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

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ABSTRACT: There have been reports of municipal solid waste landfills with waste and gas wellhead temperatures of at least 80–100 °C, which is in excess of temperatures reported at typical landfills. Landfills experiencing heat accumulation over a broad area present a number of challenges involving leachate and gas quality and quantity, and the associated collection infrastructure. The objectives of this research were to evaluate the impact of temperature on methanogenesis and fermentation in landfills, and to evaluate the extent to which microbial populations acclimate when perturbed to either lower or higher temperatures relative to their in situ temperature. Samples excavated from two landfills exhibiting elevated temperatures were utilized as inocula for small- and reactor-scale experiments. The optimum temperature for methane generation in the thermophilic range was 47.5–57.5 °C, with yields reduced by ∼37 and 75% at 62.5 and 67.5 °C, respectively. While microbial populations did not acclimate above 67.5 °C, methanogenic activity resumed when waste was cooled. 16S rRNA sequencing revealed that Methanothermobacter likely plays a key role in facilitating methane production in landfills operating in the thermophilic range and that microbial community diversity decreased at higher incubation temperatures. There was evidence that fermentation occurs up to 77.5 °C, well above the upper limit for methanogens measured in this study. Model simulations predict that fermentation reactions contribute a ∼3–10 °C rise in waste temperature.

KEYWORDS: methane, inhibition, methanogenesis, archaea, fermentation

INTRODUCTION

There have been reports of municipal solid waste (MSW) landfills with waste and gas wellhead temperatures of at least 80–100 °C.¹⁻⁴ This temperature range is well in excess of the 20–65 °C range exhibited for waste excavated from typical landfills.⁵⁻⁷ Landfills experiencing heat accumulation over a broad area have been referred to as elevated temperature landfills (ETLFs). ETLFs present a number of challenges including increased leachate strength and quantity, decreased methane generation, accelerated settlement, damage to leachate and gas collection systems, the potential for increased temperature on the geomembrane liner, and malodors.¹⁻⁴ While all adverse impacts may not be observed at every ETLF, these landfills nonetheless require increased monitoring and management. Research is ongoing to understand the mechanisms contributing to ETLFs and ultimately to avoid their occurrence. In previous numerical research, the heat from ash hydration and carbonation was shown to be a significant source of heat generation and the presence of ash correlates with elevated temperatures in Florida landfills.⁹⁰

Methane (CH₄) and carbon dioxide (CO₂) are the final products of 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 CH₄/CO₂ ratio to values that approach 0, indicating the cessation of methane generation.¹⁻⁴,¹⁰ An understanding of the extent of inhibition as a function of temperature is lacking and would be useful for at least two reasons. Models of heat generation in landfills are dependent in part on the rate of heat generation from anaerobic biological waste decomposition. Thus, information on the impact of temperature on methane generation will enable improved model parametrization. Second, under the New Source Performance Standards (NSPS), landfills with gas wells that operate above 55 °C must request a higher operating variance (HOV).¹¹ This temperature was selected on the basis of the assumption that higher temperatures are indicative of excessive vacuum on a 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.¹² However, better data are needed to
evaluate appropriate upper temperature limits for landfill gas collection wells.

The optimal temperature ranges for methane production in anaerobic digestors under mesophilic and thermophilic conditions have been established as 30–40 and 50–60 °C, respectively. In addition, different microbial populations have been reported to dominate under mesophilic and thermophilic conditions. There are, however, important differences between landfills and anaerobic digestors, including different substrate characteristics and residence time. It is thus difficult to translate the performance of anaerobic digestors to landfills, and there has been little study of temperature impacts under landfill-relevant conditions. One study showed that the optimal temperature for refuse methanogenesis was 41 °C but did not evaluate temperatures above 48 °C. A second study reported complete inhibition of methane generation at 55 °C. However, there are no data on the temperature limits for anaerobic reactions in landfills, particularly in the thermophilic range. In addition, there are no published data on the response of landfill microbial communities to elevated temperatures.

The objectives of this research were to evaluate the impact of temperature on methanogenesis and fermentation in landfills and to evaluate the extent to which microbial populations shift and acclimatize at higher and lower temperatures relative to their in situ temperatures.

### Experimental Methods

**Experimental Design.** Solid waste samples from two southeastern landfills, LFA and LFB, each reporting waste temperatures up to 90 °C, were used as inocula to explore the impact of temperature on methane generation. LFA and LFB accepted predominantly MSW, but both also accepted MSW combustion ash. Both small-scale and reactor-scale systems were used to maximize the number of samples that could be evaluated. 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 increments above and below the in situ temperature to evaluate both acclimation to higher temperatures and recovery of activity at lower temperatures (Figure S1 of the Supporting Information). On the basis of the results from LFA, the temperature range for LFB was expanded 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 initiated 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. A microbial community analysis was conducted on samples from LFA before and after incubation to identify community members and population shifts. Only samples from the small-scale perturbation experiment were selected for community analysis due to the compatible particle size.

Selected samples from each landfill were also tested in reactors (2 L) in which the entire sample, as opposed to the fines fraction, could be tested. This enabled (1) an evaluation of the extent to which the effect of temperature on methanogenic activity was similar to the fines fraction versus the unprocessed excavated waste as inocula and (2) tests to be conducted in a solid to liquid environment more representative of a landfill. Reactors were incubated in 5 °C increments between 52.5 and 72.5 °C (Figure S3). Reactors were initiated with samples excavated at 58 and 63 °C from LFA and at 61 and 71 °C from LFB. Only two samples from each landfill could be tested in single reactors (no replication) due to space constraints. 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 evaluate acclimation and recovery of activity as for the small-scale incubations.

**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 on the basis of their temperature to obtain

### Table 1. Characterization of Excavated Landfill Samples

| LF | temp (°C) | depth (m) | H₂O (%) | VS (%) | C (%)<sup>b</sup> | H (%)<sup>b</sup> | L (%)<sup>b</sup> | (C + H)/L<sup>c</sup> |
|----|-----------|-----------|---------|--------|-----------------|-----------------|-----------------|------------------|
| A  | 55        | 6         | 23.6    | 24.5   | 18.1            | 6.9             | 23.3            | 1.1              |
|    | 52        | 9         | 23.7    | 26.4   | 17.0            | 6.2             | 28.4            | 0.8              |
|    | 58        | 12        | 11.5    | 16.3   | 1.8             | 0.9             | 22.7            | 0.1              |
|    | 63        | 15        | 13.5    | 5.5    | 8.0             | 2.9             | 30.8            | 0.4              |
|    | 67        | 18        | 27.5    | 27.5   | 26.1            | 5.8             | 30.9            | 1.0              |
|    | 64        | 21        | 11.3    | 6.5    | 3.4             | 1.2             | 20.1            | 0.2              |
|    | 70        | 21        | 21.6    | n.d.   | n.d.            | n.d.            | n.d.            | n.d.             |
|    | 75        | 24        | 16.1    | 35.1   | 11.0            | 1.9             | 15.1            | 0.9              |
| B  | 52        | 9         | 28.3    | 19.9   | 10.4            | 3.5             | 26.6            | 0.5              |
|    | 60        | 9         | 26.4    | 18.5   | 21.7            | 6.2             | 34.7            | 0.8              |
|    | 61        | 12        | 21.2    | 15.8   | 29.9            | 9.1             | 31.1            | 1.3              |
|    | 67        | 12        | 24.2    | 33.6   | 31.4            | 7.8             | 27.4            | 1.4              |
|    | 70        | 15        | 25.6    | 26.1   | 18.8            | 4.2             | 26.0            | 0.9              |
|    | 71        | 15        | 28.2    | 60.7   | 30.8            | 7.1             | 24.1            | 1.6              |
|    | 74        | 18        | 19.6    | 18.8   | 12.6            | 2.9             | 17.0            | 0.9              |
|    | 79        | 21        | 25.4    | 45.5   | 28.9            | 5.4             | 20.7            | 1.7              |

<sup>a</sup>Wet weight basis (mass water/mass wet waste). A 50 g composite of each sample was used to measure MC. <sup>b</sup>C, H, and L represent cellulose, hemicellulose, and lignin, respectively. Data are expressed as a percentage of the volatile solids. The average coefficient of variation (CV) for C, H, and L analyses are 12.0, 8.8, and 9.3%, respectively. <sup>c</sup>(C + H)/L is the ratio of the major biodegradable (cellulose, hemicellulose) to nonbiodegradable (lignin) constituents.
samples ranging from 50 to 80 °C (Table 1). Samples were placed in large plastic bags, evacuated using a vacuum pump, and placed in coolers in an attempt to maintain the waste temperature close to the original excavation temperature. The fines fraction of each sample was recovered within 72 h for the use as inocula, and experiments were initiated within 1 week of excavation after ground transport.

Each sample was manually mixed and quartered to obtain subsamples for waste characterization, use in the small- and reactor-scale experiments, and community analysis. A portion of each sample was sieved through a 6.35 mm 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 was preserved for community analysis at −20 °C.

**Experimental Equipment and Operation.** For the small-scale experiments, the fines were incubated with added substrate and sterile anaerobic growth medium (Table S1) in either 30 mL pressure tubes or 160 mL serum bottles (Wheaton, Millville, NJ) that were crimp-sealed with 20 mm butyl rubber stoppers (Bellco, Vineland, NJ). An anaerobic medium was added to each tube or bottle after flushing with CO₂/N₂ (20/80, v/v). The substrate was ground (<1 mm) synthetic-MSW (Table S2). The tubes/bottles containing medium and substrate were autoclaved at 121 °C. After cooling, Na₂S solution (0.01%) was added as a reducing agent. Finally, the tubes/bottles were inoculated with the fines and resealed in an anaerobic chamber with a H₂/N₂ (5/95, v/v) headspace. The tube contents included 9 mL of media, 0.1 g of substrate, 0.1 mL of Na₂S, and 5 g of fines. To provide a larger volume for gas and liquid samples to characterize fermentation (H₂, CO, volatile fatty acids [VFAs]), the LFB samples incubated between 72.5 and 82.5 °C were tested in 160 mL bottles (Figure S2). In this case, the bottles included 100 mL of medium, 1 g of substrate, 1 mL of Na₂S solution, and 50 g of fines.

Reactor experiments were conducted in 2 L wide-mouth borosilicate glass bottles (Corning Inc., Corning, NY) that were sealed with #13 rubber stoppers. Nonfractionated landfill samples (400 g) and shredded synthetic-MSW (100 g) were mixed and added to each reactor while flushing with CO₂/N₂ (20/80, v/v). A 1 L sample of sterile 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 recirculation and for gas collection. Leachate was collected in intravenous bags (WWR, Randor, PA) to allow for recirculation and liquid sampling, while gas was collected in FlexFilms gas bags (SKC Inc., Eighty Four, PA). The reactor system was connected by 6.35 mm (ID) Masterflex FEP-lined tygon transfer tubing (Cole-Parmer, Vernon Hills, IL). Assembled reactors were tested for leaks using a vacuum pump.

All tubes, bottles, and reactors were maintained within ±1 °C of their respective temperatures in 28 L water baths.

**Monitoring.** The excavated solids were characterized on the basis of moisture content (MC), volatile solids (VS), and cellulose (C), hemicellulose (H), and lignin (L) concentrations. Gas volume and composition (CO₂, O₂, N₂, and CH₄) were measured weekly and biweekly for the reactor and small-scale experiments, respectively. CO, H₂, and VFAs were also monitored weekly and biweekly in the 2 L reactor and 160 mL bottle experiments, respectively. In the bottle experiment, the liquid sample (5 mL) was replaced with N₂ to maintain the same headspace pressure. Reactor leachate pH was measured every 2–3 days 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 community analysis once methane production began to plateau at week 20. The reactors were also monitored for 20 weeks. Except for C, H, and L, all analyses were conducted without replication.

**Analytical Methods and 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. C, H, and L were analyzed by a two-stage acid hydrolysis and measurement of the resulting sugars. VFAs were measured with a GC-FID.

Gas volume in the tubes and bottles was measured by using a water-lubricated glass syringe. An evacuated cylinder of known volume was used to measure the volume of gas in reactors as collected in gas bags. H₂ was analyzed using an SRI gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) and a 1.83 m 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).

**Microbial Community Analysis.** Samples were prepared for analysis using separate methods for the samples before (fines) and after testing (slurry) given the different physical forms. The fines fraction from the original excavated samples was blended with anaerobic phosphate buffer (23.7 mM) and centrifuged in 50 mL tubes at 3220 g for 5 min to form a pellet for DNA extraction. The postincubation slurry samples were well-mixed, poured in four 1.7 mL tubes and centrifuged at 6000g for 10 min to form pellets for DNA extraction, after which the supernatant was decanted and the pellets were frozen at −80 °C. All work was conducted in a biological safety cabinet to maintain sterility and avoid cross-contamination.

After sample preparation, DNA was extracted from the pellet following the method for the Qiagen DNeasy PowerSoil Kit (Qiagen, Carlsbad, CA) with a few modifications to minimize the high contamination levels as described in the Supporting Information. Following extraction, DNA samples were amplified using polymerase chain reaction (PCR) with modified 341F (5′-CCTAYGGGRBGCASCAG) and 806R (5′-GGACTACNNGGGTGATCTAA) primers that target the 16S V3 and V4 regions of both archaeal and bacterial 16S rRNA genes. Next-generation sequencing was performed using the Illumina MiSeq platform with a paired-end sequencing of 300 base pairs length. The results were analyzed using QIIME 2 and R packages as described in the Supporting Information. The raw sequence data were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (BioProject accession number: PRJNA660751) under BioSample accessions SAMN15959993 -SAMN15960071.

### RESULTS AND 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. Samples are identified by the landfill and temperature of each sample (e.g., a sample excavated at 52 °C from LFA is A-52). Next, the results of the microbial community analysis for LFA are presented, after
which the temperature effects data for all experiments are combined and implications for ETLFs discussed.

**Waste Characterization.** The relatively low VS concentrations indicate that many samples were likely diluted with cover soil and/or ash as even decomposed MSW would have a VS of at least \( \sim 40\% \) (Table 1). The ratio of cellulose plus hemicellulose to lignin \([ (C + H)/L ]\) is an indicator of the extent of decomposition as lignin is recalcitrant under anaerobic conditions, and the ratio eliminates the impact of dilution with inorganics. The average \([ (C + H)/L ]\) was 0.65 ± 0.41 and 1.19 ± 0.35 for samples excavated at temperatures below and above 65 °C, respectively \((p = 0.016)\). The higher \([ (C + H)/L ]\) for samples excavated above 65 °C is indicative of more undegraded substrate and suggests that in situ biodegradation was reduced, although depth and waste age are confounding factors. A temperature of 65 °C was selected on the basis of the measured impact of temperature on methane generation described below. As predicted in numerical simulations and reported for field studies, the solids temperature generally increased with depth to the center of the waste.10,37

**Small-Scale Perturbation Experiment.** The impact of temperature on methane generation for selected LFA and LFB samples is presented in Figure 1, with the remaining data presented in Figures S4 and S5. In general, methane generation decreased as incubation temperature increased. Maximum methane generation occurred between 47.5 and 57.5 °C, and methane generation occurred up to 62.5–67.5 °C, with yields reduced by \( \sim 50\% \). 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 methane production was not dependent on waste excavation temperature, as samples excavated with a range of excavation temperatures exhibited similar methane production behavior 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 (Figure 1). Thus, the results do not suggest that in situ populations acclimated to higher temperatures. However, the populations present in the samples excavated at higher temperatures produced methane without a lag when incubated at lower temperatures, indicating recovery of methanogenic activity is possible (Samples A-70, A-75, B-71, B-74, B-79 in Figures S4 and S5 and Figure S6a).

After 16 weeks, the tubes inoculated with sample A-52 were spiked with additional substrate to further evaluate microbial acclimation (Figure S6b). As in the initial incubation, substrate was consumed without a lag at 47.5–62.5 °C, but a second lag and reduced methane generation was again observed in the 67.5 and 72.5 °C treatments. Thus, microbial populations were required to reacclimate and were slow to grow even after 117 days of incubation at higher temperatures. Similarly, transitions in anaerobic digesters beyond 60 °C have been reported to disrupt overall system stability.15,22,38

The average of the maximum methane yields in the incubations with samples excavated above 65 °C, 85 ± 50 mL of methane, was significantly higher than the average yield \((30 ± 8 \text{ mL})\) in samples excavated below 65 °C \((p = 0.009)\). The yields represent those measured in the 47.5 and 52.5 °C incubations, where yields were generally the highest. This result can be explained by the higher C and H in the samples excavated at or above 65 °C (Table 1). As described above, the higher C and H in the samples above 65 °C suggests in situ inhibition. This observation is consistent with the incubation

![Figure 1](https://dx.doi.org/10.1021/acsestengg.0c00064)
experiments, which demonstrated a reduction in methane production at temperatures ≥62.5 °C.

While methanogenesis was largely inhibited at 72.5 °C, there was evidence for fermentation up to 72.5 °C, as evidenced by VFA accumulation (Figure S7). Fermentation in the absence of methanogenesis was measured consistently at 72.5 and 77.5 °C, with ~15–40% of maximum VFA concentrations present at 82.5 °C, indicating that the upper limit for biological activity in these samples is near 82.5 °C. These results are consistent with earlier studies 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.20,39,40 Figure S8 presents the methane production and VFA concentration data for LFB incubations.

Although H2 concentrations were monitored, the limit of quantitation (0.25%) was relatively high so only qualitative observations are appropriate. H2 accumulation was variable and only measured at 82.5 °C in two of eight incubations (Table S5, Figure S9). This is consistent with the VFA data that indicate that fermentation becomes inhibited between 77.5 and 82.5 °C. In all tests performed at 82.5 °C, with the exception of sample B-71, neither H2 nor VFAs accumulated throughout the 20 week incubation period. The lack of sustained accumulation of these fermentation intermediates could not be attributed to substrate limitation but may be a result of unfavorable energetics as a result of end-product (H2) accumulation. On the basis of the measured acetate, butyrate, and H2 concentrations, or assuming H2 present at the LOQ, ΔG varied from ~26 to +27 kJ/mol butyrate on the basis of a number of scenarios. Finally, intermittent CO accumulation was measured as described in the Supporting Information (Table S6). CO accumulation has been recorded at several landfills experiencing elevated temperatures and may be attributed to both chemical and biological reactions.

Reactor-Scale Perturbation Experiment. Selected samples were tested in 2 L reactors to (1) evaluate the extent to which the temperature effects observed with the fines and the bulk waste were similar and (2) conduct tests in a solid-to-liquid environment more representative of a landfill. Methane production generally decreased as temperature increased in the reactors (Figures 2 and Figures S10–S13). However, higher optimum incubation temperatures, 57.5–62.5 °C, were observed relative to the 47.5–52.5 °C observed in the small-scale incubations. Methane generation varied from 15 to 64% of the maximum at 67.5 °C, and methanogenesis was consistently inhibited at 72.5 °C. The extent of cellulose conversion was calculated from the maximum measured methane yield for each sample, the mass of refuse and the initial cellulose and hemicellulose concentration, with hemicellulose considered as cellulose. The conversion of cellulose plus hemicellulose to methane was similar across all samples at ~37–47% (Figure S14). Thus, microbial performance in all reactors was comparable despite varying amounts of substrate added.

Samples A-63 and B-61 were chosen to compare similar excavation temperatures from different landfills. The samples behaved similarly with the exception of the anomaly at 57.5 °C for sample A-63. Anomalous behavior was observed in A-63 and also in A-58 in that there was not a consistent trend with temperature (Figure 2). Unfortunately, logistical constraints precluded replication in the reactor experiments so there is no measure of natural variability.

On the basis of the LFA results, 1 LFB sample with a higher excavation temperature was tested. The optimum temperature for methane production was 52.5–62.5 °C for samples B-61 and B-71 and not apparently dependent on excavation temperature. The sample excavated at the highest temperature, B-71, was inhibited (22% of maximum) at 67.5 °C, so there is not a consistent trend of acclimation to higher incubation temperatures among samples excavated at higher temperatures. A-58 exhibited little methane production at 67.5 °C. However, there was also no evidence for fermentation (i.e.,
neutral pH, low VFAs), which is surprising (Figure S10). In contrast, in B-71, VFAs accumulated at 67.5 °C before VFA consumption was observed concurrent with a pH increase and initiation of methane generation after 80 days (Figure S13). This shift indicates that the methanogenic population was able to partially acclimate at 67.5 °C although the yield is still relatively low.

There was consistently minimal to no methane production in tests at 72.5 °C and there was VFA accumulation, which is consistent with evidence for fermentative activity. The majority of VFAs were produced in the first 2 weeks; however, unlike in the small-scale experiment, VFAs continued to accumulate. As expected, the pH in reactors with VFA accumulation remained acidic. After week 2, H₂ levels were only consistently detected for sample B-71 when incubated at 72.5 °C, ranging between 2 and 8% (Figure S13). CO was only detected at an incubation temperature of 72.5 °C, which suggests that it may only accumulate when methanogenesis is inhibited but fermentation is not. 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 H₂,
only sample B-71 had CO present consistently through week 20. In summary, the reactor experiment provided further evidence that fermentation becomes the dominant biological process at 72.5 °C. As in the small-scale experiment, samples excavated at higher temperatures readily produced methane when cooled to lower temperatures. Despite some differences in the optimum incubation temperature, the results show that 67.5 °C is about the upper temperature limit for methanogenic activity. This limit is consistent with reports on anaerobic digesters that define 70 °C as a threshold for the conversion of acetate to methane \(^{41}\) and with few isolated species of methanogens identified as capable of active growth above 60 °C.\(^{42,43}\)

**Microbial Community Structure as Influenced by Excavation and Incubation Temperatures.** The relative abundance and population shift for archaea and bacteria in LFA samples are described in this section; LFB data were not available. The impact of excavation and incubation temperatures on the microbial communities is presented in Figures 3–5 and Figures S15 and 16. *Methanothermobacter* was the predominant methanogen (63–100% of the total archaeal populations) in all eight samples excavated from LFA at 52 to 75 °C. There was no apparent relationship between excavation temperature and archaea community structure, similar to a report by van Lier et al.\(^{44}\) (Figure 3a). However, due to the lack of replication, we could not statistically evaluate the impact of excavation temperature on the community structure of the excavated samples. *Methanothermobacter* is a genus of the order *Methanobacteriales* that grows optimally from 55 to 65 °C and up to 75 °C.\(^{15,16}\) This hydrogenotrophic methanogen has been found to predominate in landfill leachate\(^{20}\) as well as in several thermophilic anaerobic digesters.\(^{48,49}\) *Methanothermobacter thermotrophicus* was also predominant in samples from an MSW landfill in a previous study, which the authors speculated was experiencing higher temperatures.\(^{38}\)

After incubation at 47.5 °C, *Methanosarcina* and *Methanosaeta* became abundant (Figure 3b). The relative abundance of *Methanosarcina* was as high as 86% (excavation temperature 55 °C), while *Methanosaeta* reached up to 58% (excavation temperature 52 °C). At 52.5 °C, *Methanosarcina* and *Methanosaeta* were still detected, but their relative abundances decreased to <60% and <37%, respectively. Both genera fall within the order *Methanosarcinales* and have been previously identified in samples from a landfill experiencing elevated temperatures (unpublished data). *Methanosarcina* can produce methane via the hydrogenotrophic and acetoclastic pathways, while all known *Methanosaeta* species are strict acetoclasts.\(^{50,51}\) *Methanosarcina* and *Methanosaeta* typically predominate in high- and low-acetate-concentration environments, respectively,\(^{19,52,53}\) and both, including some thermophilic strains, are commonly detected in anaerobic digesters.\(^{18,19,39,46,50,54}\) Thermophilic strains of *Methanosarcina* grow optimally from 50 to 57 °C but are inhibited beyond 60–62 °C.\(^{41}\) As our incubation temperatures increased to 57.5–62.5 °C, these two genera decreased in relative abundance and *Methanothermobacter* predominated (41–100%, Figure 4b). At 67.5 °C, a gradual increase in the presence of uncultured *Thermoprotei* archaeon was observed, and it was further enriched at 72.5 °C. Little information is available on this nonmethanogenic genus, but its predominance is likely due to its heat-tolerance.\(^{35,56}\) Most of the archaeal communities detected here belong to the same orders as those identified in laboratory-scale, landfill simulations conducted under mesophilic conditions and include *Methanomicrobiales*, *Methanosarcinales*, and *Methanobacteriales*.\(^{4,53,57}\) The presence of these orders suggests that a thermophilic community capable of methane production may be present in most or all MSW landfills, and increasing temperatures enrich certain populations within the community.

In the bacterial community, the genus *Bacillus* had the highest relative abundance in samples with excavation temperatures of 58, 63, 67, and 70 °C (Figure 4a), while *Tepidibacillus*, *Herbinix*, or *Lysinibacillus* predominated in samples excavated at 52, 55, 64, and 75 °C. *Bacillus* species are spore-forming cellulolytic hydrogen-producing syntrophic bacteria capable of withstanding thermophilic conditions.\(^{38,59}\) Various members of this genus have been found to predominate in several landfill studies.\(^{26,47}\) In addition, *Bacillus* were recently found to predominate in an ash layer with high pH in a Japanese landfill,\(^{60}\) which is consistent with the acceptance of ash in LFA. The predominance of non-*Bacillus* genera in four of the samples is presumably due to environmental factors other than excavation temperature. Like *Bacillus*, *Herbinix* has been identified as a cellulose-degrading bacterium. *Lysinibacillus* is known for its tolerance and biosorption of heavy metals,\(^{61,62}\) and *Tepidibacillus* is a moderate thermophile capable of anaerobic and microaerophilic fermentation.\(^{53,64}\) These genera may be more competitive than *Bacillus* species in environments with relatively low temperatures and high levels of cellulose.

There were considerable shifts in the bacterial communities after incubation (Figure 4b). At the lower incubation temperatures (47.5–52.5 °C), *Defluvitoga* and *Hydrogenispora* were abundant. Members of these genera are thermophilic fermenters that can produce acetate and hydrogen and have been identified in anaerobic digesters.\(^{65,67}\) The *Hydrogenispora* genus contains spore formers and has been added to anaerobic reactors to enhance hydrogen and acetate production,
presumably by shifting the indigenous bacterial communities. As incubation temperatures increased to 57.5–62.5 °C, *Hydrogenispora* remained in high relative abundance, and an unclassified genus named anaerobic digester metagenome (affiliated with the class Clostridia) became enriched (up to 62%).

At higher incubation temperatures (67.5–72.5 °C), multiple putative thermophilic and hyperthermophilic fermenters were observed in high abundance, including *Caldicoprobacter*, *Pseudothermotoga*, *Thermotoga*, and *Acetothermia* clone OPB14. Its presence in mesophilic landfills demonstrates the potential for its enrichment under thermophilic conditions. Bareither et al. reasoned that the hydrogen-producing *Thermotoga* phyla were linked to syntrophic growth of hydrogenotrophic methanogens, and these bacteria predominated during peak methane generation. They are also reported to form sheath-like envelopes, facilitating nutrient uptake in nutrient-poor environments.

The microbial community structure of the excavated samples changed significantly after they were incubated in the laboratory (Figure S15, p = 0.001). The excavated and incubated samples fell into two distinct clusters, indicating high dissimilarity in microbial composition. This result was somewhat expected due to physical and chemical differences between lab-scale culturing and field conditions (e.g., solids content, substrate quality, temperature). For the incubated samples, the excavation temperatures did not significantly influence the community composition (p > 0.05), but differences in community composition were observed as the incubation temperature changed. The incubated samples became significantly less diverse with increasing incubation temperatures, as evidenced by a decrease in four alpha diversity metrics (Figure S16). These indices indicate that at higher incubation temperatures, the microbial communities had fewer species (i.e., low richness), and a small number of species were highly abundant (i.e., low evenness). These results are consistent with several anaerobic digester studies that have demonstrated distinct and less diverse thermophilic microbial populations relative to mesophilic populations.

Samples incubated at the same incubation temperature closely clustered together (Figure S5), indicating that they had high similarity in microbial composition. Statistically, the community shifts associated with incubation temperatures were significant, with all pairwise p-values less than 0.05 (Table S7). Moreover, the incubation temperature was closely related to relative methane yield, with high temperatures (67.5–72.5 °C) resulting in low yields (Figure S5).

**Summary and Implications.** The impact of temperature on methane generation across all experiments is summarized in Figure 6. With one exception, ≥75% of the relative methane potential for each sample was produced at incubation temperatures of 62.5 °C, while only 10–40% relative methane potential was realized at 67.5 °C, albeit with increased variability. Methane generation was minimal at 72.5 °C. These trends are consistent across all samples and seemingly independent of individual waste characteristics. Thus, the results indicate that methane generation in landfilled waste experiencing elevated temperatures up to ~62.5–67.5 °C is possible, with inhibition above 62.5 °C. The optimum temperatures for methanogenic activity in this study, 47.5–57.5 °C, are supported by solid waste thermophilic anaerobic digester studies, including the results of Lee et al. (Figure 6). The upper temperature limits of ~67.5 °C for methanogenesis and ~82.5 °C for fermentation for landfill samples are consistent with the literature.

Our results have implications for the interpretation of landfill gas temperature data. Although gas temperatures are most typically available at full-scale landfills, gas temperatures are ~10–20 °C cooler than waste temperatures, which are not typically measured. Thus, a gas temperature of ~50 °C may represent a solids temperature of ~65 °C, and this adjustment from gas to solids temperature is imperfect. While the temperature of a solid sample represents the solids mass, this is not the case for the landfill gas temperature. Gas temperatures represent an average over the well depth, which could be ~20–75 m, and the volume of gas generated in waste at inhibited temperatures will be lower than the volume generated at favorable temperatures. Thus, the landfill gas temperature measured in a well is almost certainly lower than that of a layer of waste experiencing relatively high temperatures, and an elevated gas temperature likely reflects even hotter solids in some of the waste column. Thus, while an
HOV is currently required at landfill gas temperatures above 55 °C, this temperature should also be used as a trigger to determine the extent to which there is waste at higher temperatures.

A second implication of this study relates to modeled gas production. While landfill gas generation models are inherently uncertain, they are useful to evaluate relative impacts. To evaluate the extent to which decreases in methane generation are likely to be detected by evaluation of trends in collected gas at field-scale, simulations were conducted using the US EPA’s LandGEM model (Figure 7). Simulations were run for a base case with no inhibition at 100% 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. 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. Thus, a review of whole landfill gas volume data is not likely to detect inhibition present in 30% of the waste, though more detailed monitoring of individual landfill areas may result in detection.

While methanogenesis is the most crucial biological process in landfills, this research also evaluated the upper temperature limit for fermentation, given that fermentation reactions continue and therefore produce heat at higher temperatures than methanogenesis. To explore the influence of methanogenesis and fermentation energetics on heat accumulation in landfills, a series of simulations was explored using the batch model described in Hao et al. The Base Case assumes no fermentative activity above levels for methanogenesis, and Cases 1–3 represent a range of possible fermentation temperature inhibition functions illustrated in Figure S1. The predicted temperatures are presented in Figure 8. The results demonstrate that, if fermentative activity occurs up to 82.5 °C, while methanogenesis inhibition begins at 52.5 °C and is completely inhibited at 67.5 °C, then fermentation alone can result in an additional increase in waste temperatures of 3–10 °C. This impact should be incorporated into heat generation models.

In summary, the optimum temperature for methane generation in the thermophilic range was 47.5–57.5 °C, with reduced methane observed up to 67.5 °C. While microbial populations did not acclimate above 67.5 °C, methanogenic activity resumed when the waste mass was cooled. 16S rDNA amplicon sequencing showed that communities shifted on the basis of their incubation temperatures irrespective of the...
original sample temperature. In addition, microbial diversity decreased with increasing temperatures. Future work should be aimed at better understanding the microorganisms and their role in methane production and fermentation across the range of temperatures studied. Fermentation occurred above the upper limit for methanogenesis, suggesting the importance of fermentation in heat generation models. Although the focus of this research was on biological reactions, abiotic cellulose hydrolysis has been found to occur at temperatures as low as 72 °C (unpublished data) at which temperature fermentation reactions occur. Thus, heat evolution from fermentation reactions may stimulate an abiotic source of heat generation, but addressing this hypothesis requires further study.

■ ASSOCIATED CONTENT

* Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestengg.0c00064.

DNA extraction procedure; tables of anaerobic toxicity assay procedure, composition of synthetic MSW substrate and anaerobic growth medium, S and assay results, H₂ and CO accumulation data, analysis of variance results, and composition data for archaeal and bacterial communities; figures of the perturbation matrices, methane and hydrogen production data for additional samples, VFA concentrations, percent of the cellulose present in a reactor converted to methane, temperature inhibition function, and alpha and beta diversity (PDF)

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Notes
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