Enhancing anaerobic digestion of food waste through Biochemical Methane Potential Assays at different substrate: inoculum ratios

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Enhancing anaerobic digestion of food waste through Biochemical Methane Potential Assays at different substrate: inoculum ratios

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Point of Paper: This paper quantifies how different ratios of food waste: anaerobic inoculum affect AD performance, especially methane production.

Keywords: Anaerobic digestion, Food waste, Municipal sludge, Hydrolysis, Organic waste, Biochemical Methane Potential

Targeted Journal: Journal of Industrial Microbiology & Biotechnology

Abstract

Food waste has a high energy potential that can be converted into useful energy in the form of methane via anaerobic digestion. Biochemical Methane Potential assays (BMPs) were conducted to quantify the impacts on methane production of different food waste compositions. Anaerobic digester sludge (ADS) was used as the inoculum, and BMPs were performed at food waste: inoculum ratios of 0.42, 1.42, and 3.0 g chemical oxygen demand/g volatile solids (VS). The 1.42 ratio had the highest CH₄-COD recovery: 90% of the initial total chemical oxygen demand (TCOD) was from food waste, followed by ratios 0.42 and 3.0 at 69% and 57%, respectively. Addition of food waste above 0.42 caused a lag time for CH₄ production that increased with higher ratios, which highlighted the negative impacts of overloading with food waste. The Gompertz equation was able to represent the results well, and it gave lag times of 0, 3.6 and 30 days and maximum methane productions of 370, 910, and 1950 mL for ratios 0.42, 1.42 and 3.0, respectively. While ratio 3.0 endured a long
lag phase and low VSS destruction, ratio 1.42 achieved satisfactory results for all performance criteria. These results provide practical guidance on food-waste-to-inoculum ratios that can lead to optimizing methanogenic yield.
Introduction

Food waste is the largest contributor to municipal solid waste, comprising 21% of waste in landfills in the U.S. in 2012 (U.S. EPA, 2014a). Landfilling food waste may result in significant greenhouse gas emissions from landfills, since food waste accounts for 13% of methane emissions in landfills (EPA, 2015). The emission of greenhouse gases from food waste has led some states, such as Massachusetts, to set limits on the amount of food waste that can go to landfills (RecylingWorks Massachusetts, 2014). A corollary drawback of landfilling food waste is that its energy value is lost in proportion to the fugitive emissions that contribute to greenhouse gases.

An alternative is to anaerobically digest the food wastes and collect the produced methane. Traditionally, anaerobic digestion (AD) facilities handle organic solids from municipal wastewater treatment plants and farms, and more than 180 anaerobic digester facilities currently operate in the U.S. (EREF, 2015). Some of these facilities recently began adding food waste to the AD input. Food waste can be an excellent candidate for AD due to its high energy and moisture contents (Cirne et al., 2007; Levis & Barlaz, 2011; Moriarty, 2013). The carbohydrate, protein, and lipid fractions of food waste can be fermented to long-chain fatty acids (LCFAs) and volatile fatty acids (VFAs) that are then converted into acetate and hydrogen gas, the substrates needed by methanogens.

Digested food waste alone can inhibit methanogenesis. A high risk is that LCFAs and VFAs are produced faster than they can be consumed. Unless the alkalinity is high, this acid accumulation will cause a drop in pH that inactivates methanogens, which function well only within a near-neutral pH range (Buyukkamaci & Filibeli, 2004). The result is a
“pickled” digester that accumulates VFAs and H₂, but has minimal chemical oxygen demand (COD) stabilization to CH₄.

A promising strategy is to co-digest food waste with municipal sludge (Elbeshbishy et al., 2012; Liu et al., 2009; Neves et al., 2004). The key to success is a good ratio of food waste to methanogenic biomass. Elbeshbishy et al. (2012) investigated the impacts of the ratio of food waste to inoculum volatile solid (VS) in batch tests. With the pH held constant at 7, CH₄ production increased as the ratio of food waste to methanogenic inoculum increased. However, artificially maintaining a constant pH may not be realistic, and no studies have evaluated co-digestion of food waste without externally controlled pH. The ratio of food waste to inoculum will affect the potential to accumulate VFAs, and it also will affect the pH-buffering capacity.

The objective of this study was to assess methane production for a range of relevant ratios of food waste to methanogenic biomass. We utilized batch Biochemical Methane Potential (BMP) assays and tested three ratios of food-waste COD to VS of an inoculum of anaerobic digester sludge (ADS). To provide proof of concept and identify food-waste-to-ADS-VS ratios that are promising for further analysis, we measured TCOD, SSCOD, TS, VS, and pH at the start and end of BMP assays. Other parameters important to AD and methane production were estimated via bicarbonate alkalinity calculations and the Gompertz equation (Lay et al., 1996) for estimating lag times and maximum methane production. Our results provide guidance on ratios needed to sustain good performance by overcoming low-pH inhibition while maintaining good methanogenic yield.
Materials and Methods

Food waste recipe and anaerobic digested sludge

The food waste recipe was developed based on weekly food scrap collections at the University of Missouri campus dining operations, as outlined in Costello et al. (2015). The ingredients for the food waste recipe were purchased from a local Wal-Mart food center.

The food waste was prepared by mixing the whole food scraps first by hand, followed by grinding food scraps with 100 mL of water in a food processor (Black and Decker model FP1140BD, USA; 450-Watts) for 10 minutes on setting 2, which resulted in a paste. The food waste paste was blended (model Black and Decker BL1120SG, USA; 550-Watts) with 200 mL of water for 10 minutes on setting 4 to create a food waste slurry concentration of 110 g of food waste/L. The AD inoculum for the BMP test was obtained from Mesa Northwest Water Reclamation Plant in Mesa, Arizona.

Biochemical Methane Potential Tests/ Experimental Design

BMP tests were performed to determine the amount of CH₄ and H₂ produced from three different COD-to-VS ratios that were based on previous studies with ADS (Angelidaki et al., 2009; Elbeshbishy et al., 2012; Lisboa & Lansing, 2013; Owen et al., 1979): 0.42, 1.42, and 3.0 g COD food waste/g VS ADS. Negative controls (i.e., ADS in basal media without electron donor) were prepared for each ratio, and the methane produced by the controls was subtracted from the total CH₄ on a proportional basis to compute the methane formation from the food waste alone at the end of the BMP assays. The negative controls did not have any inhibition by low pH, but the food waste BMPs lowered pH and led to pH inhibition at different stages during the BMP test. Thus, we could not do a control subtraction until pH inhibition had been relieved, which occurred by the end of BMP tests.
in all cases. Therefore, we eliminated the impacts of differential pH inhibition by performing one-time subtraction of the gas production by the negative controls only at the end of the test (day 70). Duplicate positive controls (i.e., ADS with 30 mM acetate as a readily biodegradable electron donor) were set up to ensure that the inoculum was active in methanogenesis and verify the COD conversion to CH4.

For each ratio of COD food waste to VS ADS, 120 mL of food waste plus ADS mixture was added to 200-mL serum bottles along with 60 mL of DI water. All ratio bottles were prepared in triplicate. Table 1 shows the volumes of each component used for each experiment. All bottles were sparged with ultra–high-purity N2 for 10 minutes to ensure anaerobic conditions. Each serum bottle was sealed with a butyl rubber septum and crimped aluminum caps and placed in an incubated shaker table operated at 180 rpm and a temperature of 37 ± 1°C. Experiments continued until the daily gas production was < 1% of the cumulative gas production except for the 3 g COD FW/g VS condition, which is discussed further in results (Koch, Plabst, et al., 2015; "VDI 4630;" 2006).
Table 1: Volumes and mass of acetate used for the experiments with different ratios of g COD Food Waste (FW)/g Volatile Solid (VS) Anaerobic Digested Sludge (ADS)

<table>
<thead>
<tr>
<th>Ratios</th>
<th>FW (L)</th>
<th>ADS (L)</th>
<th>Water (L)</th>
<th>Acetate (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.42 g COD FW/ g VS ADS</td>
<td>0.014</td>
<td>0.106</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td>1.42 g COD FW/ g VS ADS</td>
<td>0.037</td>
<td>0.083</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td>3.0 g COD FW/ g VS ADS</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td>Negative Control 0.42 g COD FW/ g VS ADS</td>
<td>0</td>
<td>0.106</td>
<td>0.074</td>
<td>0</td>
</tr>
<tr>
<td>Negative Control 1.42 g COD FW/ g VS ADS</td>
<td>0</td>
<td>0.083</td>
<td>0.097</td>
<td>0</td>
</tr>
<tr>
<td>Negative Control 3.0 g COD FW/ g VS ADS</td>
<td>0</td>
<td>0.06</td>
<td>0.12</td>
<td>0</td>
</tr>
<tr>
<td>Positive Control</td>
<td>0</td>
<td>0.080</td>
<td>0.1</td>
<td>0.75</td>
</tr>
</tbody>
</table>

**Chemical analyses**

All analytical tests were performed in triplicate. Chemical oxygen demand (COD) and solids analyses were performed on the food waste, ADS, and initial and final mixtures for all BMP ratios. Total chemical oxygen demand (TCOD) and semi-soluble chemical oxygen demand (SSCOD, samples filtered through 1.2-μm glass microfiber filters (Whatman 1822-047 GF/C)) were assayed using HACH HR COD kits (TNT 821, 20-1500 mg/L). Total solids (TS) and VS were determined according to *Standard Methods* (APHA, 2012).

pH values were measured using a Cole Parmer pH meter (Vernon Hills, USA). Ammonia nitrogen (NH₃-N) was assayed with HACH kits (TNT832), which had a detection range 2-47 mgNH₃-N/L. Total alkalinity was assayed with HACH kits (TNT870), which had a detection range of 25-400 mgCaCO₃/L. Colorimetric results from all HACH kits were measured using a HACH 2800 spectrophotometer.
**Methane and hydrogen in the biogas**

Over a 70-day period, biogas production, i.e., changes in headspace volume at one atmosphere, was measured with a gas-tight glass frictionless syringe (Perfektum, NY). CH$_4$ and H$_2$ contents were analyzed using a GC-2010 gas chromatograph (Shimadzu, Japan) having a thermal conductivity detector (TCD) and Carboxen-1010 PLOT capillary column (30 m, Sigma-Aldrich). The TCD was operated with an inlet temperature of 150°C, a detector temperature of 220°C, and a current of 41 mA, and argon as carrier gas. Gas-composition analysis involved a temperature program that began at 80°C for 3 minutes and was followed by an increase in temperature of 50°C every minute until 155°C is reached, giving a total run time of 4.50 minutes. Methane and hydrogen gas volumes were calculated by multiplying the measured gas composition by the total biogas volume. Electron-equivalent energy recovery (as equivalent COD) was calculated for CH$_4$ and H$_2$ according to:

(1) \[ 1 \text{mL CH}_4 \text{ gas} = \frac{L}{10^3 \text{ ml}} \times \frac{1 \text{ mol CH}_4}{22.4 \text{ L}} \times \frac{273 \text{ K}}{313 \text{ K}} \times \frac{8 \text{ e}^- \text{ eq}}{\text{mol CH}_4} \times \frac{8 \text{ g COD}}{\text{e}^- \text{ eq}} \times \frac{10^3 \text{ mg}}{\text{g}} = 2.52 \text{ mg COD} \]

(2) \[ 1 \text{mL H}_2 \text{ gas} = \frac{L}{10^3 \text{ ml}} \times \frac{1 \text{ mol CH}_4}{22.4 \text{ L}} \times \frac{273 \text{ K}}{313 \text{ K}} \times \frac{2 \text{ e}^- \text{ eq}}{\text{mol H}_2} \times \frac{8 \text{ g COD}}{\text{e}^- \text{ eq}} \times \frac{10^3 \text{ mg}}{\text{g}} = 0.62 \text{ mg COD} \]
Bicarbonate alkalinity estimation and total alkalinity measurement

The concentration of bicarbonate alkalinity was computed from the final pH and the final CO₂ content in the headspace for each BMP bottle. Equation 3 was used to estimate the bicarbonate alkalinity:

\[
\text{pH} = pK_{a,1} + \log \left( \frac{\text{Alkalinity (bicarbonate)}}{50,000} \right) - \log \frac{\text{CO}_2(g)}{K_H}
\]

where \( pK_{a,1} = 6.33 \) for the bicarbonate system at 35°C and 1 atm, Alkalinity (bicarbonate) = bicarbonate alkalinity in the anaerobic reactor (mg/L as CaCO₃), \( \text{CO}_2(g) \) = gas phase carbon dioxide concentration in anaerobic digester headspace (atm), and \( K_H \) = Henry's law constant for carbon dioxide at 35°C and 1 atm, which is 38 atm.

The CO₂ concentration at the end of the batch BMP tests were obtained from the GC-TCD data and substituted into the equation along with pH values. Since CO₂ was being generated and out-gassed from solution, the computation may slightly under-estimate the actual bicarbonate concentration.

COD-CH₄ Normalization and Calculating Volatile Solid Destruction

COD-CH₄ normalization was used to show the conversion efficiencies of the volume of methane produced at a given day. The COD-CH₄ normalization can be calculated each day using equation 4:
\[ E = \frac{V_{\text{methane}} \cdot \text{COD}_{\text{methane}}}{\text{COD}_{\text{FW}} \cdot V_{\text{FW}}} \]

where \( E \) = conversion efficiency of COD-CH\(_4\), \( V_{\text{methane}} \) = volume of methane production, \( \text{COD}_{\text{methane}} \) = 2.52mg COD/mL CH\(_4\) at 35\(^\circ\) C at 1atm, \( \text{COD}_{\text{FW}} \) = measured chemical oxygen demand of food waste and \( V_{\text{FW}} \) = volume of food waste in the BMP test.

Volatile solid destruction (VSD) was used to measure the amount of volatile solids removed during the BMP test. The VSD was calculated by using equation 5:

\[ \%\text{VSD} = \left( \frac{V_{S_{\text{FW initial}}} + V_{S_{\text{initial}}} - V_{S_{\text{final}}} - V_{S_{\text{neg final}}}}{V_{S_{\text{FW initial}}} + S_{\text{initial}}} \right) \cdot 100 \]

where \( \%\text{VSD} \) = volatile solid destruction at day 70 (%), \( V_{S_{\text{FW initial}}} \) = VS of food waste at day 1 (g/L), \( V_{S_{\text{initial}}} \) = VS of ADS at day 1, \( V_{S_{\text{final}}} \) = VS of ratio on day 70 of the BMP assay test (g/L) and \( V_{S_{\text{neg final}}} \) = VS of negative control on day 70.

**Gompertz-equation fit to the batch BMP data**

The Gompertz equation (Lay et al., 1996) often is used to fit batch methanogenic data:

\[ M_p = \exp[-\exp\left\{ \frac{R_M}{P_M(x_o - x)e + 1} \right\}] \]

where \( M_p \) = observed cumulative methane production (mL), \( P_M \) = ultimate methane production (mL), \( R_M \) = observed methane production rate (mL/day), \( x_o \) = lag phase time.
(days), & \(x\) = time of observation (days), and \(e\) = exponential (2.718). All the parameters in the Gompertz equation were evaluated using the techniques of Parameswaran & Rittmann (2012), implemented in Microsoft Excel Solver, to determine the set of \(P_M\), \(R_M\), and \(x_0\) parameters giving the lowest sum of squares of error between the model and experimental values based on the observed experimental limitations that \(P_M\) is less than 2000 mL, \(R_M\) is less than 100 mL/day, and \(x_0\) is less than 30 days.

**Results and discussion**

**Chemical characteristics of the feed and final results for the BMP ratios**

Table 2 presents the characteristics of the food waste and the starting mixtures for each experimental ratio. Although the initial pH of the food waste alone was lower than 5, the pH values for all combined mixtures exceeded 6.3, while the 0.42 and 3.0 mixtures were in the ideal range for anaerobic digestion (Parkin & Owen, 1986), 6.8 – 7.2. SSCOD increased as the amount of food waste increased, suggesting that the organic material would be more readily bioavailable for conversion to CH₄.

**Table 2. Characteristics of food waste and the starting mixtures for the three ratios of food waste to inoculum**

<table>
<thead>
<tr>
<th>Initial Characteristics</th>
<th>0.42 g COD/g VS</th>
<th>1.42 g COD/g VS</th>
<th>3.0 g COD/g VS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCOD (g/L)</td>
<td>21.1 ± 3.75</td>
<td>23.4 ± 7.11</td>
<td>28.8 ± 3.9</td>
</tr>
<tr>
<td>SSCOD (g/L)</td>
<td>2.87 ± 0.55</td>
<td>4.13 ± 0.25</td>
<td>6.34 ± 0.4</td>
</tr>
<tr>
<td>SSCOD/TCOD (%)</td>
<td>13.6</td>
<td>17.6</td>
<td>22.0</td>
</tr>
<tr>
<td>TS (g/L)</td>
<td>33.4</td>
<td>34.1</td>
<td>34.6</td>
</tr>
<tr>
<td>VS (g/L)</td>
<td>23.2</td>
<td>24.8</td>
<td>25.0</td>
</tr>
<tr>
<td>VS:TS</td>
<td>0.69</td>
<td>0.73</td>
<td>0.72</td>
</tr>
<tr>
<td>pH</td>
<td>7.32</td>
<td>6.8</td>
<td>6.3</td>
</tr>
</tbody>
</table>
Table 3 tabulates the characteristics of the mixtures at the end of the 70-day BMP tests. Final SSCOD was lower for 0.42 and 1.42 ratios, 0.41 g/L and 0.49 g/L, respectively, compared to 1.29 g/L SSCOD for the 3.0 g COD/g VS ratio. The high final SSCOD for the 3.0 ratio implies that stabilization was incomplete at 70 days, but the 3.0 run was stopped along with ratios 0.42 and 1.42 to enable comparison within the same timeframe and, more importantly, within realistic operational timeframe for commercial AD systems (Rapport et al., 2008). Early and transient hydrogen production for ratio 3.0 indicates that inhibition based on VFA accumulation and low pH likely occurred during the first 10-15 days, after which the inhibition was overcome. The lag led to postponed methane production and, consequently, complete stabilization (shown in Figure S6). Correspondingly, the VS:TS ratio at the end of the batch BMP assays was the highest for the 3.0 ratio, which is another sign of less complete stabilization compared to ratio 0.42 and 1.42. This kind of inhibition has been seen previously when digesting food wastes: LCFA and VFAs are produced faster than they can be consumed, and the acid accumulation causes a drop in pH that inactivates methanogens, resulting in minimal chemical oxygen demand (COD) stabilization to CH₄ (Buyukkamaci & Filibeli, 2004).

Table 3. Characteristics of the mixtures at the end of the 70-day BMP assays

<table>
<thead>
<tr>
<th>Final Characteristics</th>
<th>0.42 g COD/g VS</th>
<th>1.42 g COD/g VS</th>
<th>3.0 g COD/g VS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCOD (g/L)</td>
<td>9.37 ± 0.13</td>
<td>9.41 ± 2.19</td>
<td>10.49 ± 2.10</td>
</tr>
<tr>
<td>SSCOD (g/L)</td>
<td>0.41 ± 0.14</td>
<td>0.49 ± 0.15</td>
<td>1.29 ± 0.37</td>
</tr>
<tr>
<td>TS (g/L)</td>
<td>9.88 ± 1.61</td>
<td>8.76 ± 0.59</td>
<td>7.54 ± 1.62</td>
</tr>
<tr>
<td>VS (g/L)</td>
<td>7.84 ± 1.18</td>
<td>7.12 ± 0.44</td>
<td>6.71 ± 1.32</td>
</tr>
<tr>
<td>VS:TS</td>
<td>0.79</td>
<td>0.81</td>
<td>0.89</td>
</tr>
<tr>
<td>pH</td>
<td>7.09</td>
<td>7.02 ± 0.01</td>
<td>7.36 ± 0.14</td>
</tr>
</tbody>
</table>
Methane generation during BMP tests

Figure 1a illustrates cumulative CH₄ production for the three ratios of food waste and ADS. The BMP results for the 0.42 ratio had a minimal lag time, with rapid and highest rate of methane production within the first 10 days, after which gas production slowed significantly. The lack of a lag likely was due to the high amount of AD inoculum, which provided relatively large concentrations of hydrolytic enzymes, fermenting bacteria, and acetoclastic methanogens. The activity of acetoclastic methanogens in the inoculum was confirmed by the immediate gas production in the positive control (Figure S2, Supporting Information).

After a lag of about 8 days, the BMP for ratio 1.42 began producing CH₄ gas, and the production rate was greater that achieved in the first few days for ratio 0.42. This rapid increase in CH₄ production suggests that hydrolysis and fermentation had been occurring over the first 10 days; thus, an increase in the activity of methanogen by day 10 allowed rapid conversion of the accumulated VFAs to CH₄.

Although ratio 3.0 eventually yielded the most methane from food waste only, (Figure 1b), it also had the most transient hydrogen production and longest lag time, as discussed previously (Supplemental Material Figure S6). Although ratios 0.42 and 1.42 were close to reaching saturation for methane generation, ratio 3.0 clearly had not reached saturation by the end of the experiment.
Figure 1. (a) Methane production of food waste and ADS. (b) Methane production of food waste at day 70 adjusted to subtract gas produced from negative controls. (Panel (a) does not have subtraction of the negative controls, because pH inhibition was not relieved throughout the assays and thus cannot be compared directly.) Error bars in graph represent standard deviation.

Figure 2 shows that the food waste COD-to-ADS-VS ratio affected the fraction of food waste COD removed as CH$_4$ after the cumulative one-time subtraction of negative controls at the end (day 70). The 0.42 ratio demonstrates low CH$_4$ production in Figure 1 and consequently results in low COD as CH$_4$ at 69% in Figure 2. Ratio 1.42 had the highest CH$_4$-COD conversion efficiency, 90% for food waste alone. Ratio 3.0 gave the lowest adjusted methane yield (57%), and this was caused by the long lag time and clearly incomplete conversion at the end of the test (Figure 1a).
All three ratios showed similar values for volatile solids reduction (VSD), shown in Figure 3, indicating that the hydrolysis of the particulate fraction was not limiting methanogenesis at the end of the BMP assays; hence, the differences in methanogenic yields were likely linked with VFA production and pH inhibition of VFA conversion to methane. The VSD for ratios 0.42 and 3.0 were slightly greater that the conversion of FW COD to methane (Fig. 2), which is consistent with accumulation of hydrolysis and fermentation products, including VFAs. The highest volatile solid destruction (VSD), 76% for ratio 1.42, corresponds to the highest cumulative methane production Figure 1a.
Gompertz Equation Analysis

Previous studies have shown that the Gompertz equation fits experimental data when the BMP data follow a typical pattern with initial lag, exponential, and saturation phases (Parameswaran & Rittmann, 2012). The Gompertz equation described in the methods section fit the corresponding volumetric ratios 0.42 and 1.42 experiments very well, as shown in Figure 5, which uses the parameters in Table 4. The model fit for ratio 3.0 is not as accurate, due to the ultimate methane production value being estimated from projected saturation rather than an observed value. For ratio 3.0, the model fit the data well through day 46. After the time, the experimental rate of methane production began to slow, while the modeled production rate continued to increase. Bakhov et al. (2014) also employed the Gompertz equation and were unable to provide a good representation when the food waste loading was high.
Table 4. Estimated parameters from the fit of Gompertz equation to the BMP assays of corresponding ratios.

<table>
<thead>
<tr>
<th>Substrate Ratio</th>
<th>$P_m$ (mL CH₄)</th>
<th>Ratio Pm:initial FW (mL CH₄/g COD_FW)</th>
<th>Ratio Pm:initial ADS (mL CH₄/g CODADS)</th>
<th>$R_m$ (mL CH₄/day)</th>
<th>Ratio of $R_m$:initial ADS (mL CH₄/g CODADS-d)</th>
<th>$x_0$ (days)</th>
<th>Total Sum of Squares Errors between model and actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.42</td>
<td>367</td>
<td>874</td>
<td>25</td>
<td>40.2</td>
<td>2.71</td>
<td>0</td>
<td>0.11</td>
</tr>
<tr>
<td>1.42</td>
<td>908</td>
<td>639</td>
<td>94</td>
<td>25.4</td>
<td>2.63</td>
<td>3.6</td>
<td>0.38</td>
</tr>
<tr>
<td>3.0</td>
<td>1947</td>
<td>649</td>
<td>299</td>
<td>17.2</td>
<td>2.64</td>
<td>30</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Figure 5. Cumulative CH₄ production as a function of time, where symbols represent the experimental data and lines represent the Gompertz equation model fit.

Three significant trends exist for all ratios. First, the ratio of $R_m$ to ADS inoculum had a narrow range, between 2.63-2.71 mL CH₄/g CODADS-d, for all ratios, reinforcing that the CH₄ production rate was dictated by ADS inoculum dose, which contained the bacteria.
responsible for hydrolysis. Second, the ratio of Pm to initial FW COD loading was relatively narrow, ranging from 874 mL CH\textsubscript{4}/g COD\textsubscript{FW} for the 0.42 ratio to 649 mL CH\textsubscript{4}/g COD\textsubscript{FW} for the 3.0 ratio. In contrast, the ratio of Pm to initial ADS COD loading increased steadily from 25 to 299 mL CH\textsubscript{4}/g COD\textsubscript{AD} with increasing FW:ADS ratio. These differing trends indicate that the maximum amount of CH\textsubscript{4} produced in the system was controlled by the added COD from the FW. Third, the increasing x\textsubscript{0} with increasing FW:AD clearly illuminates the delayed onset of methanogenesis due to pH inhibition from the high accumulation of VFAs from the fermentation step.

Alkalinity estimation at the end of batch BMPs

Final bicarbonate-alkalinity values, summarized in Table 5, indicate that ratio 3.0 had a lower value than ratio 1.42, possibly indicating a greater alkalinity consumption associated with the higher food waste fraction, possibly leading to a pH induced inhibition. The final NH\textsubscript{3}-N concentrations can be expressed in alkalinity equivalents by multiplying the NH\textsubscript{3}-N concentration by a factor of 50 mg as CaCO\textsubscript{3}/14 mg N. The values of alkalinity added by NH\textsubscript{3} release clearly point out that a major fraction of the bicarbonate alkalinity originated from NH\textsubscript{3}-N. The exponential phase of methanogenesis often coincides with increases in bicarbonate alkalinity and pH, both of which occurred in the 3.0 ratio at the end of 70 days. In fact, there was an abundance of NH\textsubscript{3}-based alkalinity for the ratio 1.42, which also correlates with the superior methane production performance at this ratio. On the other hand, the lower final NH\textsubscript{3}-N for ratio 3.0 may mean that the Food Waste underwent less hydrolysis, at least to the point of releasing lower NH\textsubscript{3}-N compared to the other two ratios.
Table 5. Estimated Characteristics of the mixtures at the end of the 70-day BMP assays.

<table>
<thead>
<tr>
<th>Estimated bicarbonate Alkalinity (g/L as CaCO(_3))</th>
<th>3.6</th>
<th>2.9</th>
<th>4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia nitrogen (g/L)NH(_3)-N/L</td>
<td>0.81 ±0.02</td>
<td>0.90 ±0.03</td>
<td>0.76 ± 0.08</td>
</tr>
<tr>
<td>Alkalinity originating from Ammonia-N in food waste (g/L as CaCO(_3))</td>
<td>1.4</td>
<td>2.6</td>
<td>2.1</td>
</tr>
<tr>
<td>% of Estimated bicarbonate alkalinity that came from food waste NH(_3)</td>
<td>39%</td>
<td>90%</td>
<td>47%</td>
</tr>
</tbody>
</table>

Conclusion

The effects of the food-waste-to-inoculum ratio provide insights into the performance of co-digestion with food waste. A high ratio of food waste COD to ADS VS (3.0 g FW COD/g AD VS) eventually gave greater volumetric methane production, but VFA-induced pH inhibition caused a large lag period (about 10 days). An intermediate ratio (1.42 g FW COD/g AD VS) gave the best balance of high methanogenic yield with a short lag time. A key factor was the balance of food waste COD that could be fermented to VFAs versus alkalinity in the AD and generated by NH\(_3\) release from food waste. Due to the relatively labile nature of food waste COD, the generation of VFAs could suppress the pH and inhibition methanogenesis. Thus, this work underscores the importance of measuring COD, alkalinity, VFAs, and N in food waste and in the mixture of food waste with AD.

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