Relative kinetics of anaerobic digestion under thermophilic and mesophilic conditions

H. Ge, P. D. Jensen and D. J. Batstone

ABSTRACT

With several advantages over the conventional mesophilic anaerobic digestion, such as better sludge quality and higher biogas production, thermophilic anaerobic digestion is regarded as a promising alternative for sludge digestion. Primary and activated sludges are complex materials, and historically, analysis of kinetics has been largely on whole sludge, without analysis of individual components. This paper analyses relative digestion kinetics of pure substrates designed to target main stages of sludge digestion under thermophilic and mesophilic conditions. Hydrolysis rate of cellulose was significantly influenced by temperature with hydrolysis coefficients of – at 55°C (0.7 ± 0.1 day⁻¹), 60°C (0.8 ± 0.2 day⁻¹), 65°C (1.1 ± 0.2 day⁻¹) and 70°C (1.2 ± 0.2 day⁻¹) over 38°C (0.4 ± 0.1 day⁻¹). This strongly follows the Arrhenius relationship, with an activation energy (E_A) of 31 ± 4 kJ mol⁻¹, corresponding to an increase of 1.5x for each 10°C of temperature increase.

Glucose uptake was rapid with a wide variety of fermentation products detected under mesophilic conditions, while uptake was slower under thermophilic conditions with acetate and propionate being dominant products. Propionate acetogenesis and acetate-utilizing methanogenesis kinetics were not influenced by temperatures. Hydrolysis is widely regarded as a rate-limiting step in sludge digestion, thus improvements in hydrolysis rates as measured during this study have the potential for significant improvements in overall apparent sludge digestion rates.

Key words | acetate-utilizing methanogenesis, acetogenesis, fermentation, hydrolysis, mesophilic, thermophilic

INTRODUCTION

Anaerobic digestion is widely used to treat sewage sludges and other solid organic wastes, and has four major steps: hydrolysis, fermentation, acetogenesis, and methanogenesis. Hydrolysis is generally considered as the rate-limiting step, especially in the degradation of complex substrates containing particulates such as primary and activated sludges (Pavlostathis & Giraldo-Gomez 1991). A common method to increase hydrolysis rates is to increase digestion temperature to the thermophilic range (50–70°C) (Ponsá et al. 2002). Thermophilic anaerobic digestion has been reported to result in increased biogas production and improved volatile solids destruction compared to mesophilic anaerobic digestion, which may be due to increased hydrolysis rates or increased hydrolysis extent. Song et al. (2004) showed sludge solubilisation was doubled in thermophilic anaerobic digestion (55°C) over mesophilic anaerobic digestion (35°C), indicating hydrolysis was significantly improved at thermophilic temperature. Nges & Liu (2009) evaluated the effect of thermophilic temperatures (50 and 70°C), and hydraulic residence time (HRT) of 2–3 days on sewage sludge solubilisation. They found sludge solubilisation was 22.5% at 50°C and remained similar at 70°C, but was substantially improved over solubilisation at 25°C (11.6%). A mathematical model was developed by Siegrist et al. (2002) to assess anaerobic kinetics under mesophilic (35 ± 5°C) and thermophilic (55 ± 5°C) conditions. The hydrolysis rate constant was 0.25 and 0.4 day⁻¹ under mesophilic and thermophilic conditions, respectively. Similarly, kinetics of propionate degradation was dependent on the temperature, showing a higher rate at thermophilic temperature (1.2 day⁻¹) than mesophilic temperature (0.4 day⁻¹).

While it is clear that hydrolysis is temperature dependent, in practice, it is not clear whether subsequent steps are substantially improved, or may become rate-limiting. Pure substrates have been used extensively to study kinetics...
of each stage of anaerobic digestion, however, these studies have not included assessments of the relative kinetics of each stage of anaerobic digestion or relative changes in these rates under different temperatures. In this paper, kinetics of each anaerobic process are investigated under thermophilic and mesophilic conditions using pure substrates such as cellulose, glucose, propionate, and acetate.

MATERIAL AND METHOD

Batch kinetic experiments

Batch experiments were performed in non-stirred, 160 ml serum vessels at 38, 55, 60, 65 and 70 °C, respectively. Basic anaerobic (BA) medium was used according to Angelidaki et al. (2009). Cellulose (type 20, 10 g L⁻¹), glucose (1 g L⁻¹), propionate (1 g L⁻¹) and acetate (2 g L⁻¹) were chosen as substrates for hydrolytic, fermentation, acetogenic and acetate-utilizing methanogenic assays, respectively.

Each bottle contained 10 ml inoculum and 90 ml BA medium (100 ml total liquid volume). Inoculum used in thermophilic experiments was harvested from a continuous lab-scale thermophilic reactor that had been operated as the first stage of temperature phased anaerobic digestion (TPAD) system. The TPAD was treating activated sludge collected in Australia. The HRT in the first stage was 2 days. The reactor was operated at 55 °C for over 32 days, 60 °C for over 100 days, at 65 °C for over 67 days and at 70 °C for 68 days when inoculum was harvested. Inoculum used in mesophilic experiments was harvested from a continuous lab-scale reactor operated at 35 °C (HRT 2 days). Bottles were flushed with 100% N₂ gas for 3 min (1 L min⁻¹), sealed with a rubber stopper retained with an aluminium crimp-cap and stored in temperature controlled incubators (±1 °C). Blanks contained inoculum and medium without the substrate. HCl (1 M) was used to adjust pH to 7.2. All tests were carried out in triplicates, and all error bars indicate 95% confidence in the average of the triplicate.

Gas production, composition, VFA content as well as glucose, succinate, lactate, formate and ethanol, were monitored throughout the experimental period. Gas samples and 1 ml liquid samples were taken periodically from each bottle.

Analysis

Biogas volume was recorded by measuring the gas pressure in the batch bottle, which was measured using a manometer at the start of each sampling event. Accumulated volumetric gas production was calculated from the pressure increase in the headspace volume (60 ml) and expressed under standard conditions (25 °C, 1 atm) (O’Sullivan et al. 2008). The net gas production was obtained by subtracting gas production of blanks. Changes in the headspace volume caused by liquid sampling were taken account into calculations of biogas volume.

Biogas composition (H₂, CH₄, CO₂) was measured using an auto system Perkin-Elmer gas chromatography (GC) equipped with a thermal conductivity detector (TCD), while concentrations of ethanol and VFA (acetate, propionate, iso-butryate, butyrate, iso-valerate, valerate, hexanoate) were measured by another Perkin-Elmer GC with a flame ionization detector (FID), as described in Ge et al. (2010). Concentrations of glucose, succinate, lactate, and formate were measured using high performance liquid chromatography using a diode array detector (UV/VIS) in series with a differential refractometer detector (RI).

Model based analysis

A single step model was implemented in Aquasim 2.1d (Reichert 1994) with modified inputs and dynamic processes. Initial conditions were adjusted for measured initial concentration of propionate and acetate. In the assays of hydrolysis, cellulose degradation was considered as following first order (Batstone et al. 2002), expressed as

\[ r_{X_{ch}} = -k_{hyd,ch}X_{ch} \]  

where \( r_{X_{ch}} \) is the reaction rate, \( X_{ch} \) is the particulate substrate concentration, and \( k_{hyd,ch} \) is the rate constant for substrate consumption.

Monod kinetics (Batstone et al. 2002) was applied as the dynamic process model for acetate-utilizing methanogenesis, shown as

\[ r_s = -k_{m,ac} \frac{S_{ac}}{K_s + S_{ac}} \]  

where \( r_s \) is the reaction rate, \( k_{m,ac} \) is the maximum specific growth rate, \( S_{ac} \) is the substrate concentration, and \( K_s \) is the half-saturation constant. The dynamic process of acetogenesis was also assumed to follow the first order kinetic, rather than Monod kinetic, due to the long lag phase for propionate consumption.

A 95% confidence limit was used to determine \( K_s \) for acetate, with appropriate F-values for objective function.
distribution. In assessment of hydrolysis and acetogenesis kinetics, linear error analysis was also performed. For hydrolysis, the sum of methane and soluble products was used as the fitted variable. For all others, measured substrate concentration was used as a measured variable. Data collected from the lag period was excluded from the model analysis.

RESULTS

A summary of kinetic parameters for hydrolysis, fermentation, acetogenesis and acetate-utilizing methanogenesis under thermophilic and mesophilic conditions is shown in Table 1.

Hydrolysis kinetics

Cellulose hydrolysis was assessed as the sum of soluble products (e.g. ethanol, VFAs) and cumulative methane production. Figure 1 shows the cellulose degradation at 38, 55, 60, 65 and 70 °C normalized by the final hydrolysis extent. A short lag period was observed at all test temperatures, however this was not assessed during the study. The $k_{\text{Hyd}}$ values were statistically similar at 55, 60, 65 and 70 °C and were significantly higher than that at 38 °C, as shown in Table 1. Degradation was not complete (though Figure 1 has been normalized to show relative kinetics), and degradabilities were fitted as 0.24 ± 0.03 at 38, 55 and 60 °C, and increased to 0.34 ± 0.01 at 65 and 70 °C.

The relationship of hydrolysis rate to temperature almost exactly followed the Arrhenius equation, with a doubling in rate coefficient for every 20 °C in temperature (increase of 1.5x for every 10 °C). This is effectively demonstrated in Figure 2, showing the relationship between hydrolysis coefficient and temperature. This is using activation energy ($E_a$) of 31 ± 4 kJ mol$^{-1}$ (95% confidence). While fit against the standard equation was good in the range considered given uncertainty levels, it is unlikely that temperature could be elevated substantially above 70 °C, given the likely operating limits of functional enzymes and the trend in the last two points.

Fermentation kinetics

Glucose fermentation was assessed on the basis that glucose is the base monomer in cellulose and a common intermediate produced during cellulose hydrolysis. Complete glucose

<p>| Table 1 | Kinetic parameters for the hydrolysis, fermentation, acetogenesis and acetate-utilizing methanogenesis at 38, 55, 60, 65 and 70 °C |</p>
<table>
<thead>
<tr>
<th>Parameters</th>
<th>38 °C</th>
<th>55 °C</th>
<th>60 °C</th>
<th>65 °C</th>
<th>70 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{\text{Hyd}}$ (day$^{-1}$)$^a$</td>
<td>0.4 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>$k_{\text{glu}}$ (day$^{-1}$)$^b$</td>
<td>~2</td>
<td>~1</td>
<td>~1</td>
<td>~0.7</td>
<td>~1</td>
</tr>
<tr>
<td>$k_{\text{pro}}$ (day$^{-1}$)$^c$</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>$K_w$ (mg COD L$^{-1}$)$^d$</td>
<td>&lt;900</td>
<td>&lt;900</td>
<td>&lt;2,200</td>
<td>&lt;1,300</td>
<td>&lt;500</td>
</tr>
<tr>
<td>$k_{\text{an,ac}}$ (CODS CODX$^{-1}$ day$^{-1}$)$^e$</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

$^a$Cellulose was not consumed completely at 38, 55, 60, 65 and 70 °C.

$^b$Apparent first order degradation coefficient, $t$ is the time in which 63% of the substrate is removed.

$^c$Apparent first order hydrolysis coefficient – $K_w$ could not be determined. The equivalent $K_w$ is ~4 CODS CODX$^{-1}$·day$^{-1}$.

$^d$Lower limit could not be determined.

$^e$Apparent $k_{\text{an,ac}}$ on biomass estimated from COD of inoculum.
uptake was faster at 38 °C (within half day) compared to thermophilic temperatures, as shown in Figure 3. Values in Table 1 are estimated as the apparent first order degradation coefficient, as Monod kinetics were overcorrelated. Glucose fermentation products at 55, 60, 65 and 70 °C were limited to acetate and propionate, while at 38 °C glucose fermentation yielded a range of intermediates including lactate, formate, ethanol, acetate and propionate. However, lactate, formate and ethanol were transient products and were completely consumed within two days.

**Acetogenesis kinetics**

Figure 4 shows propionate concentrations against the model fitted concentrations at 38, 55, 60, 65 and 70 °C. A long lag phase was observed at all tested temperatures; however detailed assessments of the lag phase have not been performed. Propionate consumption and methane production occurred concurrently in these tests. A first order kinetic equation was applied, as an upper limit for $K_s$ could not be determined. The apparent uptake rates $k_{m,pro}$ to the kinetics observed here would be $\sim 4 \text{ CODS COD}^{-1} \text{ day}^{-1}$. The $k_{pro}$ values shown in Table 1 were statistically similar at 38, 55, 60, 65 and 70 °C, with complete utilisation of substrate.

**ACETATE-UTILIZING METHANOGENESIS KINETICS**

Acetate consumption also had lag phases at 38, 55, 65 and 70 °C, but not at 60 °C (Figure 5). Maximum specific growth rate for acetate ($k_{m,ac}$) and half saturation constant ($K_{s,ac}$) of acetate at 38, 55, 60, 65 and 70 °C are shown in Table 1. Based on model analysis, $k_{m,ac}$ values were comparable for all tested temperatures, and the $K_{s,ac}$ values were lower-unbounded, indicating the lower limits were towards zero and could not be estimated. This is based on the surface confidence regions shown in Figure 6.

**DISCUSSION**

Hydrolysis rate ($k_{hyd}$) was nearly doubled at 55 °C compared to 38 °C, and further improved at 60, 65 and 70 °C. A similar trend was reported by Siegrist et al. (2002), who measured the hydrolysis rate of sewage sludge at 35 and 55 °C as 0.25 and 0.4 day$^{-1}$, respectively. Lynd et al. (2002) compared maximum growth rates across cellulolytic species and observed a strong dependence on growth temperature. Increased hydrolysis rates observed under thermophilic conditions are consistent with the expected increase in growth.
rate. Microbial cellulose hydrolysis is facilitated by extracellular enzymes, hydrolysis products are then transported into the bacterium for metabolism and growth. Mass transfer rates and solubility vary with temperature. Anaerobes primarily degrade cellulose using enzymes attached directly to the cell or cell-glycocalyx matrix, minimising transport requirements, and the effect of transport phenomena on the rate of microbial utilization of cellulose is not clear (Lynd et al. 2002).

Glucose was consumed twice as quickly at 38 °C compared to thermophilic conditions. Rapid consumption (on the order of 15 h) has been commonly observed at mesophilic conditions (Kaluzhnyi & Davlyatshina 1997). The profile of intermediate products varied between the mesophilic fermentations and the thermophilic fermentations. The fermentative portion of an anaerobic community may be diverse under mesophilic condition and able to utilize a wide range of common metabolic pathways to regenerate more energy for subsequent metabolism, resulting in a wide range of intermediate products (Stams 1994). The relatively limited range of intermediate products measured under thermophilic conditions indicates that fewer metabolic pathways are utilized, this may be due to thermodynamic constraints limiting the range of metabolic pathways viable or development of a temperature enriched bacterial community able to utilize fewer metabolic pathways due to lower diversity.

Statistically similar values for $k_{\text{pro}}$ at 38, 55, 60, 65 and 70 °C indicated that propionate acetogenesis was not improved at thermophilic temperatures. Propionate degradation rates were expected to improve at higher temperatures, due to the available free energy of this reaction it is mainly dependent on the temperature with the same concentrations of products and reactants. However, growth of acetogenic and methanogenic organisms is obligately syntrophic with acetogenic conversion of propionate coupled to product removal by methanogenesis (Stams 1994). Methanogenesis was not enhanced at thermophilic temperatures, thus it is possible that methanogenesis became a limiting factor in this assay.

Statistical overlaps in $k_{\text{m,ac}}$ and $k_{\text{s,ac}}$ values for acetate at 38, 55, 60, 65 and 70 °C confirm that in this study the speed of acetate uptake in anaerobic digestion was not influenced by digestion temperatures, this finding is not consistent with other works (e.g. Siegrist et al. 2002). The $K_{\text{s,ac}}$ value at 38 °C is consistent with other values reported by literatures in a wide range of 0.011–0.93 kg COD m$^{-3}$ (Pavlostathis & Giraldo-Gomez 1991). However, values for $k_{\text{m,ac}}$ were lower than levels reported in the literature (e.g. 8 CODS CODX$^{-1}$ day$^{-1}$) (Siegrist et al. 2002). The $k_{\text{m,ac}}$ is the acetate maximum specific growth rate, and thus related to the concentration of biomass in the assay. Biomass used in this study contained a broad anaerobic population rather than high portions of acetate specific microbes used in the literature (Batstone et al. 2002). In addition, the parent reactor used to harvest inoculum for this study was designed to promote hydrolysis, rather than methanogenesis and it is reasonable to conclude that the portion of methanogenic Archaea in the sludge population was lowered as a result.

Overall, the increase in hydrolysis rate was the key impact observed, and likely the most important, as it will generally determine the overall rate of anaerobic digestion (Pavlostathis & Giraldo-Gomez 1991).

**CONCLUSION**

Hydrolysis rate coefficient was strongly influenced by temperature, and was significantly higher at thermophilic conditions (55, 60, 65 and 70 °C) compared to mesophilic conditions. Other processes were either unaffected (e.g. propionate and acetate utilization), or were faster at mesophilic condition (e.g. glucose uptake). Hydrolysis may have been strongly impacted in this study because the inoculum was harvested from reactors optimized to be hydrolytic systems. However, the result is still positive for thermophilic systems, since hydrolysis is generally rate-limiting.

**ACKNOWLEDGEMENTS**

This work was funded by the Queensland State Government, under the Smart State Research-Industry Partnerships Program (RIPP), Meat and Livestock Australia,
and Environmental Biotechnology Cooperative Research Centre (EBCRC), Australia as P23 ‘Small-medium scale organic solids stabilization’. Huoqing Ge and Paul Jensen are recipients of an EBCRC postgraduate scholarship and postdoctoral award, respectively.

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First received 15 February 2011; accepted in revised form 23 February 2011