Effect of Substrate to Inoculum Ratio on Bioenergy Recovery from Food Waste, Yard Waste, and Biosolids by High Solids Anaerobic Digestion

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Abstract

Residual biosolids from wastewater treatment facilities and the organic fraction of municipal solid waste (OFMSW), such as food and yard wastes (FW and YW), are difficult to manage due to increased waste generation and recent stringent regulations. This study investigated the effect of substrate to inoculum ratio (S/I) on biochemical transformation mechanisms and biomethane production during high solids anaerobic digestion of FW, YW, and biosolids. Biochemical methane potential (BMP) assays were set up under mesophilic conditions at S/I of 1.0, 2.0, and 3.0 g OFMSW total solids (TS)/g inoculum TS. BMP with the lowest S/I (1.0) produced the highest cumulative CH₄ yield of 126 mL CH₄/(g VS_{added}), with 90% of its maximum yield by day 37, and a hydrolysis rate constant (K_{hyd}) of (0.06 day⁻¹). S/Is 2.0 and 3.0 resulted in higher volatile fatty acid release than S/I 1.0. This led to pH-induced inhibition of methanogenesis, longer lag phases (S/I 2.0=12 days and S/I 3.0=26 days), and lower cumulative CH₄ yields [S/I=2.0 at 45.6-mL CH₄/(g VS_{added}); S/I=3.0 at 6.75-mL CH₄/(g VS_{added})]. The results show that accumulation of inhibitory intermediates can cause system failure due to mass transfer limitation under low moisture conditions when S/I > 1.0 based on TS. Results from this work assist in developing best solid waste management practices for OFMSW and resource recovery.

Keywords: biochemical methane potential; biosolids; high solids anaerobic digestion; hydrolysis rate constants; organic fraction of municipal solid waste

Introduction

THE ORGANIC FRACTION of municipal solid waste (OFMSW) includes mixed organics, such as food waste (FW) and yard waste (YW), as well as paper products (Campuzano and González-Martínez, 2016). In many countries, the most common disposal alternative for OFMSW is in landfills, which have high land requirements, emit greenhouse gases, and produce leachate with high concentrations of ammonia and recalcitrant organic matter (Kjeldsen *et al.*, 2002; Aghdam *et al.*, 2019). Handling OFMSW is becoming more expensive and complex due to increased tipping fees, banning of organic waste disposal in landfills, and mandated source separation of organic wastes (Linville *et al.*, 2015; Hinds *et al.*, 2018).

Landfills are a depletable resource and need to be replaced when they become full (Ready and Ready, 1995). Community anaerobic digestion systems are being studied at many

U.S. universities such as the University of South Florida, University of California Davis, University of Michigan, and Princeton University. Policies are being developed in the United States to use anaerobic digestion systems to remove organic waste away from landfills (Edwards *et al.*, 2015). The U.S. EPA has made changes to the renewable fuel standard to include biogas as a fuel to meet renewable volume obligations. As part of zero waste goals, the cities of Madison, WI, and San Francisco, CA, restrict organic waste incineration and landfilling. Currently, there are 25 states that have bans on landfill disposal of YW (Linville *et al.*, 2015). Management of residual biosolids from wastewater treatment facilities is also a significant problem due to concerns over excess nutrients, pathogens, odors, vector attraction, and limited capacity for resource recovery from the waste (Seiple *et al.*, 2017). It is thus critical that alternative organic waste management solutions are explored, which contribute to waste minimization and resource recovery, while reducing greenhouse gas emissions.

Anaerobic digestion of OFMSW and biosolids is an alternative waste management strategy that includes recovery of CH₄ for energy production and digestate for fertilizer. There are two main types of anaerobic digesters, wet [<15% total solids (TS)] and dry (15% ≥ TS) (Brown *et al.*, 2012). In

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the United States, most anaerobic digesters are wet (EPA, 2019), but dry or high solids anaerobic digesters (HS-AD) are becoming an attractive alternative worldwide due to lower water requirements, reduced leachate production, smaller digester size, lower heating needs, and reduced maintenance requirements (Kayhanian, 1995; Chynoweth *et al.*, 2001; Karthikeyan and Visvanathan, 2013; Chen *et al.*, 2014). OFMSW and biosolids are a readily available source of organic substrate and co-digestion of these wastes can improve the economics of HS-AD projects (Ergas *et al.*, 2018).

Anaerobic digestion of readily biodegradable organic matter, such as FW, can result in rapid production of long-chain fatty acids and volatile fatty acids (VFAs) that can exceed the utilization rate of methanogens, resulting in decreased reactor pH and microbial inhibition (Khanal, 2011). This is a particular concern in HS-AD, as the low water content and heterogeneous mixing in these reactors result in low mass transfer rates of intermediates to microbial communities, resulting in formation of microniches where VFAs can accumulate (Karim *et al.*, 2005).

The selection of an appropriate substrate to inoculum ratio (S/I) is critical for preventing VFA accumulation during anaerobic digestion with the intention of maximizing methanogenic yield. Too high of an S/I overloads microbial populations, while an S/I that is too low results in large reactor volume requirements and less CH₄ (Hinds *et al.*, 2018). S/I of FW to anaerobic digested sludge at 0.3 in Hobbs *et al.* (2018) study was close to reaching CH₄ saturation by the end of the test, but produced the least amount of CH₄ compared to 1.0 and 2.1 during wet AD. Higher S/I of OFMSW may lead to increased production of CH₄ due to OFMSW buffering capacity.

The first-order kinetic phenomena for municipal solid waste can be described for wet AD of OFMSW (Boulanger *et al.*, 2012; Trzcinski and Stuckey, 2012). Trzcinski and Stuckey (2012) studied the effect of S/I on wet anaerobic digestion of FW, paper waste, and garden waste at ratios of 0.4, 0.2, and 0.07 based on VS and observed the highest CH₄ yield at an S/I of 0.4. The highest S/I 0.4 had a hydrolysis constant of 0.112 day⁻¹ compared to 0.151 and 0.221 day⁻¹, compared to the other two ratios (Trzcinski and Stuckey, 2012). Although HS-AD studies have assessed S/I ranging from 0.25 to 16.67 for multiple substrates investigating parameters that influence performance (Boulanger *et al.*, 2012; Trzcinski and Stuckey, 2012; Li *et al.*, 2018), literature is limited on hydrolysis of HS-AD of FW, YW, and biosolids and S/I that may yield higher methane. Thus, a study focusing on the effects of S/I for OFMSW in HS-AD and its hydrolysis may provide insight for managing the largest waste products in the United States.

This research is centered around the impact of S/I ratio on mesophilic high solids anaerobic co-digestion of FW, YW, and biosolids in Hillsborough county, FL. Currently, the county utilizes the substrate to substrate ratio. The overall goal of this work is to contribute to the understanding of the reactor's parameters required to maximize bioenergy production from HS-AD of OFMSW and biosolids. Biochemical methane potential (BMP) assays were carried out with varying S/I. Measurements of chemical parameters, including pH, VFA, NH₃-N, and alkalinity concentrations, were used to elucidate biochemical mechanisms. Hydrolysis rate constants were calculated to interpret kinetics based on cumulative CH₄ production (Koch *et al.*, 2015).

Results from this study have implications for reducing OFMSW and biosolids disposal in landfills, increasing CH₄ produced for bioenergy generation, and producing fertilizer or soil conditioner from digestate.

Materials and Methods

Materials

The inoculum used in this study was dewatered sludge from a wet anaerobic digester at the City of Clearwater Northeast Advanced Wastewater Treatment Facility (Clearwater, Florida). The facility digests a mixture of primary and waste activated sludge (WAS) under mesophilic conditions at a solid retention time of 21 days. The inoculum was centrifuged at the plant to achieve ~20% TS content. Before the start of the experiment, inoculum was acclimated for 21 days at 35°C with the experimental substrates and solid-phase alkalinity sources (described below).

Synthetic FW and YW were used in this study to obtain repeatable results (Table 1). The FW mixture was based on a typical FW composition (Ariunbaatar *et al.*, 2014). YW composition was based on feedback from a waste management facility in Hillsborough County Florida (United States). The primary composition of YW was oak leaves, pine needles, grass cuttings, and wood. FW ingredients were processed using a Hamilton Beach model 70725A series A5351CE food processor (Miami Lakes, FL) for 30–60 s, and then sieved through a 3 × 3-mm mesh. YW was prepared as described by Hinds *et al.* (2016) and sieved through a 3 × 3-mm mesh.

Municipal biosolids consisted of dewatered (by screw press) WAS from the Falkenburg Advanced Wastewater Treatment Plant (WWTP) (Tampa, FL). Biosolids were dewatered at the facility with the addition of SNF Polydyne SE-1012 Polymer. A mixture of NaHCO₃ and crushed oyster shells was added to achieve 3 g/L of added alkalinity (as CaCO₃). Crushed oyster shells were purchased from a local feed store and sieved through a 3 × 3-mm mesh. The mixture was based on preliminary experiments (Ergas *et al.*, 2018) that showed a mixture of 33% NaHCO₃ (Arm & Hammer Baking Soda, Princeton, NJ) and 67% crushed oyster shells provided a good mix of fast acting and slow-release alkalinity.

BMP assays

BMP assays were performed based on methods as described by Angelidaki *et al.* (2009) and Chynoweth *et al.*

TABLE 1. FOOD AND YARD WASTE COMPOSITION BY MASS FRACTION

Substrate	Component	Wet mass fraction (%)
FW	Fruits and vegetables	72.7
	Meat	8.8
	Dairy products	5.5
	Bread and bakery	6.6
	Pasta and rice	6.4
YW	Grass clippings	25
	Oak leaves	25
	Pine needles	25
	Wood debris	25

FW, food waste; YW, yard waste.

TABLE 2. EXPERIMENTAL SETUP BY TOTAL AND VOLATILE SOLIDS

S/I	Inoculum (g)		FW (g)		YW (g)		Biosolids (g)	
	TS	VS	TS	VS	TS	VS	TS	VS
1.0	12.1	9.1	3.1	3.0	6.8	6.4	2.2	1.9
2.0	9.0	6.2	4.6	4.4	9.9	9.3	3.3	2.8
3.0	7.0	5.3	5.5	5.3	11.9	11.2	3.9	3.3
Inoculum only (blank)	18.8	14.1	0.0	0.0	0.0	0.0	0.0	0.0

S/I, substrate to inoculum ratio; TS, total solids.

(1993). Substrates were mixed with acclimated inoculum and deionized water to achieve 15% TS and added to 250 mL glass serum bottles to yield S/I shown in Table 2. For the different S/I ratio mixtures, the substrate, inoculum, and water amounts changed to yield the same total mass (100 g wet weight) and TS content (15% TS) added to each of the reactors. Note that inoculum-only blanks were adjusted from the “as received” value to 19% TS on day 10. S/I values used in this study were based on a literature review of previously studied S/I for similar substrates (Chen *et al.*, 2014; Liao *et al.*, 2014; Hobbs *et al.*, 2018). BMP assays were carried out in triplicate along with five inoculum-only blanks run in parallel. Blanks were used to determine CH₄ production from the inoculum. The inoculum was degassed to remove residual material. After the S/I material was added, bottles were flushed with N₂ gas, to remove headspace gases, and placed in a 35°C thermostatically controlled room for 24 h.

Analytical methods

Biogas samples were collected every 2 days from each digestion bottle headspace using a 50-mL frictionless syringe (5157; Cadence Science, Inc.) equipped with a 25-gauge needle (Z192406-100; BD Precisionglide). Digestion bottles were shaken vigorously before each measurement to dislodge trapped gas bubbles. The CH₄ content of the biogas was measured by dissolving 20 mL of the collected biogas into a 3N NaOH barrier solution and measuring the remaining gas volume (ASTM D1827-92, 2002). Note that this method assumes that biogas consists of CH₄ and CO₂, with negligible quantities of other inert gases (e.g., N₂ H₂). Biogas and CH₄ volumes were adjusted to standard temperature and pressure (STP: 273.2 K and 101.3 kPa).

BMP experiments ran for 47 days, starting with nine reactors per S/I ratio. Solid and liquid chemical analyses were performed weekly on the BMP digestate using one sacrificed BMP assay from each S/I digestion set. Inoculum-only blanks were sacrificed for analysis every 2 weeks. TS and VS were measured according to *Standard Methods* (2540B and E) (APHA *et al.*, 2012). Liquid extracts were prepared with 15 g of wet sample diluted with 30 mL of deionized water, vortexed for 3 min, and centrifuged at 4,500 rpm for 10 min using an Eppendorf 5810 Centrifuge (Hauppauge, NY) (EPA Method 9045D; USEPA (Agency, 2007). *Standard Methods* (APHA *et al.*, 2012) were used to measure the liquid extract pH (4500-H+B), soluble chemical oxygen demand (sCOD) (5200B), and alkalinity as CaCO₃ (2302B). HACH test kits

(HACH, U.S. TNT 872) and a HACH DR2800 Spectrophotometer (Loveland, CO) were used to measure total VFA as acetic acid (HAc). A Timberline Instruments model TL-2800 Ammonia Analyzer (Boulder, CO) was used to measure total NH₃-N (TAN).

Data analysis

CH₄ yield was calculated by dividing the cumulative CH₄ production (mL) (corrected to STP) by the mass of VS:

$$CH_4 \text{ yield} = \frac{\text{Cumulative } CH_4 \text{ production (corrected to STP)(mL)}}{\%VS \text{ Sample} \left(\frac{g}{g}\right) * \text{Sample Wet Weight}(g)} \quad (Eq.1)$$

CH₄ production data were analyzed using paired *t*-tests comparing the means of the replicate data (significance level is $\alpha=0.01$) (Washington *et al.*, 2010). As samples were sacrificed throughout the experiment, the degrees of freedom for the experimental sets changed, which were taken into consideration to determine significance.

Hydrolysis of complex organic matter to soluble compounds can be a rate-limiting step for CH₄ production (Pavlostathis and Giraldo-Gomez, 1991; Bolzonella *et al.*, 2005). First-order hydrolysis model was used to interpret the hydrolysis kinetics from the cumulative CH₄ production:

$$Y = Y_{\max} [1 - \exp(-k_{hyd}t)] \quad (Eq.2)$$

For this equation, *Y*=cumulative CH₄ production from the BMP assay at time *t* (mL), *Y*_{max}=ultimate CH₄ yield from the BMP assay at the end of the incubation time (mL), *t*=time (day), and *k*_{hyd}=first-order hydrolysis rate constant (day⁻¹). [Eq. (2)] can be re-written as follows:

$$-k_{hyd}t = \ln \left[1 - \left\{ \frac{Y}{Y_{\max}} \right\} \right] \quad (Eq.3)$$

In this equation, the slope is the hydrolysis rate constant (*k*_{hyd}).

The differences between S/I *k*_{hyd} were statistically analyzed using the paired *t*-test in Microsoft Excel 2019.

Results and Discussion

Cumulative CH₄ generation and yield

CH₄ generation from the inoculum-only blank demonstrated that acetoclastic methanogens (methanogenesis from acetate) were present, since no soluble organic molecules (sugars, amino acids, and/or fatty acids) were added (Fig. 1a). A lag time of at least 9 days was observed, after which gas production for S/I=1.0 increased linearly and S/I=2.0 increased exponentially. Ninety percent of the maximum CH₄ production for S/I=1.0, 2.0, and 3.0 was obtained on days 37, 45, and 22, respectively. Over the length of the experiment, ratio 1.0 produced significantly greater overall CH₄ volume (2.320 mL CH₄) and a significantly higher methanogenic yield (126 mL CH₄/(g VS_{added})) than the other to S/Is (Fig. 1b). In contrast, S/I=2.0 produced 824 mL CH₄ [(45.6 mL CH₄/(g VS_{added}))] and S/I=3.0 produced 159 mL CH₄ [(6.75 mL CH₄/(g VS_{added}))] by day 47 of the experiment. Ratio 2.0's CH₄ production increased on day 31. Ratio 3.0 never transitioned into a rapid phase of CH₄ production.

FIG. 1. BMP assays at S/I=1.0, 2.0, and 3.0 g substrate TS/g inoculum TS. (a) Cumulative CH₄ generation; (b) Methanogenic yield. BMP, biochemical methane potential; S/I, substrate to inoculum ratio; TS, total solids.

BMPs with S/I=1.0 transitioned into a rapid phase of CH₄ production after 9 days. This performance is likely due to the high amount of inoculum, which resulted in reactor parameters in the optimal range for methanogenic activity. BMPs with S/I=2.0 eventually began to yield CH₄, but had a long lag phase, incomplete conversion, and did not reach saturation by the end of the experiment. Results from S/I=3.0 imply that a low inoculum concentration results in substrate overload. Due to the CH₄ measurement method used, there may have been some H₂ present that was accounted for as CO₂. Kim *et al.* (2004) and Lay *et al.* (1999) observed H₂ production at high S/I and minimum CH₄ production. The results show that increased CH₄ production is achieved with more balanced S/I. The findings are similar to Lopes *et al.* (2004) and Hobbs *et al.* (2018) who studied S/I for wet anaerobic digestion.

Chemical characteristics of S/Is

Initial pH readings for the inoculum-only blank, S/I 1.0 mixture, 2.0, and 3.0 were 8.70, 8.08, 7.82, and 7.50, respectively. As S/I increased, final alkalinity and pH decreased (Fig. 2). This trend indicated that greater alkalinity consumption occurred with higher substrate fractions. Consequently, this led to pH-induced suppression of methanogenesis for ratio 3.0, which negatively impacted CH₄ production (Fig. 1a, b). The high alkalinity and pH for ratio

FIG. 2. Final pH and alkalinity for BMP assays at S/I=1.0, 2.0, and 3.0 g substrate TS/g inoculum TS.

1.0 coincide with the greatest amount of CH₄ production compared to ratio 2.0 and 3.0 (Fig. 2).

pH measurements can be used as indicators of acid-induced microbial inhibition that causes reactors to produce negligible amounts of biogas and CH₄. Guan *et al.* (2015) stated that HS-AD microbial inhibition occurs in anaerobic digesters at pH values <6.0.

Figures 3 and 4 illustrate VFA and TAN concentrations throughout the duration of the experiment. From day 0 to 6, all S/I VFA and TAN concentrations increased due to the high quantity of easily degradable substrates present. Through day 20 until the end of the test, VFA concentrations remained high for S/I values of 2.0 and 3.0, while concentrations of VFAs decreased for ratio 1.0.

When the S/I is too high, it can lead to the rapid degradation of carbohydrates and overload the system with high concentrations of VFAs. For example, S/I=3.0 had the highest VFA concentration and produced the least amount of CH₄ over the length of the experiment. On the contrary, ratio 1.0 had the least amount of VFAs and produced the most CH₄. This is most likely due to the high concentration of the inoculum's active methanogens. In addition, although high TAN concentrations result in the inhibition of methanogenesis (Hinds *et al.*, 2018), levels of TAN below 3.5 g/L are tolerable for HS-AD as NH₃ contributes buffering capacity

FIG. 3. Variations in VFA concentrations versus time for BMP assays at S/I=1.0, 2.0, and 3.0 g substrate TS/g inoculum TS. VFA, volatile fatty acid.

FIG. 4. TAN versus time for BMP assays at S/I = 1.0, 2.0, and 3.0 g substrate TS/g inoculum TS. TAN, total ammonia nitrogen.

and metabolic stability (Ahring and Westermann, 1988; Bujoczek *et al.*, 2000).

Brown and Li (2013) stated that pH and VFA/alkalinity ratios are common stress indicators in the AD process. Khanal (2011) determined that VFA concentrations exceeding 10,000 mg/L can cause microbial inhibition. HS-AD systems sometimes experience acidic microbial deficiencies when the microbial communities in the system are not balanced or when an easily degradable substrate, such as FW, is introduced into the system, resulting in VFA accumulation (Forster-Carneiro *et al.*, 2008).

Figure 5 shows the weekly sacrificed reactors sCOD concentrations and VFA:sCOD ratios. As the S/I increased, the initial sCOD concentrations increased, which highlighted the substrate's bioavailability. Final sCOD was lower for S/I 1.0 at 0.82 g/L compared to 1.74 and 1.73 g/L sCOD for S/Is 2.0 and 3.0. These final values imply that stabilization was incomplete for S/I 2.0 on day 48 and S/I 3.0 experienced accumulation of VFA. VFAs were 33%, 16%, and 24% of the sCOD on day 48 for S/I = 1.0, 2.0, and 3.0 respectively. At S/I 3.0, VFA were produced faster than consumed. This acid accumulation for S/I 3.0 corresponds with the low pH value,

FIG. 5. Initial and final sCOD and VFA:sCOD ratio for BMP assays at S/I = 1.0, 2.0, and 3.0 g substrate TS/g inoculum TS. sCOD, soluble chemical oxygen demand.

FIG. 6. Linear regression fit for estimating hydrolysis constant for BMP assays at S/I = 1.0, 2.0, and 3.0 g substrate TS/g inoculum TS.

leading to minimal CH_4 yield and fermentation products. The S/I 3.0 VFA:sCOD demonstrates that this BMP was most likely still in the acidogenesis phase at the end of the study.

Hydrolysis constant

A first-order hydrolysis model with respect to CH_4 was simulated to estimate K_{hyd} using the various S/I BMP assay data (Fig. 6). The simulated data showed a relatively good fit to experimental data from CH_4 with $R^2 > 0.8$ for S/I 1.0 and 3.0. The R^2 for S/I 2.0 was 0.68 lower than the other two S/Is. The goodness of fit for S/I 2.9 was affected by increased production toward the end of the test, causing incomplete CH_4 stabilization on day 47. A lag phase was observed for all S/I values indicating that the rate-limiting step was likely hydrolysis based on previous studies (Pavlostathis and Giraldo-Gomez, 1991; Bolzonella *et al.*, 2005; Bakhov *et al.*, 2014). S/I 1.0 had significantly higher K_{hyd} of 0.06 day^{-1} compared to S/I 3.0 at 0.007 day^{-1} (p -value, 0.03) and the K_{hyd} for S/I 2.0 (0.02 day^{-1}) was an order of magnitude higher than S/I 3.0. For comparison, Tandukar and Pavlostathis (2015) reported K_{hyd} of $\sim 0.10 \text{ day}^{-1}$ for dewatered fat, oil, and grease and gum waste. Interestingly, as the S/I ratios increased, the K_{hyd} decreased. This suggests that too high sCOD depresses bioavailability. In this study, S/I 3.0 produced high concentrations of VFA that the inoculum could not remove properly, hence producing low CH_4 .

Conclusions

HS-AD facilities may be a viable option for processing and managing OFMSW in the United States due to its ability to decrease substrates and produce resources. This study identified the feasibility of co-digesting OFMSW with biosolids and processes that must be considered for achieving high CH_4 yields under HS-AD. Results from S/I 1.0, 2.0, and 3.0 g of substrate TS/g inoculum TS demonstrate that concentrations of VFA and TAN initially increased due to high quantity of easily degradable substrates present. However, at high S/I of 2.0 and 3.0, VFA accumulation and low pH led to limitations that resulted in long lag phases, and negatively impacted CH_4 yields. While sCOD may be higher for S/I 2.0 and 3.0, high concentrations of VFAs impeded progression to

methanogenesis. S/I 1.0 corresponded with high CH₄ yield from HS-AD along with a high K_{hyd} and a short lag time. The stability of HS-AD and components depends on the production of sCOD, pH, and VFAs along with alkalinity generated by TAN from the substrates. The conditions are required to maximize bioenergy production from co-digesting OFMSW and biosolids are important to successful HS-AD operation. This research suggests that S/I 1.0 may be a viable ratio for digestion of OFMSW in municipal facilities. More research should be done to test the financial feasibility to construct more HS-AD and its life cycle impacts. Implications of this study diversify current waste management strategies and assist municipalities in meeting goals of diverting organic wastes from landfills and incinerators and expanding energy mixes.

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