.

The methane production for sample B-67 appeared inhibited by a factor unrelated to temperature (Table 1, Figure S6d). However, the ATA results did not provide any evidence for toxicity in the solids (Table S4). In addition, the low Total S in all samples eliminates the possibility that the presence of residuals from the $H_2S$ removal system were responsible. Therefore, the results for this sample are considered an anomaly.

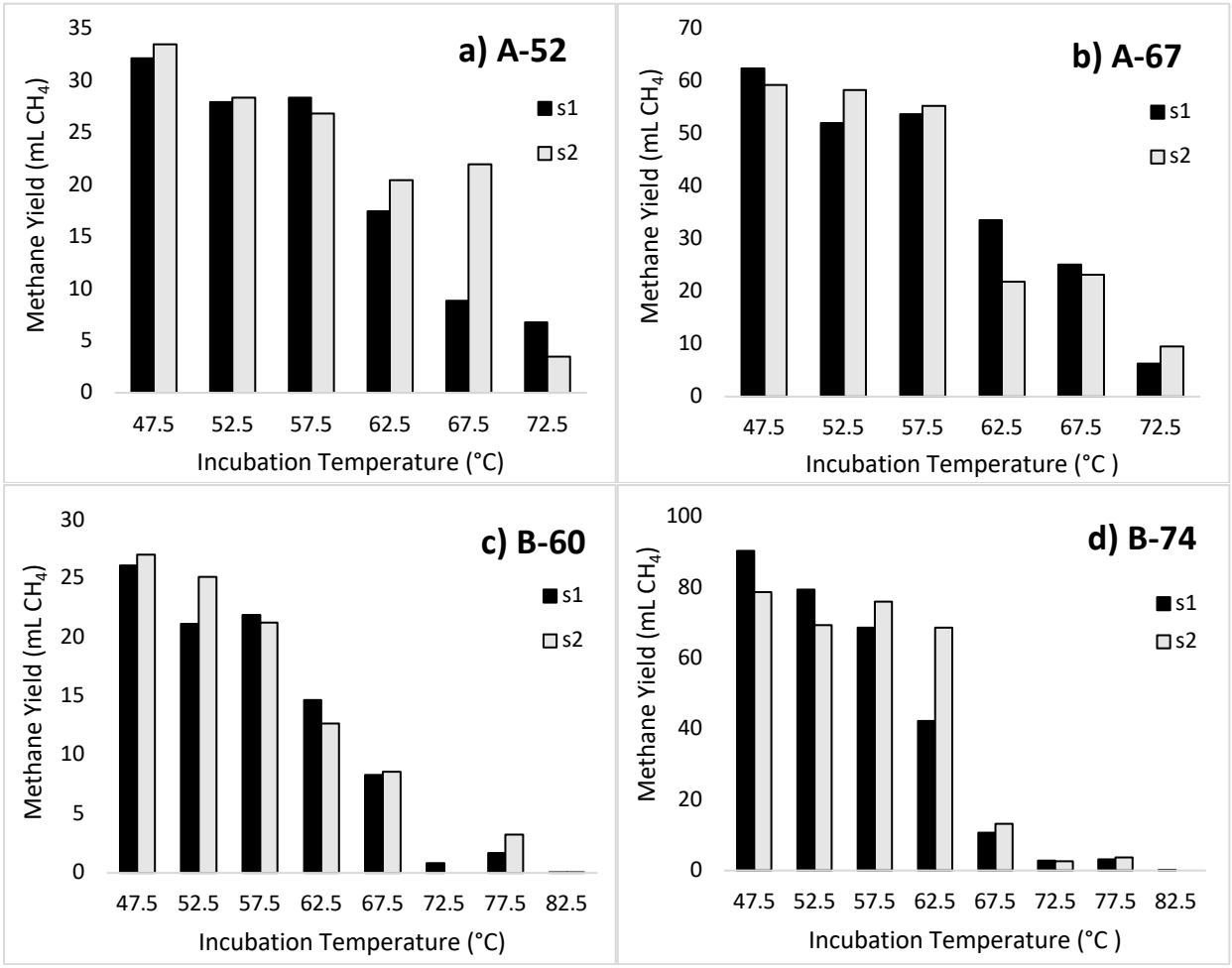


**Figure 1.** Methane yield in tube/bottle tests by incubation temperature for samples excavated from LFA at: **(a)** 52 °C, **(b)** 67 °C and from LFB at: **(c)** 60 °C and **(d)** 74 °C after 20 weeks of incubation. s1 and s2 represent duplicate samples with added substrate. Maximum methane generation in this experiment refers to the maximum amount of methane produced relative to each sample's methane potential in the temperature range tested. Each sample has different yields dependent on how much organic substrate (C & H) was present in the excavated waste as presented in Table 1. The remaining results for all samples tested, along with background yield results (blanks) are presented in Figures S5 – S6.

While methanogenesis was largely inhibited at 72.5 °C, there was evidence for fermentation up to 77.5 °C. VFA accumulation in leachate is a good indicator of fermentative activity. A summary of VFA concentrations in the small-scale bottle experiments incubated from 72.5 to 82.5 °C is presented in Figure 2. Fermentation in the absence of methanogenesis was measured consistently at 72.5 and 77.5 °C. The production of VFAs was significantly reduced at

82.5 °C across all samples, indicating that the upper limit for biological activity in these samples is between 77.5 and 82.5 °C. These results align well with previously published work on the upper temperature limit for biological activity in anaerobic systems, as well as the ability of fermentative bacteria to tolerate higher temperatures than methanogens (Zinder, 1993; Hogland & Marques, 2003, Lee et al., 2008).

Figure 3 presents the $CH_4$ production and VFA concentrations for representative samples chosen from LFB. As illustrated in Figure 3a, VFAs were produced and then presumably consumed by methanogens corresponding with an increase in $CH_4$ production around Day 40. In Figure 3b, we observe that maximum VFA accumulation occurred at 77.5 °C, at which temperature there was also $CH_4$ production. While $CH_4$ production occurred in several samples, it was on average less than 10% of its maximum which is consistent with significant inhibition. As observed in the methane production results, each sample has different VFA yields dependent on how much organic substrate (C & H) was present in the excavated waste as presented in Table 1.

The maximum $H_2$ content measured in the small-scale bottle experiments at week 20 was 2.75 % and no significant accumulation was observed in any treatment beyond week 2. The majority of the $H_2$ levels measured were at or below the limit of quantification (<0.25%) and are presented in Table S5. Qualitatively, $H_2$ was detected in six of the eight samples from LFB at 72.5 and 77.5 °C. Only one sample, B-71, produced measurable $H_2$ over time when incubated at 82.5 °C. These results align with our conclusion based on the VFA data that fermentation becomes inhibited between 77.5 and 82.5 °C.

Although there was some accumulation of VFAs over time, the majority were produced within the first two weeks of monitoring for all samples (Figure S8). This is consistent with the typical acid phase in anaerobic biodegradation. If we assume all of the hydrogen produced during

the acid phase was consumed by acetogens in the first two weeks, as methanogens appear to be inhibited, it is evident that while subsequent fermentative activity was not inhibited due to temperature, it was reduced due to lack of substrate controlled by associated syntrophic relationships. Therefore, it is speculated that the partial pressure of $H_2$ in the bottle headspace made it energetically unfavorable for syntrophs to continue to produce $H_2$, and therefore acetate, as there was no available biological avenue for consumption given the observed inhibition of methanogens.

In an attempt to provide further evidence for fermentation at 72.5 – 82.5 °C, the headspace in the bottles was sparged with $N_2$ to check for $H_2$ gas re-accumulation after 22 weeks. Additional $H_2$ production was measured in about half of the samples, however, the concentration was below the standard detection limit. This was again assumed to be due to the limiting partial pressure of hydrogen, $10^{-4}$ atm, being too low for any measurable evidence of activity. Although elevated pH (>8) was observed in some of the bottles, the reactor-scale experiment corroborates the fermentation results observed in this experiment with expected pH levels.

Our laboratory results for $H_2$ contrast with the elevated levels that have been observed in ETLFs (Stark et al., 2011; Martin et al., 2012, Jafari et al. 2017a, Benson, 2017). In some cases, hydrogen production can be attributed to chemical reactions, but in other instances it has been reasoned that accumulation is attributable to fermentation decoupled from methanogenesis (Sadri et al., 2008). Inter-microbial distances between acetogens and $H_2$-scavenging microorganisms have been shown to influence specific growth rates (Batstone et al., 2006). This relationship and the rate of gas migration that could continue to keep $H_2$ partial pressure low within the waste mass may be an important difference not controlled for in our lab-scale study as opposed to in landfills. Further research is necessary to confirm the mechanism for biological hydrogen accumulation in ETLFs.

Finally, carbon monoxide was also monitored in the bottle experiment and the maximum level recorded for any sample at week 20 was 350 ppm (Table S6). While most of the values measured were close to the detection limit (100 ppm), three of the eight samples accumulated CO at 72.5 to 82.5 °C through week 20. In several instances, CO fluctuated over time. The level of CO production during methanogenesis inhibition was similar to that observed by Haarstad et al. (2006) in a lab-scale solid waste reactor experiment. These results demonstrate that CO accumulation may be possible in landfill systems up to 82.5 °C.

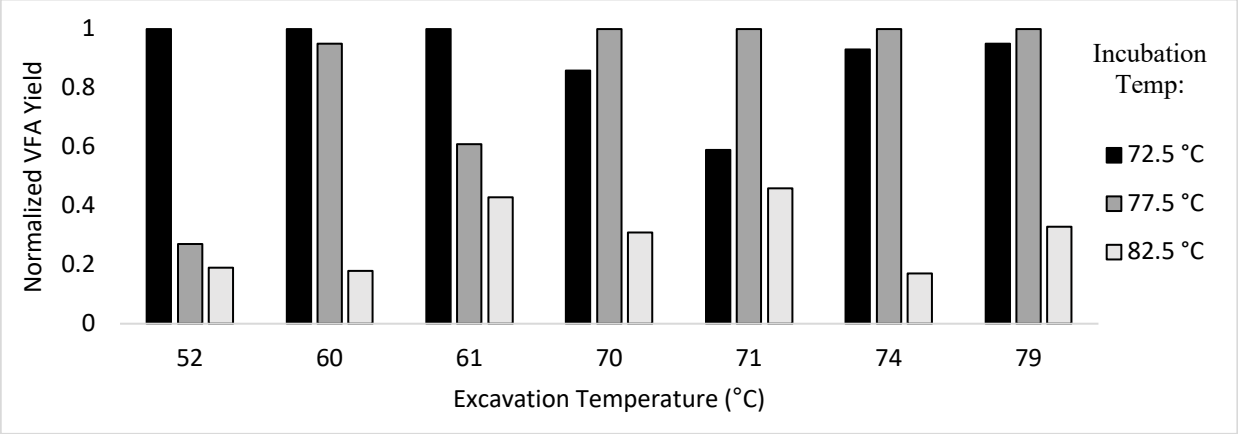


**Figure 2.** Summary of Fermentation Results. The concentration of VFAs (as mg/L acetate) were normalized to each sample (0.0 – 1.0) based on the maximum VFA concentration for the sample. The plotted bars represent the average of the eight samples for the LFB bottle experiments at each incubation temperature tested (72.5 – 82.5 °C).

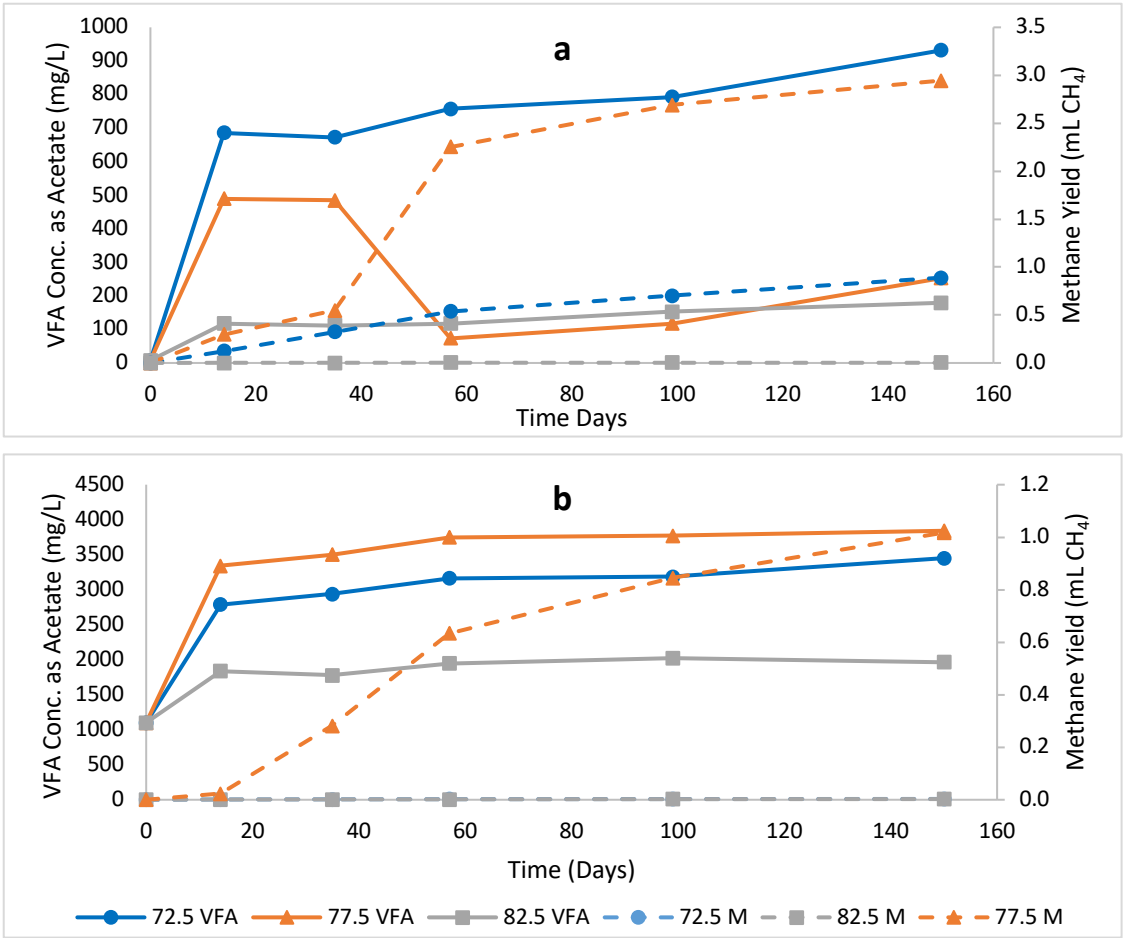


**Figure 3.** Methane (M) production and VFA concentrations in tube/bottle tests by incubation temperature for samples excavated from LFB at: (a) 52 °C, (b) 70 °C. The remaining figures for all samples are presented in Figure S8.

**Reactor-Scale Perturbation Experiment**

The results for the reactor experiment are presented in Figures 4, 5, and S9 – S11. As observed in the small-scale experiment, methane production generally decreased as temperature increased (Figure S9a). However, higher optimum incubation temperatures, 57.5 – 62.5 °C, were observed in the reactor-scale experiment relative to the results from the small-scale experiment, 47.5 – 52.5 °C, for the same sample (Table 1). Results varied for methane production at 67.5 °C for each sample while methanogenesis was consistently inhibited at 72.5 °C. The conversion of cellulose and hemicellulose to methane was similar across all samples with ~37 to 47 % conversion of the initial cellulose (Figure S9b). Thus, the microbial performance in all reactors is comparable despite varying amounts of substrate available in each sample.

For sample A-63, maximum methane production occurred at 62.5 °C, with 52.5 °C also near optimal, while the 57.5 °C samples showed ~40% reduction in methane production. Similarly, optimal methane generation occurred at the excavation temperature, 57.5 °C, for sample A-58, with ~50 % reduction in $CH_4$ at 52.5 and 62.5 °C. The higher methane yield at temperatures above 52.5 °C was anomalous and similar behavior was not observed in the small-scale experiments. Unfortunately, there was no replication in the reactor experiments and therefore no measure of natural variability. In contrast to the small-scale experiment, these data suggest that the microbial populations were well acclimated to the temperature at which they were excavated (up to 62.5 C) and the larger sample sizes in this experiment may have been a contributing factor. Based on the LFA reactor results, LFB samples with higher excavation temperatures were selected for testing. In contrast to LFA, for samples B-61 and B-71, the optimum temperature for methane production was in a range of 52.5 – 62.5 °C and not apparently dependent on excavation temperature.

Significant but reduced methane production at an incubation temperature of 67.5 °C was observed for samples A-63 and B-61. In contrast, for sample A-58, there was minimal methane produced at 67.5 °C. However, there was also no evidence for fermentation, which was an anomaly (Figure S10). In Figure 5, it is observed for sample B-71 that VFAs first accumulate at 67.5 °C before a significant drop is observed concurrent with a pH increase and initiation of methane generation after an 80-day lag. This shift indicates that the methanogenic population was able to acclimate at 67.5 °C.

There was consistently minimal to no methane production in reactor tests at 72.5 °C and there was a VFA accumulation, which is consistent with evidence for fermentative activity. The majority of VFAs were produced in the first two weeks, however unlike in the tube perturbation experiment, VFAs continued to accumulate. As expected, the pH in reactors with VFA build-up remained acidic. Low pH and the peak in $H_2$ content in the first 2-weeks of reactor incubation at all temperatures is consistent with the acid or fermentation phase of anaerobic decomposition preceding methanogenesis. Beyond week 2, $H_2$ levels were only consistently detected for sample B-71 when incubated at 72.5 °C, ranging on average between 2 – 8 % (Figure 5). This experiment had similar limitations to the small-scale experiment for monitoring hydrogen accumulation over time. CO was only measured at an incubation temperature of 72.5 °C for any of the reactors which suggests that it may only accumulate when methanogenesis is inhibited. Maximum CO levels of 140, 210, 190, and 516 ppm were recorded for samples A-58, A-63, B-61, and B-71, respectively. As with $H_2$, only sample B-71 had CO present consistently over time through week 20.

In summary, the reactor experiment provided further evidence that fermentation becomes the dominant active biological process at 72.5 °C. As for the upper temperature limit for methanogenic activity, 67.5 °C likely marks an upper limit for even inhibited $CH_4$ production. It

is also important to note, that as for the results of the small-scale experiment, samples excavated from hotter temperature conditions had no trouble re-acclimating when cooled to lower temperature conditions. Despite some differences in the optimum incubation temperature for maximum thermophilic methane production between samples tested in the small-scale versus reactor experiments, the results show that 67.5 °C is about the upper temperature limit for methanogenic activity.

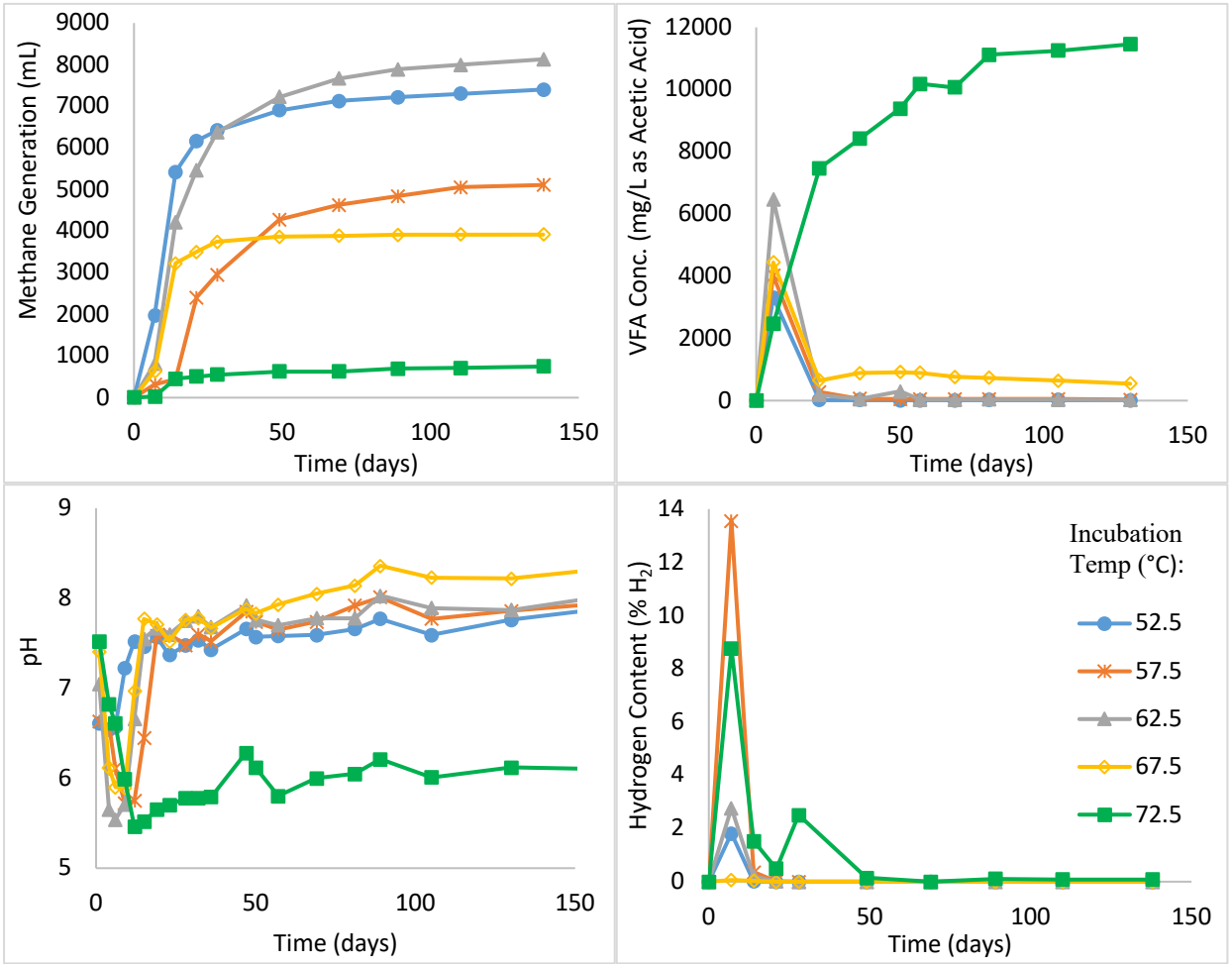


**Figure 4.** Methane Production, VFA concentrations, pH and $H_2$ in a reactor system for sample excavated at 63 °C from LFA incubated between 52.5 and 72.5 °C.

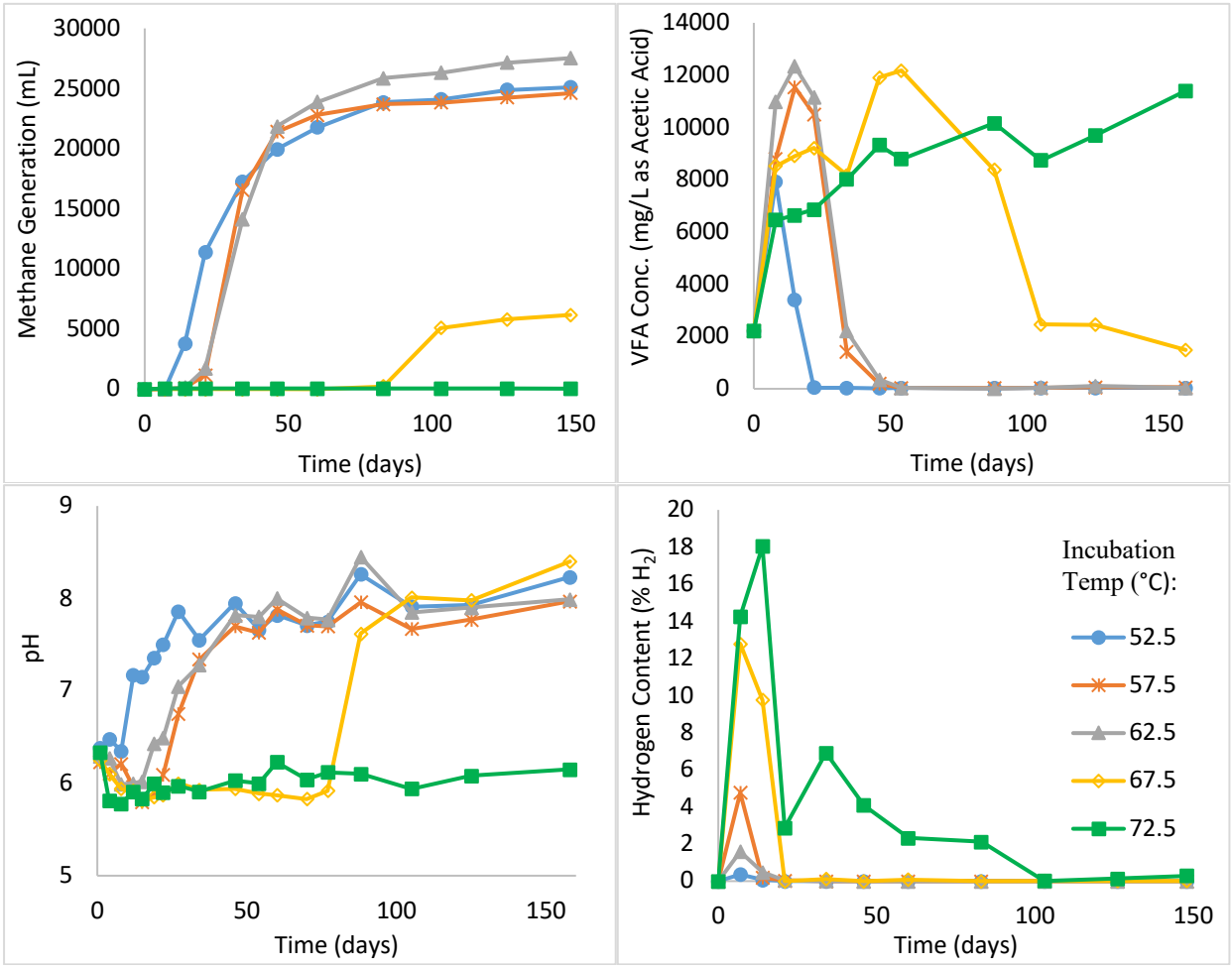


**Figure 5.** Methane Production, VFA concentrations, pH and $H_2$ in a reactor system for sample excavated at 71 °C from LFB in reactors incubated between 52.5 °C to 72.5 °C. There was a leak detected in the gas bag around Day 100 which could explain the decrease in $H_2$ in the 72.5 °C incubation.

**Microbial community structure as influenced by excavation and incubation temperature**

Only the methanogenic populations from LFA are discussed due to the availability of data at the time of this thesis. The impact of excavation temperature and incubation temperature on *Archaeal* community structure are presented as averages in Figures 6, 7, and S12. Recall that the samples from the small-scale LFA experiment were tested as duplicates in a range of 47.5 to 72.5 °C and only $CH_4$ production was monitored.

As presented in Figure 6, *Methanothermobacter* was the dominant methanogen in all 8 samples excavated from LFA over the range of 52 – 75 °C and there was little diversity in the *Archaeal* population, especially at hotter temperatures. *Methanothermobacter* is a genera of the *Methanobacteriales* order that grows optimally from 55 – 65 °C and up to 75 °C (Zeikus & Wolfe, 1972; Enzmann et al., 2018). This hydrogenotrophic methanogen has been found to dominate in landfill leachate (Song et al., 2015) as well as in several thermophilic anaerobic digester studies (Illmer et al., 2014; Pap et al., 2015). *Methanothermobacter thermautotrophicus* was identified as dominant in solid waste samples taken from an MSW landfill which the authors speculated was experiencing higher temperatures (Chen Ann-Cheng et al., 2003).

When the excavated samples were incubated at temperatures ranging from 47.5 – 72.5 °C, a more diverse methanogenic population emerged at the lower incubation temperatures (47.5 – 52.5 °C) (Figure 7). In addition to *Methanothermobacter*, *Methanosarcina* and *Methanosaeta* were also abundant at incubation temperatures of 47.5 to 52.5 °C. *Methanosarcinales* is the only methanogenic order capable of acetate-utilization, but *Methanosarcina* can also produce methane via the hydrogenotrophic pathway. *Methanosarcina* and *Methanosaeta* genera typically dominate in high- and low- acetate concentration environments, respectively (Karakashev et al. 2005; Montero et al., 2008; Staley et al., 2011b). *Methanosarcina* have been found to be largely

responsible for controlling methane production in anaerobic digesters (Zinder, 1993; Shaw et al., 2017). Thermophilic strains of *Methanosarcina* grow optimally from 50 – 57 °C but are inhibited beyond 60 – 62 °C (Zinder, 1990). Several thermophilic strains of *Methanosaeta* have been characterized and have been found to also dominate in digester studies (McMahon et al., 2004; Karakashev et al., 2005; Enzmann et al., 2018). Both *Methanosarcina* and *Methanosaeta* were identified in a similar laboratory study that took samples from a landfill experiencing elevated temperatures (Sadri et al., 2008). Liu et al. (2011) studied sediment in leachate collection ponds and determined that *Methanosaeta* dominated in aged landfills. *Methanoculleus*, a member of the *Methanomicrobiales* order and a $H_2$-utilizer, was found in relatively low abundance in a couple of the samples, despite several studies reporting its dominance in landfill and thermophilic AD systems (Weiss et al., 2009; Song et al., 2015).

As incubation temperatures increased to 57.5 – 62.5 °C a shift in the *Archaeal* community dominance to almost exclusively *Methanothermobacter* was observed. This is consistent with the populations in the excavated samples before incubation and the microorganism's ideal temperature environment. Finally, starting at 67.5 °C we observe a gradual increase in the presence of *uncultured Thermoprotei archaeon*, which appears to then dominate at 72.5 °C. Little information is available to describe this non-methanogen species. This result is likely explained by the fact that acidogens have been found to dominate over methanogens at temperatures of 73 °C and beyond (Lee et al., 2008).

The communities observed in the thermophilic range tested in our experiment all belong to the same orders as those identified in lab-scale landfill refuse studies done under mesophilic conditions: *Methanomicrobiales*, *Methanosarcinaceae*, and *Methanobacteriales* (Staley et al., 2011b, 2018; Bareither et al., 2013). The presence of these methanogens in MSW samples as well

as our samples excavated from a landfill experiencing elevated temperatures indicates that the thermophilic population capable of methane production likely already exists in most MSW landfills and can be selected for as temperatures change over time. The shift toward a higher abundance of *Methanothermobacter* at temperatures above 52.5 °C indicates an increased role of the hydrogenotrophic pathway for methane production at elevated temperatures in landfills. However, it should be noted that the relative abundance of a species does not necessarily reflect its activity within the system.

The metagenomic analysis revealed that incubation temperature had a significant impact on the community composition independent of excavation temperature and that microbial diversity decreased with increasing temperatures for incubated samples. The results from the microbial analysis aid in confirming the trends that were observed for methane production. As discussed previously, waste excavation temperature did not impact performance in terms of methane yield. This was also observed in the microbial communities – regardless of the excavation temperature and sample composition, similar trends in communities were observed across all samples at the varying incubation temperatures (Figure 7). The decrease in microbial diversity associated with increasing temperatures corresponds with a decrease in methane production (Figure S12). A sparser microbial population at thermophilic conditions in comparison to mesophilic conditions has been observed in several anaerobic digestion studies (Karakashev et al., 2005; Lee et al., 2008; Shaw et al., 2017). It is logical that an even smaller community is capable of withstanding temperatures beyond the thermophilic range and this likely explains the significant reduction in methane for the samples incubated at 72.5 °C where *uncultured Thermoprotei archaeon* is more abundant than any methanogens.

Our results demonstrate that a microbial community derived from a landfill can be shifted according to temperature changes as the populations in the tubes evolved considerably during the incubation period. However, this does introduce some limitations for application to elevated temperature landfills. Our laboratory experiment controlled for all factors other than temperature, and microbial populations in landfills will be influenced by several environmental conditions in addition to temperature (e.g., available substrate type). Nevertheless, this research identified potential key methanogens abundant in a landfill experiencing elevated temperatures, most notably *Methanothermobacter*, and linked methane production to the abundance of methanogens and overall community diversity. Future work will further elucidate the microbial ecology of elevated temperature landfill samples and explore *Bacterial* contributions.

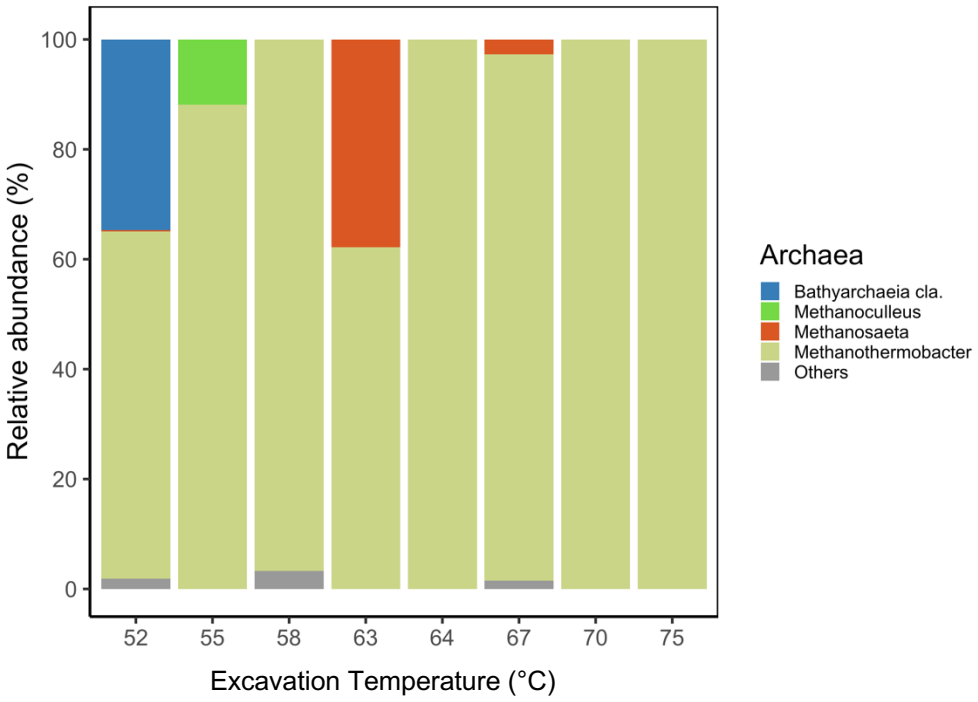


**Figure 6.** Impact of excavation temperature on Archaeal diversity.

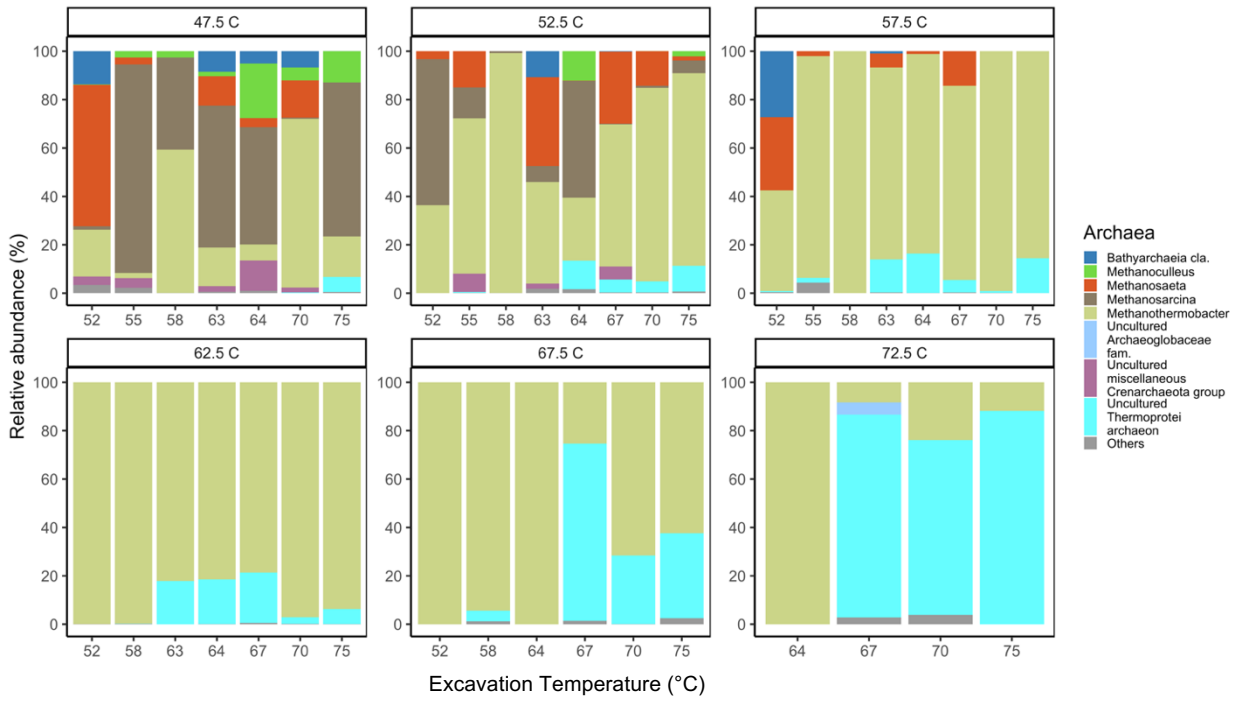


**Figure 7.** Impact of incubation temperature on microbial diversity. The incubation temperature is the value of the top of each plot. The smaller number of samples included at the higher incubation temperatures is due to the exclusion of samples that resulted in low quality DNA sequences.

**Upper Limit of Biological Activity and Application to Elevated Temperature Landfills**

Methane generation across all experiments is summarized in Table 1 and Figure 8. For the majority of samples maximum methane generation occurred between 47.5 and 57.5 °C. In general, ≥75 % of the relative methane potential for each sample was produced at incubation temperatures of 62.5 °C, except for in the small-scale experiment for LFB. Approximately ten percent or greater of the methane potential was produced at 67.5 °C, albeit with increased variability. There was practical cessation in methane generation (< 10% of the maximum) at 72.5 °C and above. These trends were consistent across all samples and seemingly independent of individual waste characteristics. Therefore, we suggest that methane generation is possible in landfilled waste experiencing elevated temperatures up to ~62.5 to 67.5 °C, with considerable inhibition above 62.5 °C. The optimum temperature conditions for methanogenic activity observed in this study is supported by the reported thermophilic range for microbial activity in anaerobic digestors (Pfeffer, 1974; Cecchi et al., 1993; Tchobanoglous et al., 1993; Lee et al., 2008) and the results of Lee et al. align well with this study (Fig. 8).

There are two issues to consider in translating this study's results to the field. First, it has been reported that gas temperatures are ~10 – 20 °C cooler than waste temperatures (Martin et al., 2012; Jafari et al., 2017a). Recognizing that gas temperature is monitored at landfills, a gas temperature of ~45 °C may represent a solids temperature of ~60 °C. The difference between gas and solids temperatures can be explained by the fact that gas temperatures represent an average over the well depth (20 – 150 m) and the volume of gas generated in waste at inhibited temperatures will be lower than the volume generated at favorable temperatures. Furthermore, our results show that methane production resumes when waste is cooled. This is consistent with the similar composition of methanogenic communities observed in all samples independent of

excavation temperature. This recovery is important at field-scale because even if anaerobic biodegradation of waste is inhibited to some extent by elevated temperatures, over time if the landfill mass cools, then methane production can be expected to resume.

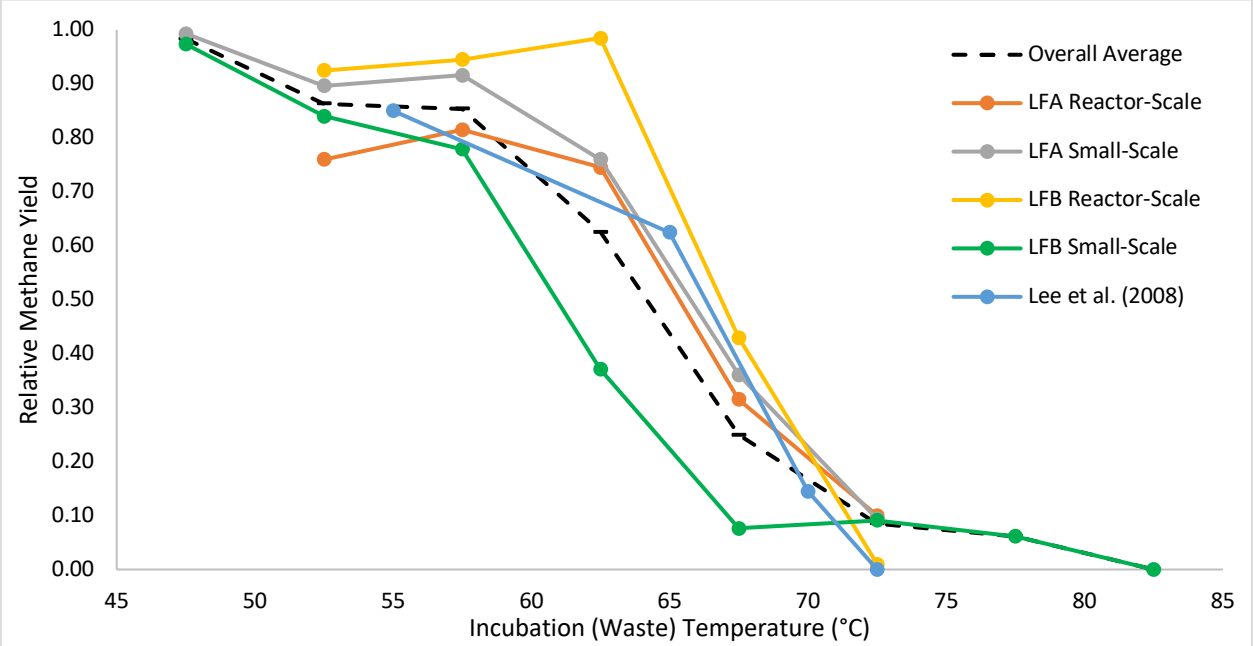


**Figure 8**. The effect of methane generation as a function of temperature across all experiments. Methane yields were normalized to the maximum for each sample (0.0 – 1.0). The points represent the average of the eight samples for the tube experiments and 2 samples for the reactor experiment at each incubation temperature. The data from Lee et al. (2008) is included for comparison.

A second implication of this study relates to modeled gas production. While such models are inherently uncertain (Wang et al., 2015), they are useful to evaluate relative impacts. To evaluate the extent to which changes in $CH_4$ generation are likely to be detected by evaluation of trends in collected gas, we ran a series of simulations of the US EPA's LandGEM model (Figure 9). Simulations were run for a base case with no inhibition at perfect and 75% gas collection efficiency, recognizing that landfill gas data represent collected as opposed to produced gas, as well as cases in which 30% of the buried waste experienced varying levels of inhibition. In keeping with common engineering practice, only the methane potential ($L_0$) was varied to capture the

temperature effects although our results demonstrated that the decay rate (k) is also likely impacted. The results show that when 30 % of the waste mass experiences 90 % inhibition (i.e., T > 70 °C), modeled gas generation is comparable to the 75 % collection efficiency case. This suggests that looking at whole landfill gas volumes only, the assumed inhibition will not likely be detected, though more detailed monitoring of individual landfill areas may result in detection of inhibition.

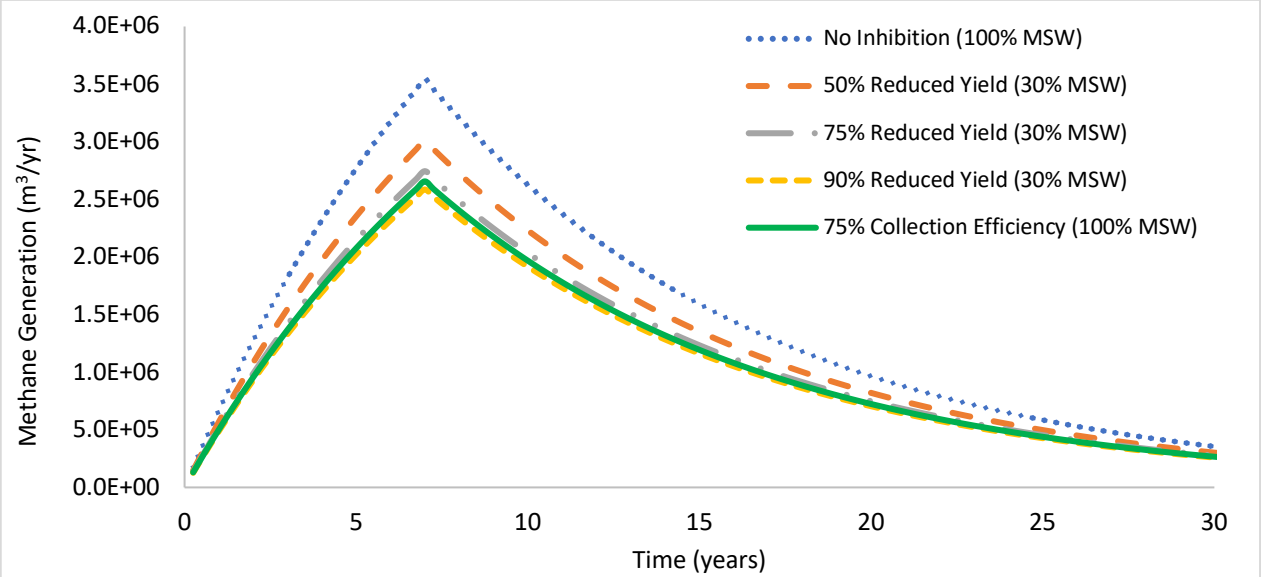


**Figure 9.** Impact of temperature on methane generation at field scale. LandGEM inputs: decay rate (k) = 0.1 $yr^{-1}$, methane potential ($L_0$) = 100 $m^3$ $CH_4$/Mg, mass (M) = 100,000 Mg/yr for 7 years. The blue-dashed lined shows no inhibition of MSW with 100 % collection efficiency. The solid green line shows no inhibition of MSW but with 75 % collection efficiency. The orange, gray, and yellow dashed lines show 50, 75, and 90 % reduced methane yields for 30 % of the MSW with 100 % collection efficiency. A 30% waste mass area was chosen because typically elevated temperatures only impact part of a landfill.

Our results suggest that an overarching 55 °C temperature limit in gas wells imposed by NSPS regulations is simplistic. However, the aggressive monitoring and analysis required to fully capture the temperature effects at any given site are also unrealistic. In recognizing that a gas well temperature of 55 °C could correspond to solids at 65 °C, some inhibition of methane

generation is likely in wells with gas temperature above 55 °C. However, the extent of inhibition is on the order of 50% and likely not observable at field-scale.

While methanogenesis may be viewed as the most crucial biological process for landfill operation, this research also evaluated the upper limit of fermentation given that fermentation reactions produce heat at higher temperatures than methanogenesis. To explore the influence of methanogenesis and fermentation energetics on heat accumulation in landfills, various simulations were explored using a batch model developed by Hao et al. (2017). The Base Case assumes no fermentative activity above levels for methanogenesis and Cases 1 – 3 represent a range of possible fermentation temperature inhibition functions as illustrated in Figure S13. The predicted temperature from this model are presented in Figure 10. The model results demonstrate that if fermentative activity continues up to 77.5 °C, and accounting for the inhibition of methanogenesis above 67.5 °C, this anaerobic reaction alone can result in an additional increase in waste temperatures from ~3 to 10 °C.

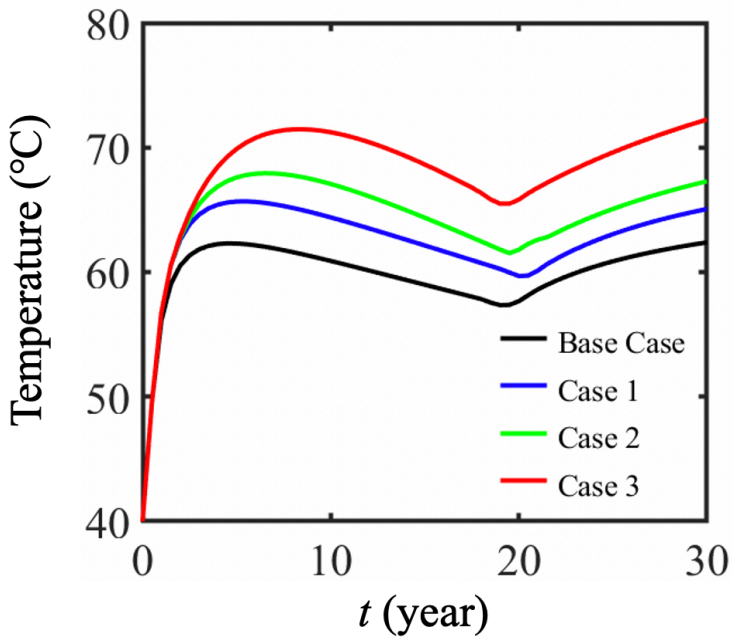


**Figure 10.** Impact of fermentation reaction on temperature increase in batch model of elevated temperature landfills. The base case assumes no fermentative activity above levels for methanogenesis and cases 1 – 3 represent a range of possible fermentation temperature inhibition functions. The heat associated with the fermentation reaction is captured here in the extent that it continues past methanogenesis and is quantified for simplicity as methane yield. Case 1 represents an inhibition function derived from the methane yield data from this experiment. Case 2 uses a linear relationship of the inhibition function between 60 and 82.5 C. Case 3 represents a scenario where methane production is only reduced to 50 % between 60 and 77.5 C followed by exponential decay. All cases account for heat loss.

# CHAPTER 5 - CONCLUSIONS

This research took the unique approach of monitoring samples excavated directly from landfills exhibiting elevated temperature to evaluate the temperature range for biological activity and the potential for microbial community adaptation to temperature shifts. It is concluded that:

1. methane generation in possible in landfilled waste experiencing elevated temperatures up to about 62.5 to 67.5 °C. However, the yields are consistently lower in comparison to the measured methane generation at incubation temperatures of 47.5 to 57.5 °C, which may be presumed to be the optimum for thermophilic anaerobic biodegradation in landfills.
2. While the microbial populations were not capable of acclimation beyond 67.5 °C, there was evidence that methanogenic activity resumes when the waste mass is cooled. DNA analysis confirmed that the microbial communities shifted based on their incubation temperatures regardless of the original excavation temperatures. In addition, microbial diversity decreased with increasing temperatures, which supported our experimental results for methanogenic activity inhibition.
3. Fermentation in the absence of methanogenesis was measured from 72.5 to 77.5 °C, signifying the upper limit for biological activity in landfills is near 82.5 °C. This reaction can cause an additional 3 to 10 °C temperature increase in the landfill waste mass.

These results are largely corroborated by the extensive literature available for anaerobic biological activity in regard to solid waste biodegradation but this study is the first to provide actual data for landfills currently experiencing elevated temperatures. In terms of field scale applicability, this research will provide data to support HOVs for continuance of gas well operations in instances where methane production appears uninhibited at temperatures in excess of 55 °C. This research will also provide data for the temperature limit for anaerobic reactions necessary for accurate

predictive models for heat accumulation. Future work will provide a more in-depth analysis of the microorganisms responsible for methane production, as well as fermentative activity, in the temperature range of interest.

**Recommendations**

This research highlights the importance of evaluating the temperature range of biological activity in landfills as the results have shown how fundamental the effects can be for both methane production and heat accumulation on field scale. Further research is recommended to:

1. Compare the impact of mesophilic versus thermophilic landfill conditions on methane production. While the goal of this study was to determine an upper limit for biological reactions, an interesting related study would be to determine if the rate and yield trends for biological reactions according to temperature in landfills are similar to that of anaerobic digestion.
2. Complete an in-depth analysis of microbial ecology of elevated temperature landfill samples. This would include exploring *Bacterial* contributions in relation to methanogenic and quantifying activity of microorganisms in addition to abundance to further understand their individual roles. Characterization of fresh refuse samples would also be useful to determine the initial relative abundance of *Methanothermobacter* in waste. This would be an extensive project due to the combined limitations of both landfill sampling and application of lab-scale results to the field.
3. Elucidate the impact of abiotic processes in landfills experiencing elevated temperatures now that an upper limit to biological activity around 82.5 °C has been established. If a landfill continues to experience waste temperatures in excess of 80 °C, potentially harmful

abiotic processes, such as pyrolysis or cellulose hydrolysis, may begin to take place (Hogland & Marques, 2003; Stark et al., 2011; Hao et al., 2017). Lab-scale studies are recommended to quantify the impact of the processes that can be used in model simulations.

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## SUPPORTING INFORMATION (SI)

Figures S1 to S3 present the experimental design for the small-scale perturbation experiments for LFA, LFB, and the reactor-scale experiments, respectively. Tables S1 and S2 present the composition of the anaerobic growth medium and synthetic MSW, respectively. Figure S4 illustrates the reactor design schematic. Table S3 provides the Total S results for the samples. The methods for the anaerobic toxicity are described and the results are discussed and presented Table S4. This is followed by the sample preparation procedure for DNA extractions including the modified Qiagen procedure.

Figures S5 and S6 present the results for the small-scale perturbation experiments for each landfill. The results for two samples from each landfill, A-52, A-67, B-60, and B-74, were presented in the text, but are presented again here with the inclusion of data from the blank treatments. Figure S7 presents the rate data for sample A-75 (a) and from the spike experiment to test for microbial acclimation in sample A-52 (b). Figure S8 presents the VFA results for the small-scale experiment. The results for two samples, B-52 and B-71, are presented in the text and not repeated here. Tables S5 and S6 present the $H_2$ and CO results for the small-scale bottle experiment, respectively.

Figures S9 to S11 presents methane production for the reactor experiments. Figures S12 presents the alpha diversity results from the microbial community analyses. Figure S13 presents temperature predictions using the batch reactor heat accumulation model (Hao et al., 2017).

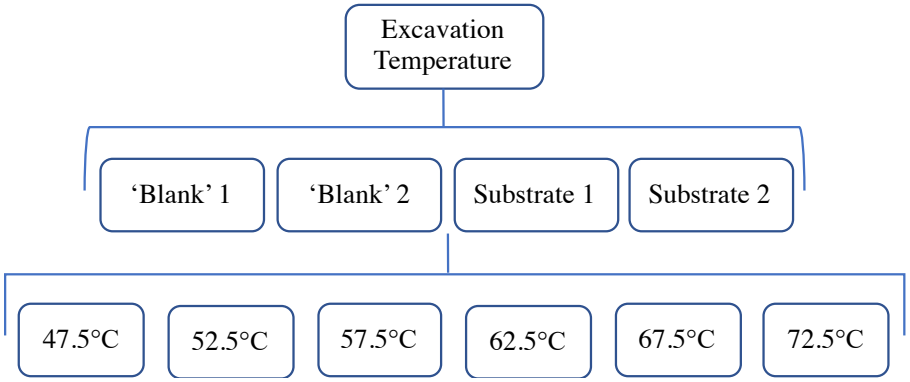



Figure S1. Tube perturbation matrix for samples excavated from Landfill A. 192 tubes in total were monitored over a 20-week period (8 samples x 6 temperatures x 4 tubes per sample).

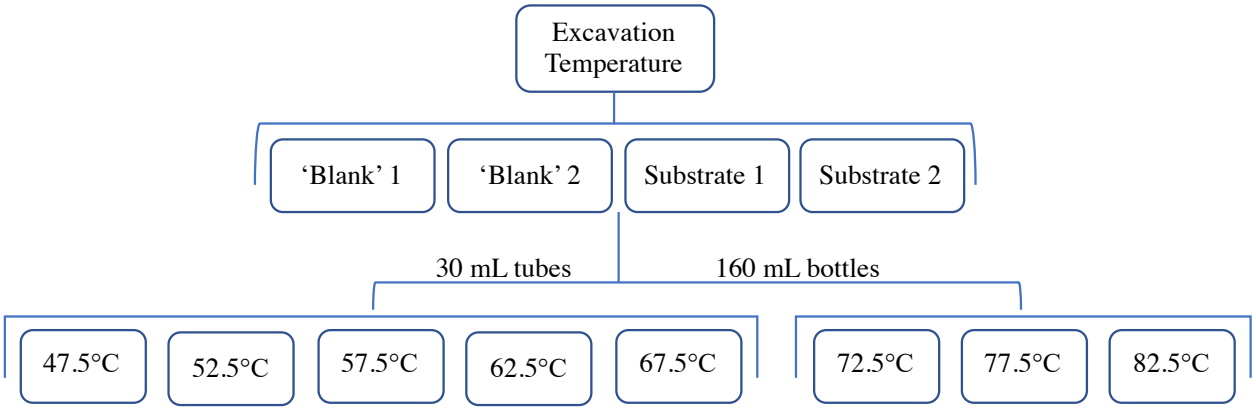



Figure S2. Tube and bottle perturbation matrix for samples excavated from Landfill B. 160 tubes and 96 bottles in total were monitored over a 20 week period (8 samples x 8 temperatures x 4 tubes per sample).

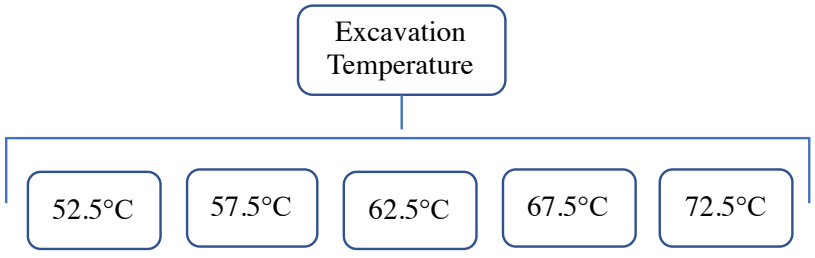



Figure S3. Reactor perturbation matrix for samples excavated from Landfill A and B. 20 reactors in total were monitored over a 20-week period (4 samples x 5 temperatures). A narrower temperature range and no duplicates or blanks were tested due to space and electrical constraints.

Table S1. Composition of anaerobic growth medium[a]

| Component | per liter |
|---|---|
| $PO_4$ solution | 100 mL |
| $M_3$ solution | 100 mL |
| Trace Mineral solution | 10 mL |
| Vitamin solution | 10 mL |
| Resazurin (0.1%) | 2 mL |
| Distilled water | 768 mL |
| $NaHCO_3$ | 3.5 g |

[a] Adopted from Wang et al., 1994.

Table S2. Synthetic MSW Composition

| Component | | Estimated Amount to Add (kg) | Actual Wt. added (kg) |
|---|---|---|---|
| Paper | Newsprint | 3.08 | 3.08 |
| | Office Paper | 1.42 | 1.42 |
| | Magazines | 0.75 | 0.75 |
| | Corrugated Containers | 4.00 | 4.00 |
| | Other Paper | 19.91 | 20.98 |
| Metals | Aluminum Cans | 0.66 | 0.67 |
| | Steel Cans | 0.64 | 0.64 |
| | Other Metals | 11.40 | 10.81 |
| Plastics | PET Containers | 2.29 | 2.29 |
| | HDPE Containers | 1.91 | 1.91 |
| | Other Plastics | 29.97 | 30.08 |
| Glass | Glass Containers | 7.07 | 7.08 |
| | Other Glass | 2.64 | 2.64 |
| Other Wastes | Rubber and Leather | 7.50 | 7.51 |
| | Textiles | 14.85 | 14.85 |
| | Food Waste | 40.77 | 40.89 |
| | Yard Waste | 15.74 | 15.75 |
| | Wood | 15.39 | 15.39 |
| **Total** | | 180.00 | 180.75 |

Elemental Analysis:

| Component | Content (wt.%) |
|---|---|
| Carbon (C) | 52.0 |
| Hydrogen (H) | 7.6 |
| Nitrogen (N) | 1.6 |
| Oxygen (O) | 38.8 |

A synthetic MSW was used for all the experiments. It was comprised of plastic, wood, metal, paper, fabric, and other wastes, consistent with the average MSW composition in the United States as described in EPA waste characterization reports. The density of the uncompacted waste that was charged into the reactors was about 180 kg/m$^3$. The determined biochemical methane potential of this substrate is approximately 165 mL $CH_4$/g. This substrate was comprised of 39.3% cellulose, 10.3% hemicellulose, and 26.2 % lignin.

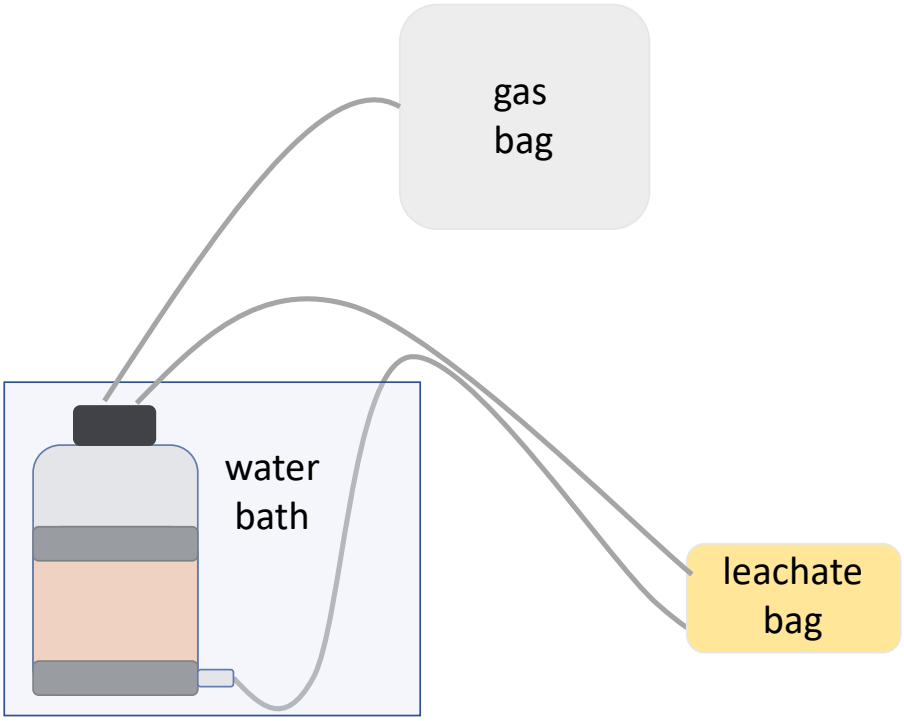



Figure S4. Reactor schematic.

Table S3. Total S Results

| Sample ID | S (% dry wt.) |
|---|---|
| A-55 | 0.84 |
| A-52 | 1.01 |
| A-58 | 0.11 |
| A-63 | 0.23 |
| A-67 | 0.47 |
| A-64 | 0.23 |
| A-70 | n.d. |
| A-75 | 0.33 |
| B-52 | 0.58 |
| B-60 | 0.84 |
| B-61 | 0.34 |
| B-67 | 0.98 |
| B-70 | 0.76 |
| B-71 | 0.61 |
| B-74 | 0.30 |
| B-79 | 0.50 |

## Anaerobic Toxicity Assay

The methane production for sample B-67 appeared inhibited by a factor unrelated to temperature (Table 1, Figure S6d). A modified anaerobic toxicity assay (ATA) was performed to determine if the conversion from cellulose to methane was inhibited in the presence of an increasing mass of fines to liquids ratio as described previously (Wang et al., 1997). The methods for testing methanogenic activity using treatments containing fines as inoculum, substrate, and medium were followed as detailed for the small-scale perturbation experiment. The fines portion of 5 g used in the 30-mL tube experiment was maintained, but additional treatments diluted the solids to liquid ratio by 50 and 100 % with additional media (Table S3). Treatments were tested in triplicate. Tests were conducted at 52.5 °C and incubated for 90 days. Gas composition and volume were measured every 30 days. Methane yields in the ATA test bottles were corrected to standard temperature and pressure. The sample with suspected inhibition, B-67, and two control samples, B-52 and B-60, were tested to ensure the validity of the test.

In theory, if there was a toxic substance present in the fines, an increase in media volume would dilute the toxicity and therefore increase the methane yield. As shown in Table S3, the ATA results did not provide any evidence for toxicity in the sample suspected to have inhibition. The diluted fines sample for B-67 actually produced the lowest methane yield. Unlike in the small-scale tube perturbation experiment, methanogenesis did not appear inhibited at all in the sample when incubated at 52.5 °C. In addition, there was considerable variability in the yields produced for sample B-67 in comparison to the controls. While toxicity within the fines cannot explain the observed discrepancies, it was determined that the results for sample B-67 are not representative and should not be included in further interpretations of results for this study.

Table S4. ATA Assay Results for LFB

| Sample | Dilution | Fines (g) | Media (mL) | Substrate (g) | $CH_4$ produced in 90 days (mL)[a] |
|---|---|---|---|---|---|
| Control 1: B-52 | None | 5.0 | 9.0 | 0.1 | 14.86 (1.16) |
| | 50% | 5.0 | 13.5 | 0.1 | 13.98 (0.93) |
| | 100% | 5.0 | 18.0 | 0.1 | 15.46 (1.63) |
| Control 2: B-60 | None | 5.0 | 9.0 | 0.1 | 22.05 (2.68) |
| | 50% | 5.0 | 13.5 | 0.1 | 17.33 (1.39) |
| | 100% | 5.0 | 18.0 | 0.1 | 17.86 (0.51) |
| Test for Toxicity: B-67 | None | 5.0 | 9.0 | 0.1 | 29.73 (15.04) |
| | 50% | 5.0 | 13.5 | 0.1 | 34.45 (6.33) |
| | 100% | 5.0 | 18.0 | 0.1 | 22.04 (10.32) |

[a] The values presented are the average of triplicates with the standard deviation given parenthetically.

## DNA Extraction Procedure

Sample Preparation

The fines fraction from the original excavated samples were prepared following the 'Direct Phosphate Buffer Method' described by Staley et al. (2011a). The fines which had been maintained at -20 °C were combined with chilled 23.7 mM $PO_4$ buffer in a Waring blender and homogenized. The mixture was immediately transferred to sterile 50 mL tubes and centrifuged at 3220 x g for 5 minutes. The supernatant was decanted to avoid freeze/thaw lysing and samples were stored for a short period of time at -80 °C before amplification.

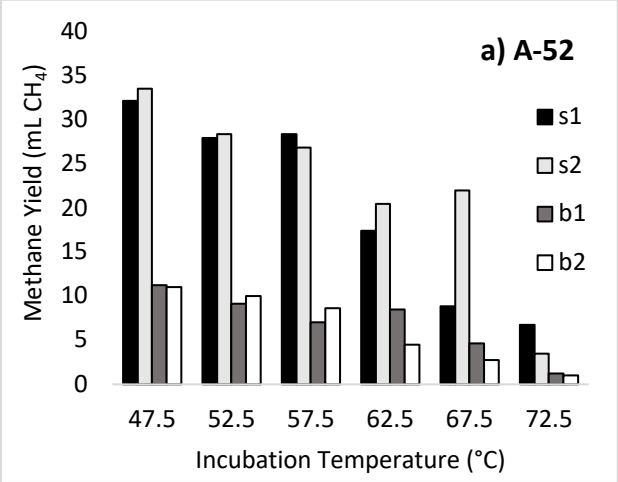
The slurry samples from the tubes (a mixture of the same fines, media, and substrate) for LFA and LFB were taken down on Day 200 and 170, respectively. This was done to preserve the population at the same timepoint across all temperature ranges. The slurry samples from the LFB bottles were taken down on Day 210, as additional experiments ($H_2$ and VFAs) were necessary first. The tube/bottle contents after perturbation were moved into a biological safety cabinet to maintain sterility and avoid cross-contamination of samples. After thorough mixing, the rubber stopper was then removed and the slurry was poured in four 1.7 mL centrifuge tubes. The tubes were then centrifuged at 6000 x g for 10 mins. The supernatant was decanted to avoid freeze/thaw lysing and samples were stored for a short period of time at -80 °C.

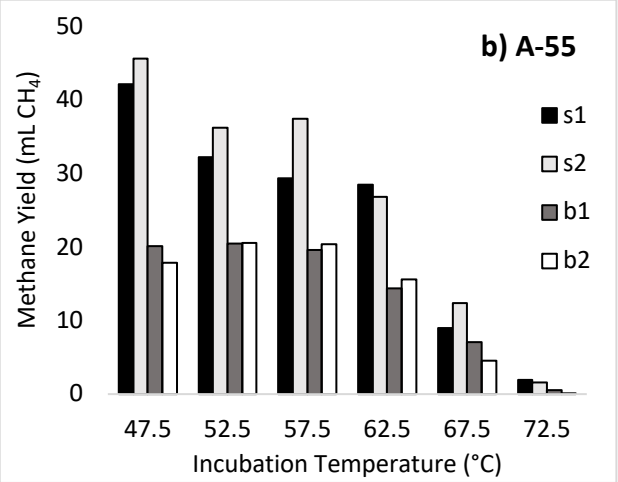
Qiagen DNeasy PowerSoil Modified Methods

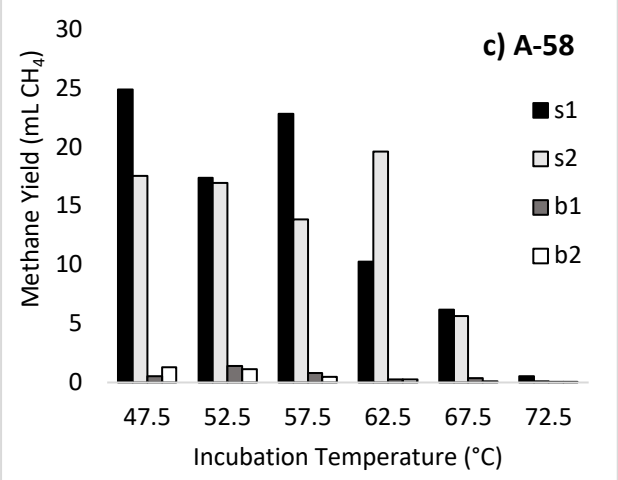
The Qiagen DNeasy PowerSoil Kit manufacturer protocol was followed, except a few steps were modified steps to minimize the high contamination levels of our samples. Initially, the pellets from two microcentrifuge tubes were transferred to the PowerBead tube provided in substitution for the 0.25 g of soil called for in the procedure. The next modification was an extra centrifugation step

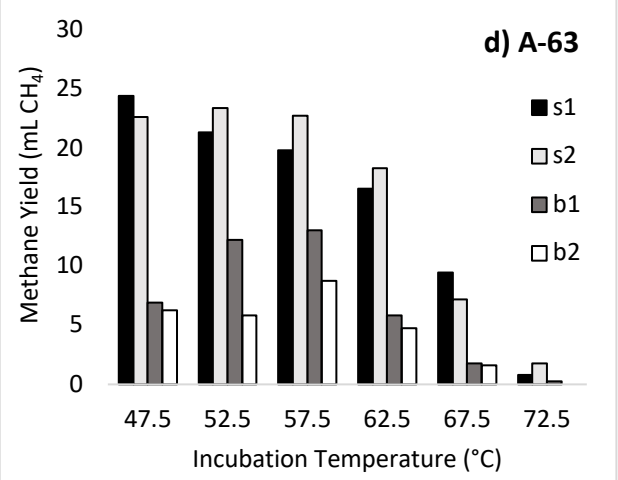
after bead beating the samples to further purify the supernatant. Additionally, the ethanol-based wash solution step in the MB Spin Column was repeated to further clean the DNA bound to the silica membrane. The purity of the DNA extracted was tested using a Nanodrop-1000 and frozen at -80 °C before further NGS testing at GSL.

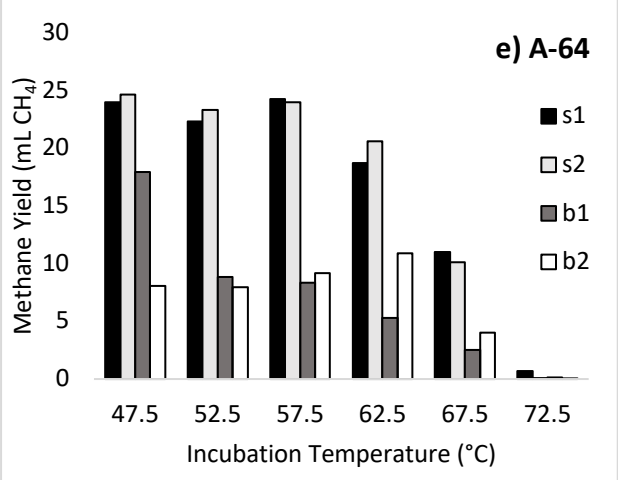
**Figure S5.** Cumulative methane yield in small-scale perturbation tests by incubation temperature for samples excavated from LFA at: (a) 52 °C, (b) 55 °C, (c) 58 °C, (d) 63 °C, (e) 64 °C, (f) 67 °C, (g) 70 °C, and (h) 75 °C after 20 weeks of incubation. s1 and s2 represent duplicate samples to which additional substrate was added. b1 and b2 represent duplicate treatments that only included the excavated fines and media. The inclusion of the blank data in these figures demonstrates the varying organic matter available for conversion to methane based on the C & H available in each excavated waste sample. Only the samples were substrate added were analyzed in consideration of the experimental objectives.

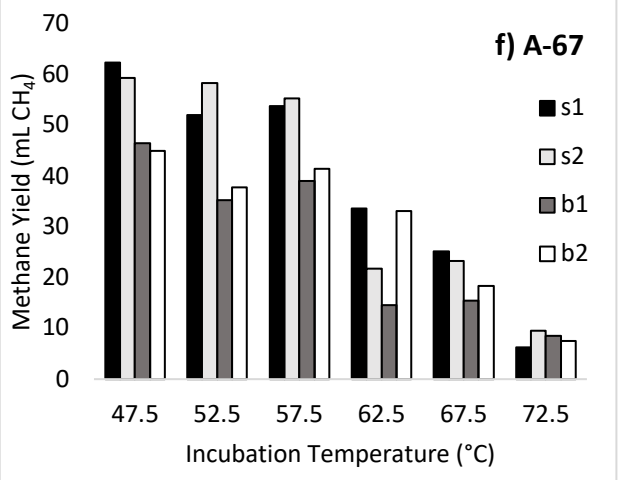

a) A-52
Methane Yield (mL CH4)
0
5
10
15
20
25
30
35
40
s1
s2
b1
b2
47.5
52.5
57.5
62.5
67.5
72.5
Incubation Temperature (°C)

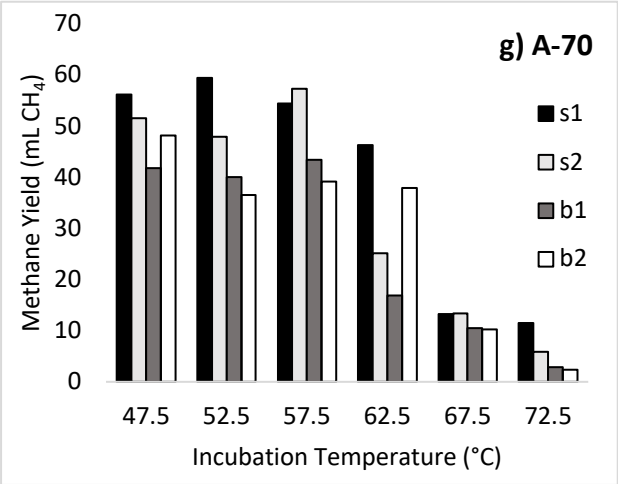

b) A-55
Methane Yield (mL CH4)
0
10
20
30
40
50
s1
s2
b1
b2
47.5
52.5
57.5
62.5
67.5
72.5
Incubation Temperature (°C)


c) A-58
Methane Yield (mL CH4)
0
5
10
15
20
25
30
s1
s2
b1
b2
47.5
52.5
57.5
62.5
67.5
72.5
Incubation Temperature (°C)


d) A-63
Methane Yield (mL CH4)
0
5
10
15
20
25
30
s1
s2
b1
b2
47.5
52.5
57.5
62.5
67.5
72.5
Incubation Temperature (°C)


e) A-64
Methane Yield (mL CH4)
0
5
10
15
20
25
30
s1
s2
b1
b2
47.5
52.5
57.5
62.5
67.5
72.5
Incubation Temperature (°C)


f) A-67
Methane Yield (mL CH4)
0
10
20
30
40
50
60
70
s1
s2
b1
b2
47.5
52.5
57.5
62.5
67.5
72.5
Incubation Temperature (°C)


g) A-70
Methane Yield (mL $CH_4$)
70
60
50
40
30
20
10
0
s1
s2
b1
b2
47.5
52.5
57.5
62.5
67.5
72.5
Incubation Temperature (°C)

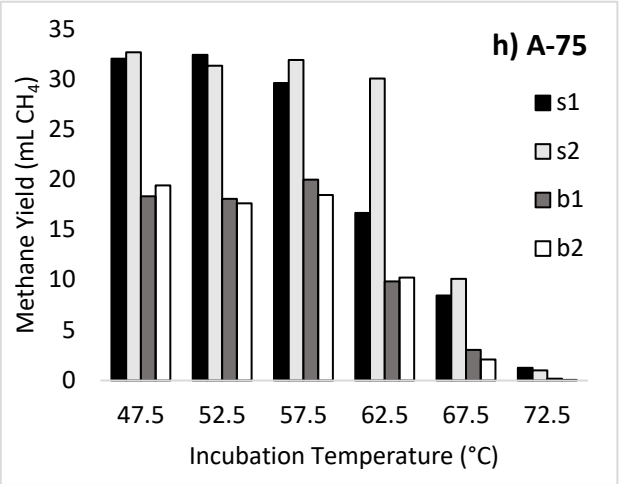
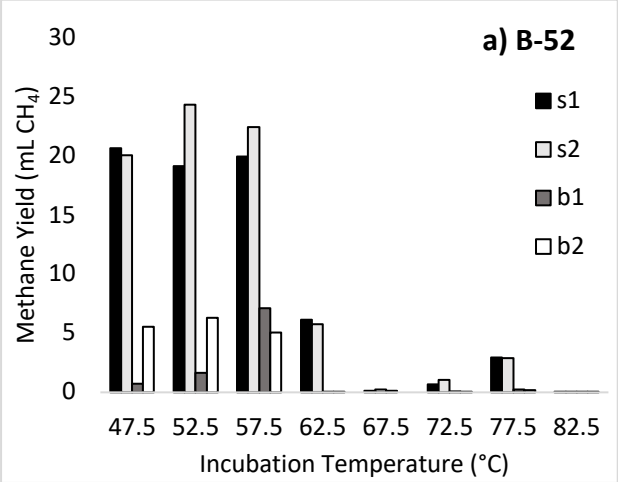
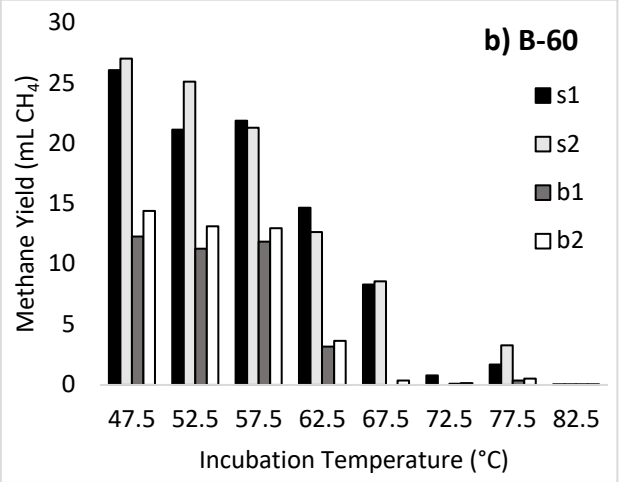
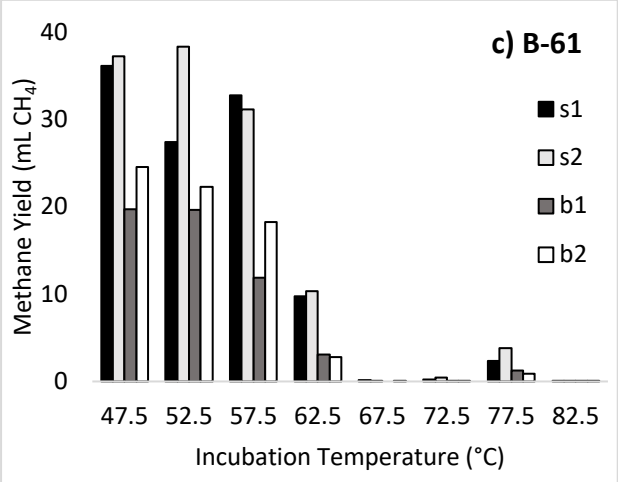
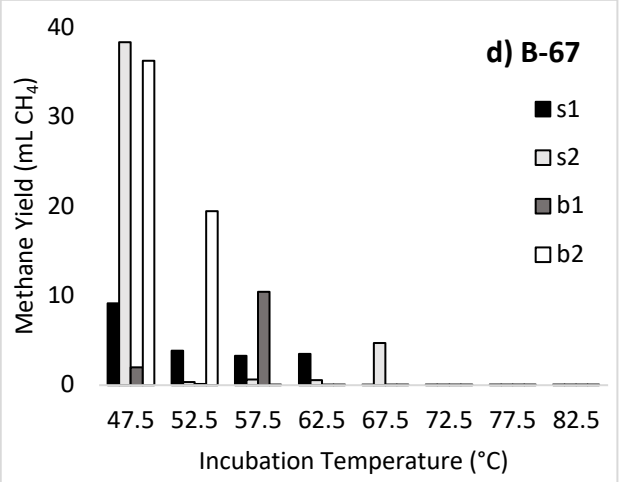

h) A-75
Methane Yield (mL $CH_4$)
35
30
25
20
15
10
5
0
s1
s2
b1
b2
47.5
52.5
57.5
62.5
67.5
72.5
Incubation Temperature (°C)

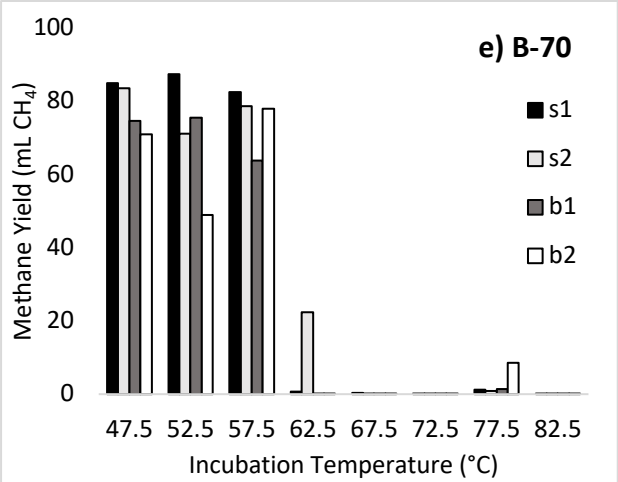
**Figure S6**. Cumulative methane yield in small-scale perturbation tests by incubation temperature for samples excavated from LFB at (a) 52 °C, (b) 60 °C, (c) 61 °C, (d) 67 °C, (e) 70 °C, (f) 71 °C, (g) 74 °C, and (h) 79 °C after 20 weeks of incubation. The treatments incubated at temperatures from 72.5 to 82.5 °C were tested in 160 mL bottles rather than 30 mL tubes as described in the methods. The methane yields for the bottles have been divided by 10 to reflect the lower mass of fines in the tubes and so the results are comparable. s1 and s2 represent duplicate samples to which additional substrate was added. b1 and b2 represent duplicate treatments that only included the excavated fines and media. The inclusion of the blank data in these figures demonstrates the varying organic matter available for conversion to methane based on the C & H available in each excavated waste sample. Sample B-67 is suspected to have experienced inhibition due to a factor unrelated to temperature (d). The lack of methane produced at 67.5 °C is an anomaly for sample B-79 (h). Only the samples were substrate added were analyzed in consideration of the experimental objectives.

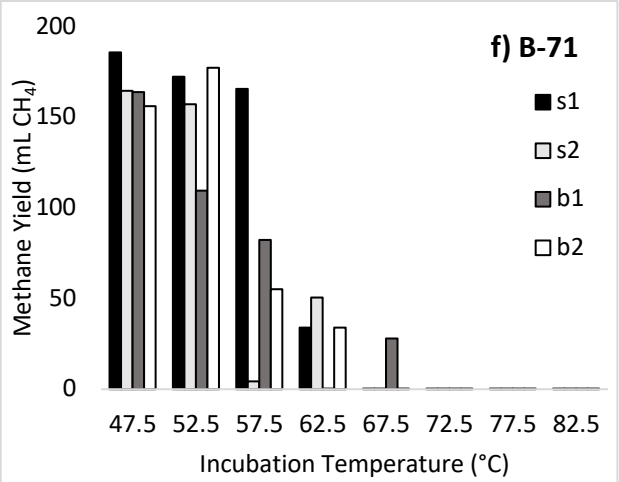

a) B-52
Methane Yield (mL $CH_4$)
30
25
20
15
10
5
0
s1
s2
b1
b2
47.5 52.5 57.5 62.5 67.5 72.5 77.5 82.5
Incubation Temperature (°C)

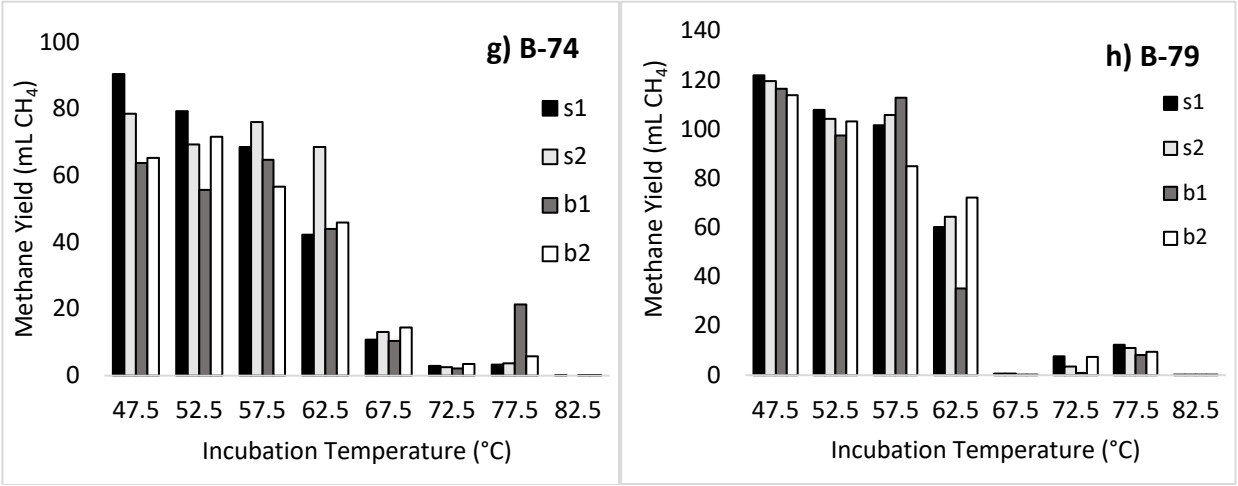

b) B-60
Methane Yield (mL $CH_4$)
30
25
20
15
10
5
0
s1
s2
b1
b2
47.5 52.5 57.5 62.5 67.5 72.5 77.5 82.5
Incubation Temperature (°C)


c) B-61
Methane Yield (mL $CH_4$)
40
30
20
10
0
s1
s2
b1
b2
47.5 52.5 57.5 62.5 67.5 72.5 77.5 82.5
Incubation Temperature (°C)


d) B-67
Methane Yield (mL $CH_4$)
40
30
20
10
0
s1
s2
b1
b2
47.5 52.5 57.5 62.5 67.5 72.5 77.5 82.5
Incubation Temperature (°C)


e) B-70
Methane Yield (mL $CH_4$)
100
80
60
40
20
0
s1
s2
b1
b2
47.5 52.5 57.5 62.5 67.5 72.5 77.5 82.5
Incubation Temperature (°C)


f) B-71
Methane Yield (mL $CH_4$)
200
150
100
50
0
s1
s2
b1
b2
47.5 52.5 57.5 62.5 67.5 72.5 77.5 82.5
Incubation Temperature (°C)


g) B-74
Methane Yield (mL CH4)
100
80
60
40
20
0
s1
s2
b1
b2
47.5
52.5
57.5
62.5
67.5
72.5
77.5
82.5
Incubation Temperature (°C)
h) B-79
Methane Yield (mL CH4)
140
120
100
80
60
40
20
0
s1
s2
b1
b2
47.5
52.5
57.5
62.5
67.5
72.5
77.5
82.5
Incubation Temperature (°C)

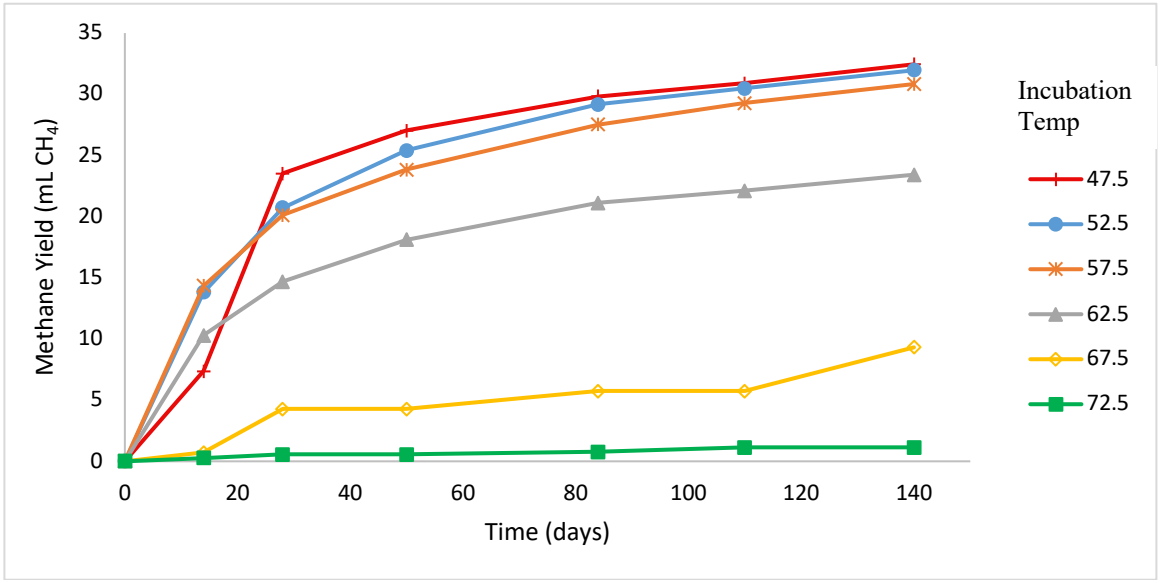



Figure S7a. Methane production for sample A-75. No lag in $CH_4$ production rate was observed.

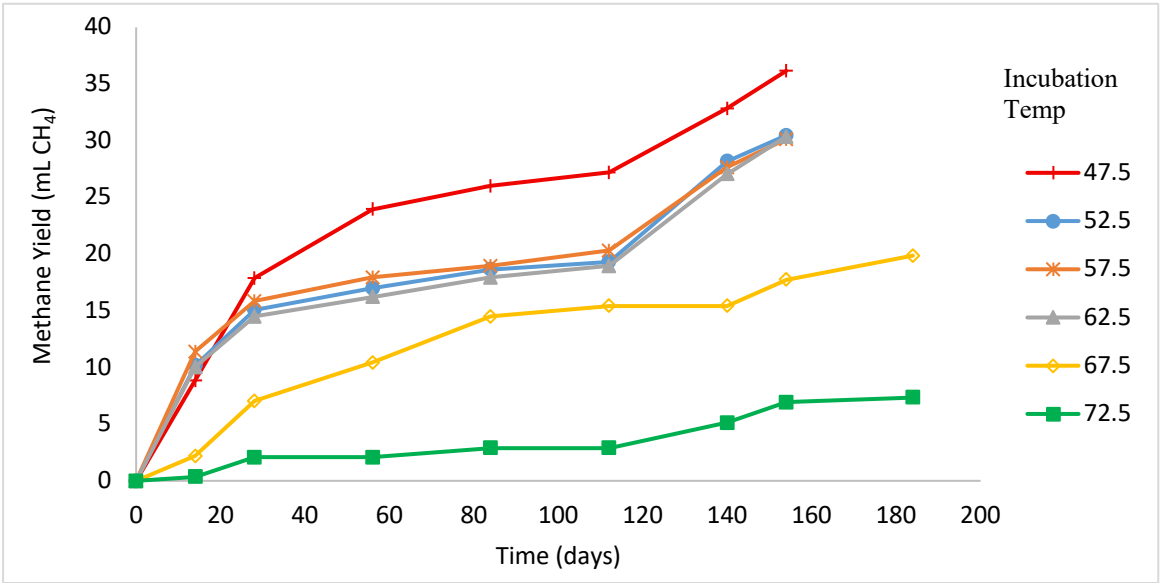
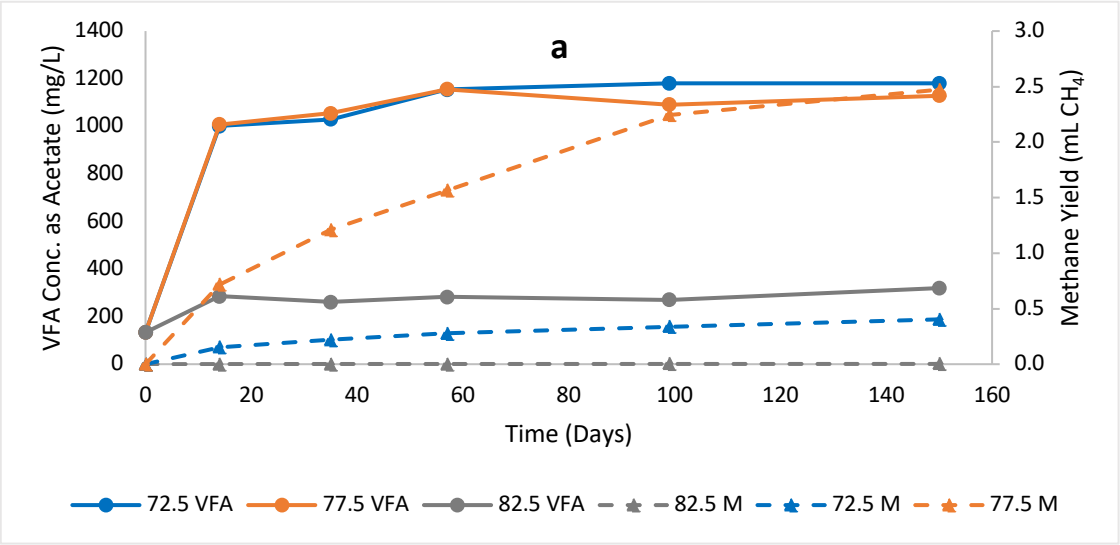
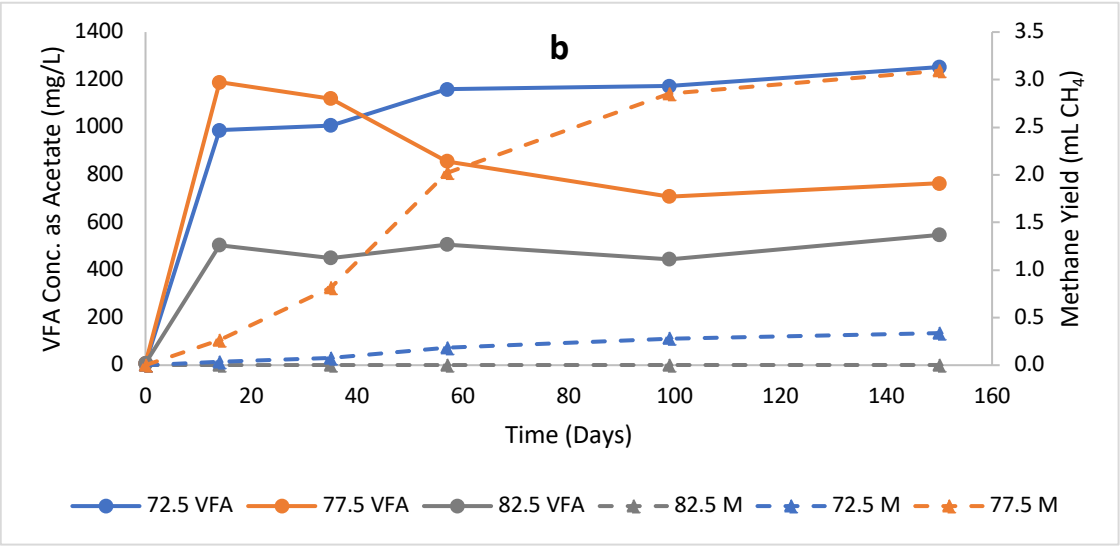
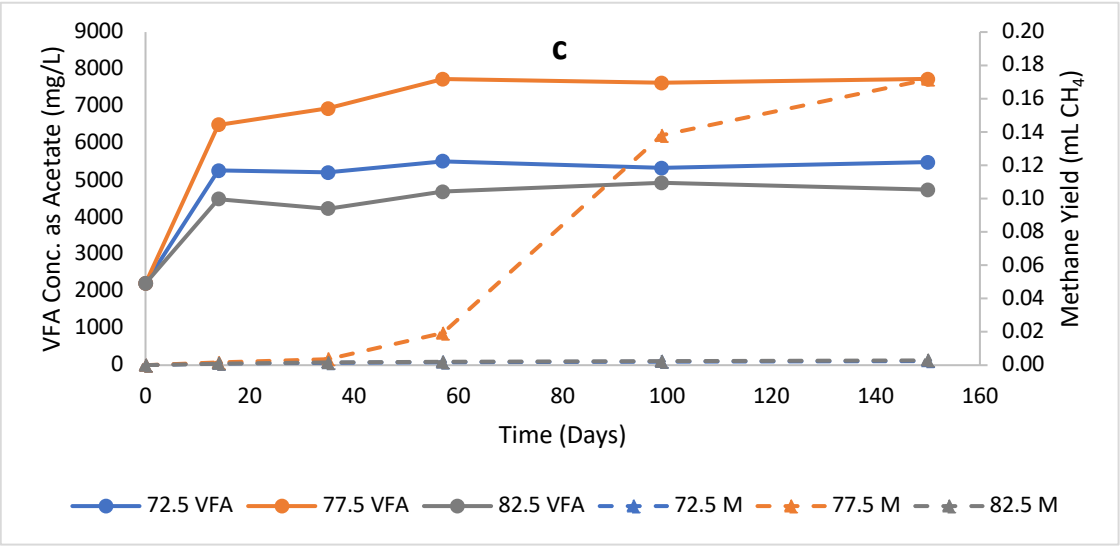



Figure S7b. Methane production for sample A-52. This sample was spiked with additional substrate (synthetic MSW) on Day 117 to test for acclimation potential.


a
VFA Conc. as Acetate (mg/L)
Methane Yield (mL CH4)
Time (Days)
72.5 VFA
77.5 VFA
82.5 VFA
82.5 M
72.5 M
77.5 M


b
VFA Conc. as Acetate (mg/L)
Methane Yield (mL CH4)
Time (Days)
72.5 VFA
77.5 VFA
82.5 VFA
82.5 M
72.5 M
77.5 M


c
VFA Conc. as Acetate (mg/L)
Methane Yield (mL CH4)
Time (Days)
72.5 VFA
77.5 VFA
82.5 VFA
72.5 M
77.5 M
82.5 M

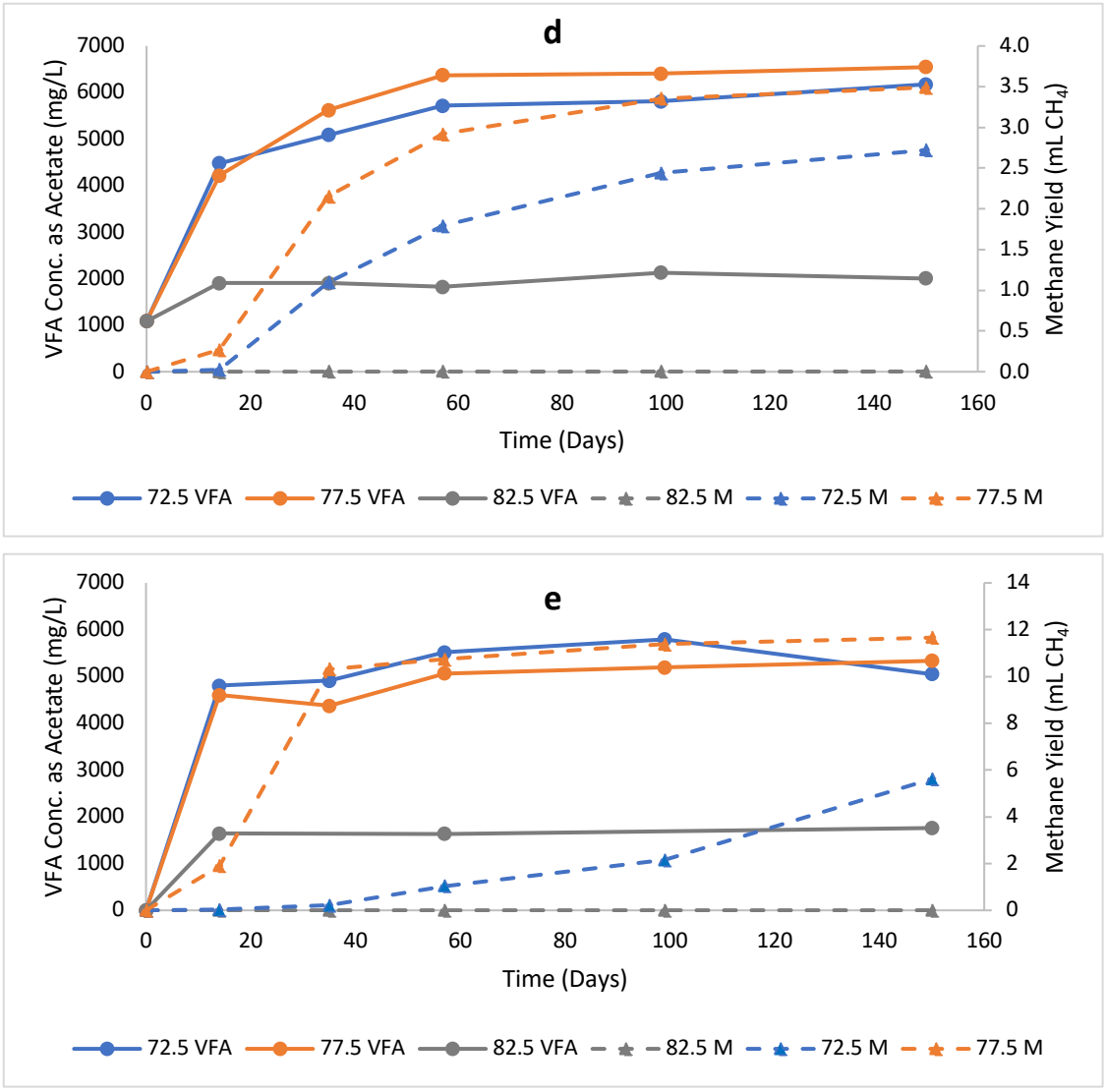



**Figure S8 a-e**. Methane (M) production and VFA concentrations in tube/bottle tests by incubation temperature for samples excavated from LFB at: (a) 60 °C, (b) 61 °C, (c) 71 °C, (d) 74 °C, (e) 79 °C. Sample 67 °C is not included because leachate samples precipitated out after acidification and could not be measured.

Table S5: $H_2$ accumulation (reported in %)[a] detected in samples through week 20 for LFB.

| | Incubation Temperature (°C) | | |
|---|---|---|---|
| Sample | 72.5 | 77.5 | 82.5 |
| B-52 | <0.25 | <0.25 | n.d. |
| B-60 | n.d. | n.d. | n.d. |
| B-61 | <0.25 | <0.25 | n.d. |
| B-67 | n.d. | n.d. | n.d. |
| B-70 | 2.76 | <0.25 | n.d. |
| B-71 | 1.61 | 0.72 | 0.92 |
| B-74 | <0.25 | 0.29 | n.d. |
| B-79 | <0.25 | <0.25 | n.d. |

[a] The value presented is the maximum level observed for the samples tested in duplicate. For samples that were detected below the limit of quantification, the value is reported as <0.25 (%). For samples that could not be detected by the instrument, the value is reported as n.d.

Table S6: CO accumulation detected in samples through week 20 for LFB (ppm) [a].

| | Incubation Temperature (°C) | | |
|---|---|---|---|
| Sample | 72.5 | 77.5 | 82.5 |
| B-52 | 0 | 0 | 0 |
| B-60 | 0 | 0 | 0 |
| B-61 | 0 | 0 | 151 |
| B-67 | 0 | 263 | 0[b] |
| B-70 | 118 | 0 | 0[b] |
| B-71 | 350 | 151 | 205 |
| B-74 | 126 | 129 | 0[b] |
| B-79 | 0 | 0 | 180 |

[a] The value presented is the maximum concentration observed for the samples tested in duplicate. All values obtained from manually integrating the area under the curve.

[b] Value >100ppm were recorded for these samples at week 12. It is unclear why the monitored CO dropped to 0 ppm on week 20.

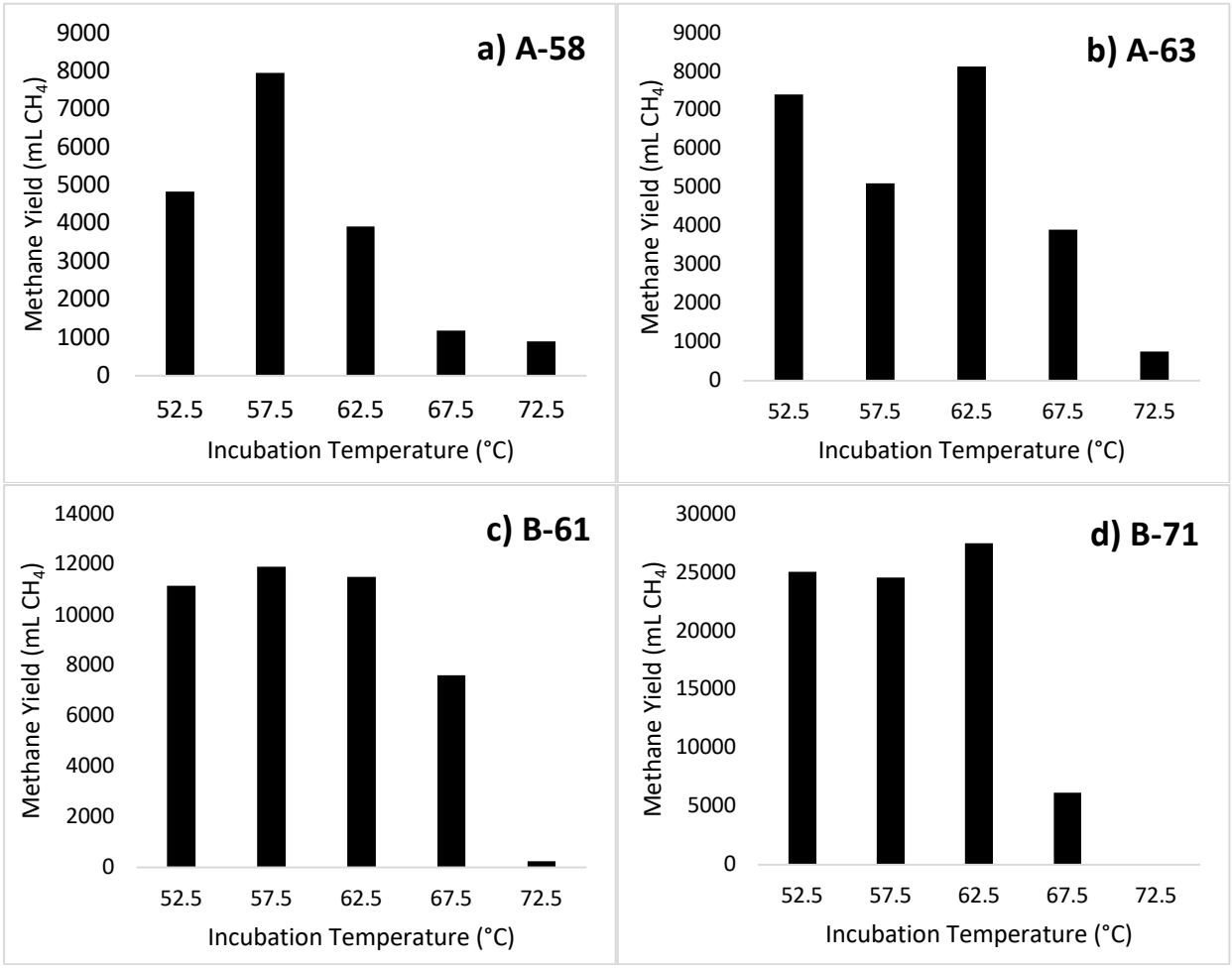



Figure S9a. Cumulative methane yield in reactor tests by incubation temperature for samples excavated from LFA at: (a) 58 °C and (b) 63 °C, and from LFB at: (c) 61 °C and (d) 71 °C, after 20 weeks of incubation.

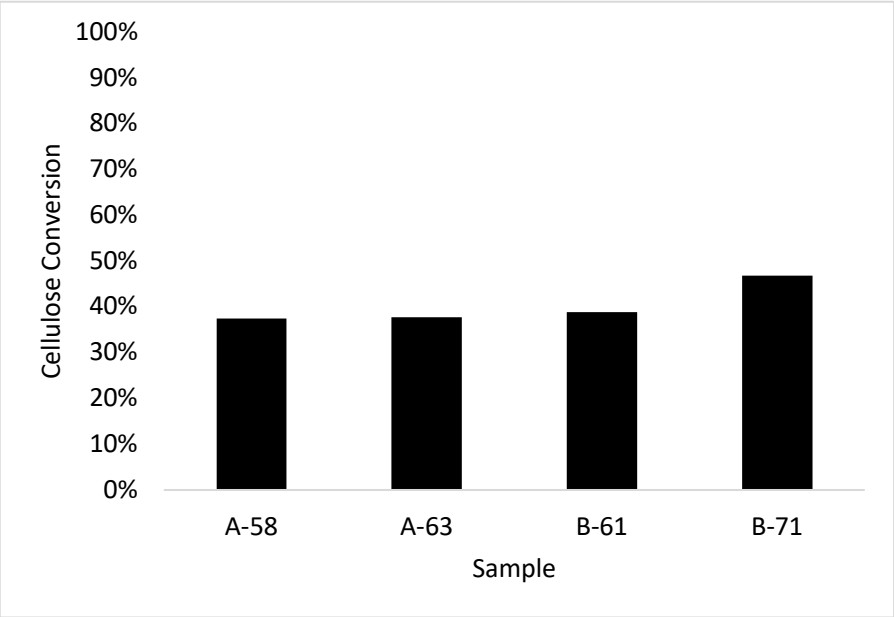



Figure S9b. The percent of the cellulose present in a reactor that was converted to methane based on the stoichiometry of cellulose to methane (414.8 ml $CH_4$/gm cellulose). For this calculation, the hemicellulose was also treated as cellulose. The mass of cellulose was based on the measured cellulose and hemicellulose content of the excavated sample and the added synthetic refuse.

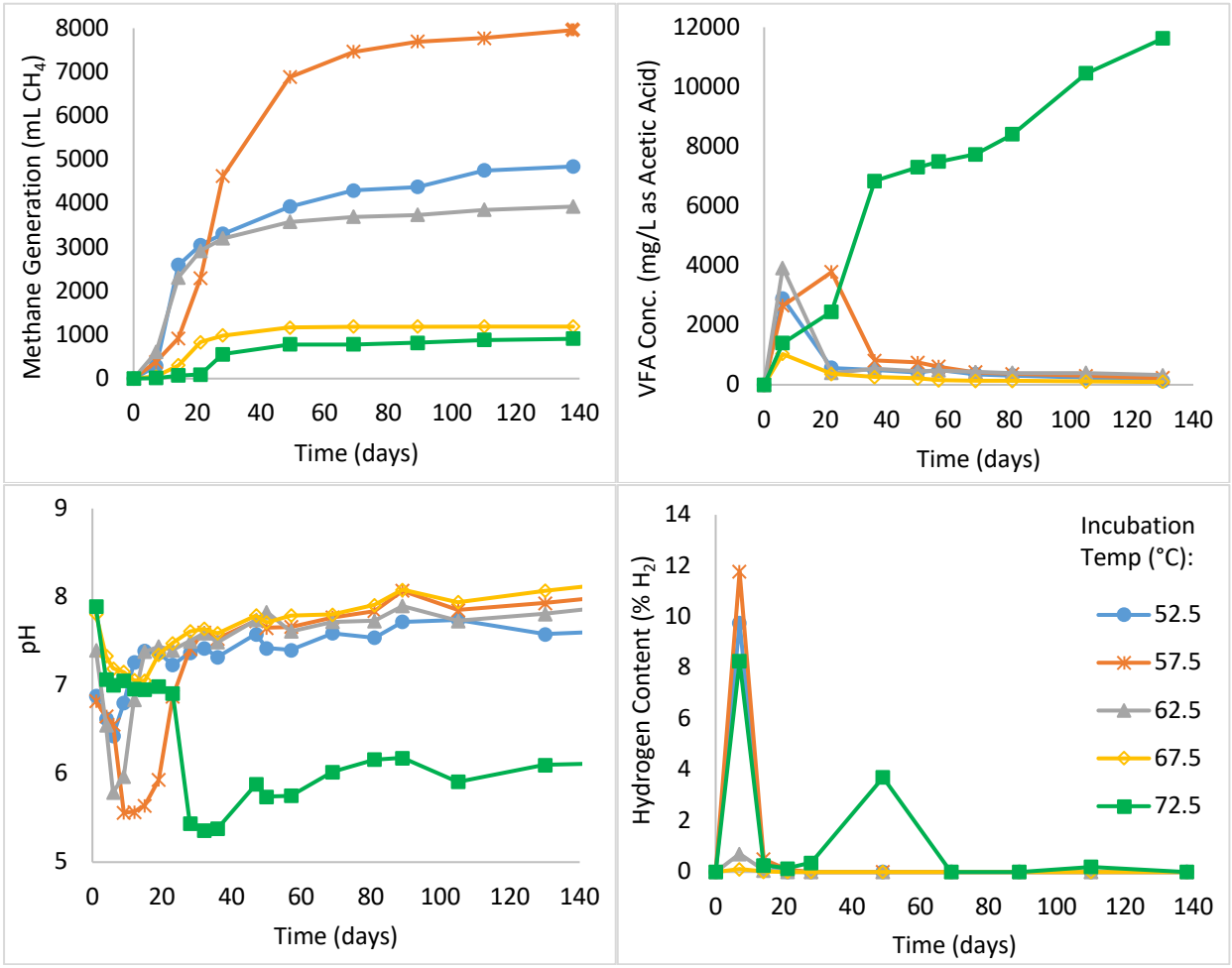



Figure S10. Methane Production, VFA concentrations, pH and $H_2$ in a reactor system for sample excavated at 58 °C from LFA in reactors incubated between 52.5 and 72.5 °C.

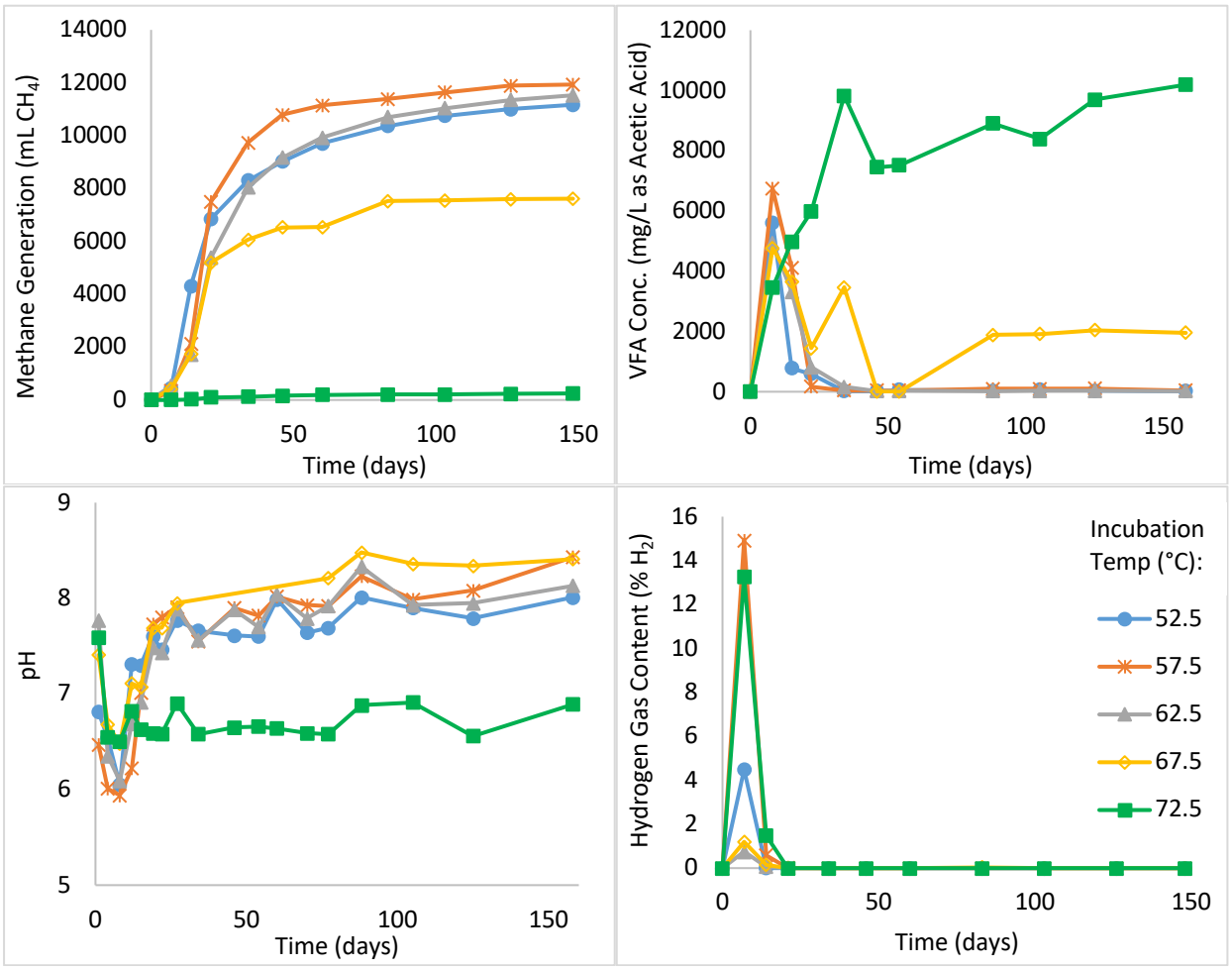



Figure S11. Methane Production, VFA concentrations, pH and $H_2$ in a reactor system for sample excavated at 61 °C from LFB in reactors incubated between 52.5 and 72.5 °C.

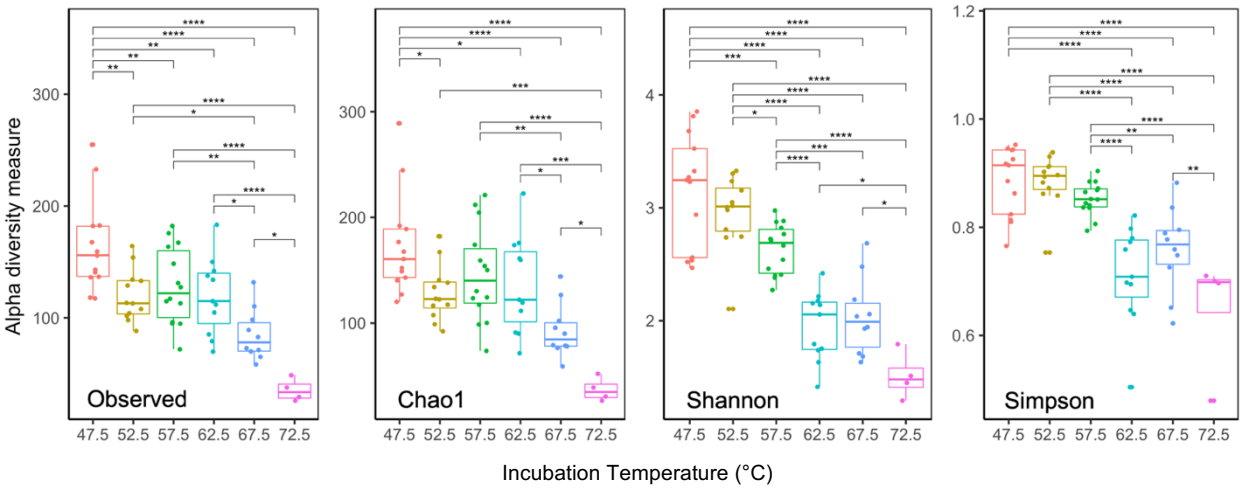



Figure S12. Alpha diversity in samples from LFA incubated between 47.5 to 72.5 C using various quantification metrics. The number of stars represents the statistical significance based on p-values: ****(<0.0001), ***(<0.001), **(<0.01), *(<0.05).

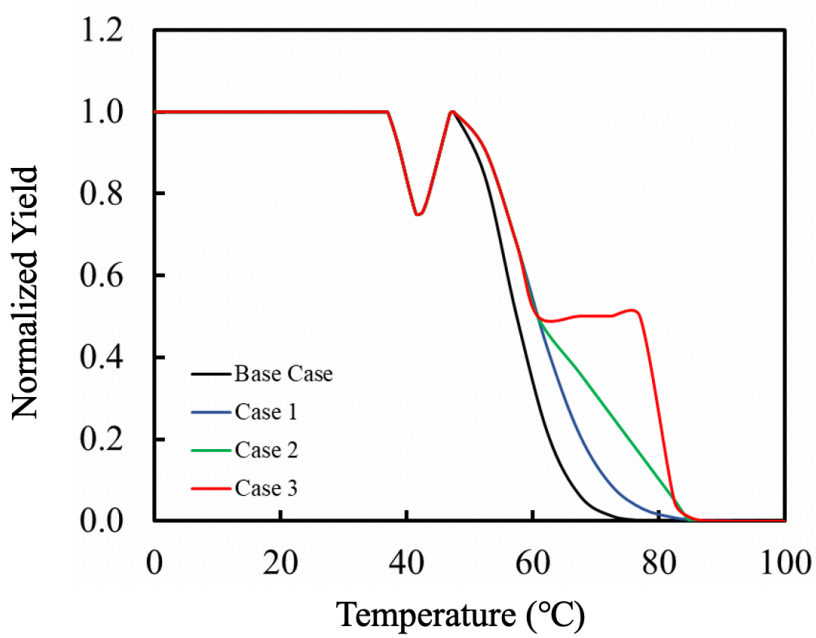



Figure S13. Impact of temperature on methane production. The base case assumes no fermentative activity above levels for methanogenesis and cases 1 – 3 represent a range of possible fermentation temperature inhibition functions. The heat associated with the fermentation reaction is captured here in the extent that it continues past methanogenesis and is quantified for simplicity as methane yield. Case 1 represents an inhibition function derived from the methane yield data from this experiment. Case 2 uses a linear relationship of the inhibition function between 60 and 82.5 C. Case 3 represents a scenario where methane production is only reduced to 50 % between 60 and 77.5 C followed by exponential decay.