The Effect of Temperature on the Performance and Stability of Thermophilic Anaerobic Digestion

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Abstract: Sustainable operation of an anaerobic sewage sludge digester requires the effective shuttling of carbon from complex organic material to methane gas. The accumulation of intermediates and metabolic products such as volatile fatty acids and hydrogen gas not only reveal inefficiency within the digestion process, but can be detrimental to reactor operation at sufficiently high levels. Eight anaerobic digesters (1 mesophilic and 7 thermophilic) were operated in order to determine the effect of steady-state digestion temperature on the operational stability and performance of the digestion process. Replicate reactors operated at 57.5°C, the highest temperature studied, were prone to accumulation of volatile fatty acids (4052 and 3411 mg/L as acetate) and gaseous hydrogen. Reactors operated at or below 55°C showed no such accumulation of intermediate metabolites. Overall methanogenesis was also greatly reduced at 57.5°C (0.09 L CH4/g VS fed) versus optimal methane formation at 53°C (0.40 L CH4/g VS fed). Microbial community assessment and free energy calculations suggest that the accumulation of fatty acids and hydrogen, and relatively poor methanogenic performance at 57.5°C are likely due to temperature limitations of thermophilic aceticlastic methanogens.

Keywords: Acetate; methane; hydrogen; propionate; temperature; thermophilic anaerobic digestion

INTRODUCTION

Stringent pathogen regulations required for the land application of domestic wastewater biosolids have revitalized interest in thermophilic anaerobic digestion. Since biological activity at these temperatures requires specialized organisms equipped with thermostable cell components and enzymes, thermophilic anaerobic digestion has recently been recognized as holistically unique biological process, rather than a close variation of mesophilic anaerobic digestion. As such, much of the fundamental discussion regarding anaerobic digestion technology, keeping mesophilic anaerobic digestion in mind, is inadequate for the development of a thorough understanding of the design parameters and operational variables that greatly affect thermophilic performance.

Much of the recent research regarding thermophilic anaerobic digestion deals with previous reports of digester instability and the accumulation of organic intermediates as a result of reactor perturbation. Several previous studies have looked at the effect of intentional shifts in digester temperature and intermittent temperature fluctuations on the stability of a thermophilic anaerobic digester. While these studies are important in assessing the response and adaptation of a pre-existing microbial community to an alternate condition, few studies have focused on the effect of steady-state temperature on the development of a unique digester community. The objectives of this study were to determine the effect of different temperatures within a chosen thermophilic range (49°C-57.5°C) and to assess the role of digestion temperature in determining the steady-state performance of an anaerobic digester.
METHODS

Anaerobic digester setup and operation

High-density polyethylene batch fermentation reactors supplied by Hobby Beverage Equipment Company (Temecula, California) were chosen for this study. The conical bottom of these vessels was thought to be advantageous in terms of mixing and suspension of grit, similar to the full-scale application of egg-shaped anaerobic digesters. The nominal volume of each vessel was 25 litres (L) and was operated with an active volume of 22.5 L. The reactor vessels were modified to accept a threaded stainless steel thermometer, also supplied by Hobby Beverage. Digesters were operated at 35°C, 49°C, 51°C, 53°C, 55°C, and 57.5°C. Replicate digesters at 53°C and 57.5°C were operated in order to investigate the reproducibility of the results of this study. For the purpose of this paper, the reactors are named according to their operating digestion temperature. Replicate reactors of the same temperature are designated by appropriate subscripts. The digesters were heated using an external circulating water bath (Haake DC10-W19) attached to 13 mm i.d. vinyl tube water jacket around each digester. This heating method controlled the internal temperature of the digesters within ± 0.2°C of the nominal temperature.

A conical stainless steel batch fermentation reactor produced by Blichmann Engineering, LLC was used in the operation of reactor 49°C. The reactor had similar dimensions and the same nominal volume as the HDPE reactors produced by Hobby Beverage and was therefore determined to be an equivalent reaction vessel for anaerobic digestion. Unfortunately, the reactor lid consisted of a stainless steel to stainless steel joint and leaking of digester gas from the reactor was unpreventable. Compositional analysis of the digester headspace gas did not yield significant quantities of ambient air (N₂ plus O₂), so the digester was thought to be functioning in a manner similar to the other units. Seeding, start-up, daily feed preparation, and mixing were performed as described previously (Wilson et al., 2006). Stabilization of reactor operation typically occurred within 30 days of commencement of regular feeding and wasting. In the case of the 49°C digester from which quantification of biogas production was not possible, monitoring of pH was used as the primary indicator of steady-state performance.

Gibbs free energy change

The Gibbs free energy for the reactors under actual reactor conditions (ΔG') was calculated on the basis of previously published standard Gibbs free energies for the individual reactions involved in syntrophic propionate oxidation (McCarty and Smith, 1986; Rossini et al., 1952). The calculation of ΔG' included temperature corrections to standard Gibbs free energy (ΔG') for the various reactor conditions by applying the constant enthalpy from of the Van’t Hoff equation as well as measured concentrations of appropriate reactants and reaction products.

Headspace Analyses

Methane and Carbon Dioxide. Headspace methane (CH₄) and carbon dioxide (CO₂) were analyzed on a Shimadzu Gas Chromatograph (Model GC-14A) with a thermal conductivity detector (TCD). The column was constructed from a 4 metre length of copper tubing with a 6.35 mm inner diameter. The column was coiled to fit in the GC-14A oven and packed with Haysep Q media (Supelco, Bellefonte, PA). Helium was used as the carrier gas at a flow rate of approximately 17 ml/min.

Headspace Hydrogen. Headspace hydrogen (H₂) concentrations were analyzed using a reduction gas detector (Trace Analytical RGA5). Nitrogen was used as the valve actuator at 414 kPa and carrier at a flow rate of 50 ml/min. The RGA5 was operated isothermally with a bed temperature of 265°C and a column temperature of 80°C.

Volatile fatty acid analysis

Volatile fatty acids (VFA) were measured weekly on the solution phase of each digester. Samples for VFA analysis were passed through a 0.45 μm nitrocellulose membrane filter and frozen prior to analysis. VFA were measured using a Shimadzu gas chromatograph (Model GC-14A) with flame ionization detector (FID). Species separation was performed using a Nukol™ fused silica 15 m x 0.53 mm capillary column with 0.5 μm
film thickness. A Shimadzu computer integrator (Model CR501 Chromatopak) was used for data analysis. Helium was used as the carrier gas at a flow rate of 17 ml/min. Additional gases are as follows: Hydrogen, 45 ml/min; Air, 450 ml/min; Nitrogen, 13 ml/min. Volatile fatty acids are expressed as mg/L of individual species (C2-C7 fatty acids). Individual acid concentrations are converted to acetate on a theoretical oxygen demand basis and summed to report Total VFA as mg/L as acetate.

PCR-DGGE analysis
Samples were collected aseptically from the digesters and feed source, and shipped on dry ice to Bucknell University (Lewisburg, PA) for community analysis. Prior to analysis samples were stored at -50°C to prevent changes in community structure. DNA extraction and amplification (Ovreas et al., 1997) and denaturing gradient gel electrophoresis (DGGE) (LaPara et al., 2000) were performed as previously described. DGGE was carried out for bacterial and archaeal analyses at run times of 5.5 hours and 3.5 hours, respectively, and with an applied voltage of 200V as previously described (Nakatsu et al., 2000). Primers PRBA338f and PRUN518r were used for community analysis of organisms in the bacterial domain, and primers PARCH340f and PARCH519r were used for community analysis of the archaeal community (Chen et al., 2005).

Statistical analysis
Statistical analyses of reactor pH and methane production rate were performed to determine repeatability of replicate reactors and to determine significant difference in reactor operation at various temperatures. These parameters were chosen since they were used to define steady-state reactor operation and have been classically been viewed as indicators of reactor upset and performance. Statistical analyses were performed using NCSS statistical software package (Kaysville, Utah). All statistical analyses were carried out at the 95% confidence level (α = 0.05). Reactor pH data (n ≥ 25) were found to be normally distributed over the period of steady-state data collection based; random variation in methane production rates caused these data (n ≥ 21) to be non-normal. Since it was desired that the same statistical analysis be used for pH and methane data, a nonparametric test was required for this condition. The Kruskal-Wallis Z-test was used in this case since it did not require the assumption of data normality.

Other analyses
Total and volatile solids concentrations (Method 2540-G) as well as total alkalinity (Method 2320-G) were analyzed as specified in Standard Methods for the Examination of Water and Wastewater (APHA, 1995).

RESULTS
An observed optimal thermophilic temperature range for methanogenesis in terms of both methane partial pressure (PCH4, Figure 1a.) and average daily methane production (ADPCH4, Table 1) is approximately 53-55°C. These data correspond with the classic definition of thermophilic anaerobic digestion (generally 55°C). There are insufficient data to determine whether suboptimal temperatures (49°C, 51°C) resulted in significantly reduced methane production; however, operation of replicate digesters at 57.5°C showed that ADPCH4 was reduced by up to 77% at digestion temperatures only slightly above optimal. While random variation in ADPCH4 by digesters operated at 51-55°C did not allow us to make any statement as to the significance of varied ADPCH4 in that range, both digesters operated at 57.5°C showed ADPCH4 that was significantly (p < .05) less than each of lower temperature digesters. It is interesting to note that similar changes to these can be seen in PCO2 and ADPCO2 at 57.5°C. PCO2 increased from an average 32.9 ± 1.4 kPa at temperatures ranging from 49-55°C to 39.1 at 57.5°C. Across the same temperature change, ADPCO2 was observed to decrease by 36-67%.

The accumulation of volatile fatty acids (VFA) has long been associated with anaerobic digester upset conditions (Harper and Pohland, 1986). Such accumulation has been observed to be more pronounced at thermophilic temperatures (Gray et al., 2006; Speece et al., 2006), likely due to temperature sensitivity of aceticlastic methanogens under elevated thermophilic temperatures (Ahring et al., 2001; Chen, 1983; Leven et al., 2007). In this study, operation of digesters at 57.5°C resulted in the accumulation of C2-C7 fatty acids.
Specifically, acetate and propionate accumulated to maximum concentrations of 624 and 1485 ppm as acetate in digester 57.5°C, respectively, accounting for approximately 62% of total VFA in that reactor. In digester 57.5°C, acetate and propionate showed substantial accumulation relative to lower temperature reactors, however these individual acids only comprised 41% of total VFA. The reason for the preference for longer chain fatty acids (C4-C7, individual VFA species data not shown) in digester 57.5°C has not been determined, but the overall accumulation of relatively simple organic acids in both reactors operated at 57.5°C suggests that some form of digester upset is being induced at this temperature.

While the presence of VFA within an anaerobic digester is representative of inefficient conversion of complex organic substrates to CH₄, fatty acids can also be particularly damaging to the digester environment in themselves. The accumulation of VFA serves as a pool of weak acid, of which, dissociation would cause the release of free hydrogen ions into solution, thus lowering the reactor pH. Since the pKa values of acetate, propionate, and butyrate are all less than 5.0, these important VFA species tend to exist in a predominantly deprotonated form at near neutral pH (Rittmann and McCarty, 2001). The effect of VFA in this context is that the steady-state operating pH of digesters operated at 57.5°C (= 7.13) was significantly lower (p < .05) than that of digesters operated at 55°C or below (= 7.43 ± 0.04), contributing to the relative instability of the higher temperature thermophilic digesters (Table 1). Reactor pH again follows the previous discussion of relatively good reactor performance at temperatures ranging from 49-55°C, and relatively poor reactor performance at 57.5°C. Over the course of the study, the steady-state pH of each reactor tended to fluctuate very little, which suggests that these reactors were quite well buffered in light of relatively large variations in VFA concentration. Stability of an anaerobic digester is often defined as the reactor's -value. That is, the ratio of the concentration of total organic acids (VFA) to that of total alkalinity within a digester. Digesters 57.5°C and 57.5°C have -values of 1.07 and 0.87, respectively, showing a decreased capacity for resisting additional perturbation (e.g. toxicants, organic slug loading, temperature and mixing fluctuations). Digesters operating at 49-55°C proved to have much lower -values (0.13-0.36) and are considered to be more stable systems.

Table 1: Observed reactor conditions at various temperatures.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>pH</th>
<th>Alkalinity (ppm as CaCO₃)</th>
<th>VSR (%)</th>
<th>Daily Gas Production (litres per day)</th>
<th>VFA Concentrations (ppm as Acetate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CH₄</td>
<td>CO₂</td>
</tr>
<tr>
<td>37°C</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>7.0 ± 0.3</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>49°C</td>
<td>7.45 ± 0.04</td>
<td>3391 ± 115</td>
<td>57.9 ± 3.9</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>51°C</td>
<td>7.38 ± 0.04</td>
<td>4180 ± 143</td>
<td>48.8 ± 1.6</td>
<td>10.8 ± 3.0</td>
<td>6.1 ± 1.7</td>
</tr>
<tr>
<td>53°C₁</td>
<td>7.40 ± 0.06</td>
<td>4269 ± 112</td>
<td>57.8 ± 4.9</td>
<td>12.9 ± 1.2</td>
<td>6.8 ± 0.8</td>
</tr>
<tr>
<td>53°C₂</td>
<td>7.46 ± 0.05</td>
<td>4819 ± 96</td>
<td>56.8 ± 1.8</td>
<td>14.1 ± 1.1</td>
<td>7.6 ± 0.7</td>
</tr>
<tr>
<td>55°C</td>
<td>7.46 ± 0.03</td>
<td>4695 ± 73</td>
<td>48.4 ± 3.7</td>
<td>13.0 ±1.3</td>
<td>6.3 ± 2.4</td>
</tr>
<tr>
<td>57.5°C₁</td>
<td>7.13 ± 0.07</td>
<td>3722 ± 123</td>
<td>45.4 ± 5.1</td>
<td>3.2 ± 0.5</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>57.5°C₂</td>
<td>7.13 ± 0.04</td>
<td>3965 ± 89</td>
<td>45.5 ± 1.9</td>
<td>4.8 ± 0.5</td>
<td>3.9 ± 0.5</td>
</tr>
</tbody>
</table>

¹ Data not available for reactor ² Average ± Standard Deviation ³ Below detection limit (<10 ppm)
Figure 1: Effect of digestion temperature on (a.) headspace gas composition and free energy release associated with (b.) propionate metabolism, (c.) methane formation, and (d.) acetate metabolism.

Hydrogen has been found to play a key role in the conversion of VFA to acetate, and eventually CH₄. A relatively narrow window of hydrogen concentrations exists (0.1-10 Pa) within which propionate can be successfully metabolized (McCarty and Smith, 1986). This can be partially attributed to the potentially large molar quantities of hydrogen produced during propionate metabolism causing feedback inhibition on the oxidation of propionate and other organic acids (de Boek et al., 2001; Seeliger et al., 2002). Hydrogen in digester 57.5°C accumulated to a concentration of approximately 11.4 Pa, only slightly higher than the previously published upper limit for efficient metabolism of propionate. Such concentrations of hydrogen in themselves are unlikely to result in a substantial buildup of Pr, however past studies focused on the development of online early warning systems for digester upset used hydrogen partial pressures as low a 6.5 Pa to indicate potential onset of overload conditions (CordRuwisch et al., 1997).

Bioenergetics and thermodynamics can be used in order to assess the implication of reactor conditions as observed in this study, and in conjunction with previous literature describing the microbiology of thermophilic anaerobic digestion, can help to establish a mechanism to explain previous reports of temperature induced instability. The concentrations of H₂, CH₄, CO₂, H⁺, acetate, and propionate were used to calculate actual Gibbs free energies for reactions directly involved in propionate and acetate metabolism (listed in Table 2) under methanogenic conditions at a selection of temperatures (Figures 1c.-1d.). The mesophilic reactor was not considered in this analysis since propionate was below detectable levels.

Previous studies have shown that the minimal energy quantum required to sustain microbial life corresponds to 1⁄3 of the quantum required for the synthesis of 1 mol ATP (~70 kJ/mol ATP) (Ahring and Westermann, 1988; Scholten and Conrad, 2000). This means that for a microorganism to survive by carrying out any of the metabolisms listed in Table 2, the reaction must provide a minimum free energy release of greater than 23 kJ/reaction ($G = -23 \text{ kJ/mol}$).

The results of this analysis show that, under actual reactor conditions, propionate oxidation, hydrogenotrophic methanogenesis, and acetoclastic methanogenesis are all near the theoretical threshold of viable energy gain from their respective metabolism. It has been well documented that hydrogenotrophic methanogens and acid-oxidizing bacteria occur in syntrophic clusters that enhance the intercellular transport of hydrogen (de Bok et al., 2004). As such, the energy produced through these two reactions (syntrophic propionate oxidation) is observed to be much greater than the energy available to either community in itself (Figure 1b.). Additionally, past research has shown that in complex competitive environments, the actual energy requirements for microbial life may indeed be slightly lower (Hoehler et al., 2001). In their study, Hoehler et al. found that hydrogenotrophic methanogenesis was able to proceed with a free energy release as low as $G = -10.6 \text{ kJ/mol}$.  

![diagram](https://via.placeholder.com/150)
While data from previous studies regarding syntrophic metabolism and energetic limits for microbial life may provide an explanation as to how effective methanogenesis can occur in the thermophilic reactors operated in this study at or below 55°C, strictly looking at microbial energetics alone does not explain the poor methanogenic performance of the digesters operated at 57.5°C. Microbial community assessment via PCR-DGGE analysis was used to observe changes in overall microbial community structure at the various digester temperatures. The archaean community analysis (Figure 2b.) provided useful insight into methanogenic inhibition occurring at high digestion temperatures. In an anaerobic digester, analysis of total archaean population is considered to be representative of the methanogenic population (Chen et al., 2005). Analysis of reactors 35°C, 49°C, and 53°C yielded the presence of distinct bands, representing individual populations of methanogenic organisms. Interestingly, fewer archaean bands were identified in the mesophilic reactor, signifying less microbial diversity among methanogens. Similar results in previous research have been interpreted as a community lacking the biological diversity to resist significant environmental stressors; however, data from this study suggest that the limited archaean community observed at 35°C is indeed effective in regulating fatty acids and H₂ within the digester. Methanogenic communities observed in reactors 49°C and 53°C were robust, having multiple well-defined bands. Reactor 57.5°C, however, failed to produce any well-defined bands representing methanogenic DNA. The minimal production of CH₄ by this digester suggests that the poorly defined shadows seen in Figure 2b. at 57.5°C represent some quantity of methanogenic biomass.

Table 2: Energetic reactions involved in acetate and propionate metabolism during anaerobic digestion

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Equation</th>
<th>ΔG° (kJ/reaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogenotrophic Methanogenesis</td>
<td>H₂ + ½CO₂ → ½CH₄ + ½H₂O</td>
<td>-130.7</td>
</tr>
<tr>
<td>Aceticlastic Methanogenesis</td>
<td>CH₃COO⁻ + H⁺ → CH₄ + CO₂</td>
<td>-31.0</td>
</tr>
<tr>
<td>Syntrophic Propionate Oxidation</td>
<td>CH₃CH₂COO⁻ + ½H₂O → CH₃CH₂COO⁻ + ½CH₄ + ½CO₂</td>
<td>-26.0</td>
</tr>
<tr>
<td>Propionate Oxidation</td>
<td>CH₃CH₂COO⁻ + 2H₂O → CH₃COO⁻ + 3H₂ + CO₂</td>
<td>68.4</td>
</tr>
<tr>
<td>Acetate Oxidation</td>
<td>CH₃COO⁻ + 4H₂O → 2HCO₃ + 4H⁺ + H²</td>
<td>104.6</td>
</tr>
</tbody>
</table>

Past research has shown aceticlastic methanogens are typically more temperature sensitive than hydrogenotrophic methanogens or acetotrophic bacteria. Ahring et al (2001) found that an increase in reactor temperature from 55°C to 65°C actually resulted in a marginally increase in the activity of hydrogenotrophic methanogens, while aceticlastic activity and overall methane yield decreased.

The results of this study with regard to methane production, as well as the accumulation of acetate and propionate are consistent with the findings of previous research. The greatly simplified methanogenic community observed at 57.5°C may be the result of temperature inhibition of aceticlastic methanogens, resulting in the reliance on hydrogenotrophic methanogenesis as the dominant source of methane gas.

The implication of this hypothesis on overall carbon flow within the 57.5°C reactors is that acetate produced during the fermentation of organic acids and other complex substrates must be channeled around aceticlastic methanogenesis in order to produce methane gas. Thermophilic species of bacteria capable of carrying out acetate oxidation as shown in Table 2 have been recognized (Hattori et al., 2000; Lee and Zinder, 1988); however, acetate oxidation is primarily thought to dominate at lower acetate concentrations than were observed during this study (Shigematsu et al., 2004). The highly exergonic condition for acetate oxidation shown in Figure 1d. clearly exhibits the competitive advantage that acetate-oxidizing bacteria would have under the conditions within our thermophilic digesters. Perhaps the elimination of a functional aceticlastic methanogenic population would naturally result in the channeling of acetate through acetate oxidation. Community analysis of the bacterial population (Figure 2a.) present at 49°C, 53°C, and 57.5°C shows that the overall bacterial community in not greatly affected by the digestion temperature, and supports the hypothesis that bacterial oxidation of acetate is an important metabolism in our digesters.
CONCLUSION
The observed mechanism of temperature induced instability observed in this study agrees with previous discussions of high-temperature methanogenic environments. The accumulation of fatty acids corresponded with low observed methane output, and suggests that the temperature limitation of acetoclastic methanogenesis may be responsible for reactor instability and a significantly depressed pH at 57.5°C. Hydrogen accumulation was observed to gradually increase throughout the range of thermophilic temperatures, revealing that the onset of temperature induced instability was not merely a binary phenomenon at 57.5°C, but the result of a gradual change in energy flow that became apparent only once an inhibitory reactor condition was reached. This study supports past efforts to establish on-line hydrogen monitoring as an early warning detector of digester upset in instability. Following the protocol established by CordRuwisch et al (1997), the reactor operating a 55°C would have signaled digester upset conditions, and the type of failure observed at 57.5°C would potentially be avoided.

A better understanding of the role that digestion temperature plays on the accumulation of anaerobic digestion intermediates is critical to the design of high-temperature digestion systems to achieve sludge hygienisation. Additional microbial community and kinetic studies are required in order to test the proposed mechanism of digester instability, and to determine the importance of acetate oxidizing bacteria during high-temperature anaerobic digestion.

REFERENCES


