March 2018

Impact of Substrate to Inoculum Ratio on Methane Production in High Solids Anaerobic Digestion (HS-AD) of Food Waste, Yard Waste, and Biosolids

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by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Civil Engineering
Department of Civil and Environmental Engineering
College of Engineering
University of South Florida

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Date of Approval:
March 21, 2018

Keywords: alkalinity, bioenergy production, methane yield, municipal solid waste, sewage sludge

ACKNOWLEDGEMENT

Thank you goes to my co-advisors, Drs. James Mihelcic and Sarina Ergas, for guiding me patiently through the challenge of identifying novel research topics and completing successful experiments. Special thanks to Drs. Eunyoung Lee and Meng Wang for providing guidance on the research and focusing me throughout this process. Thanks to the operators at the Falkenburg and Northeast Wastewater Treatment Plants in Tampa and Clearwater, FL. Facilities individuals helped to provide me with the materials there were needed throughout my research journey.

I would like to acknowledge the help of everyone who has assisted me with this research to date. This includes my office and lab mates, the undergraduate team (Aleem Waris, Dhwani Khambhati, Eduardo Jimenez, Jana Tripp, Patrick Lacoff, and Paula Bittencourt), Costa Rica international research team (Bethany Loya, Denver Gordon, Fabricio Camacho, Jose Montero, and Kevin Orner), microbiology team (Madison Davis), formatting team (Charlotte Haberstroh and Dongqing Zhang), committee members (Drs. Joseph, Scott, Tejada, and Zhang), and everyone who supported me throughout the journey.

Funding for this research was provided by the William W. “Bill” Hinkley Center for Solid and Hazardous Waste Management. Additional funding was provided by the National Science Foundation (NSF) Partnerships for International Research and Education program (PIRE; Grant #1243510). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation.

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ABSTRACT

High solids anaerobic digestion (HS-AD) is an alternative for managing the organic fraction of municipal solids waste (MSW), which produces mainly methane (CH_4) and fertilizer as byproducts. HS-AD offers a potentially more economically and environmentally sustainable option compared with landfilling or incineration waste-to-energy facilities. However, HS-AD is a complex process requiring specific microbial communities working together symbiotically. Previous studies have found that the substrate to inoculum (S/I) ratio affects CH_4 production and yield in HS-AD reactors by affecting substrate mass and energy transfer as well as microbial activity. In this thesis, biochemical methane potential (BMP) assays were used to investigate the effect of S/I ratio on CH_4 production and chemical properties during the digestion of food waste, yard waste, and biosolids. The results indicate that the S/I ratio of 1.0 based on total solids (TS) content was the optimum ratio for the mixtures, compared to 2.0 and 3.0 based on TS as well as an inoculum only blank. Specifically, the S/I ratio of 1.0 based on TS had the greatest cumulative CH_4 production of 2,320-mL and maximum cumulative CH_4 yield of 126 mL-CH_4/ (g VS_{added}) over 47 days while reducing total TS and VS in the reactors. Weekly chemical analyses showed that the optimum values were produced in BMPs with S/I of 1.0 because this set was the least influenced by pH, volatile fatty acid (VFA), total ammonia nitrogen (TAN) induced microbial inhibition. Overall, these findings may assist in the design and operation of HS-AD systems with greater CH_4 volume and CH_4 production for the digestion of the organic fraction of MWS.

CHAPTER 1 INTRODUCTION

In the U.S., municipal solid waste (MSW) is primarily disposed of in landfills (53%) or processed at incineration waste-to-energy facilities (13%) (EPA, 2015). In addition, municipal biosolids or waste activated sludge (WAS) is primarily processed by anaerobic digestion (AD), landfilling, land application, and/or incineration waste-to-energy facilities (EPA, 1994). However, these conventional disposal methods have limited capacities or are being phased out, which accelerates the necessity for better management of MSW and municipal biosolids (EPA, 2015).

Anaerobic digestion (AD) is a natural process where organic matter is broken down without oxygen by microorganisms to produce biogas composed primarily of methane (CH_4) and carbon dioxide (CO_2) with some trace gases (Frigon & Guiot, 2010). According to the chemical properties, CH_4 is the most reduced from of carbon and CO_2 is the most oxidized (Angelidaki & Sanders, 2004). AD offers a way to manage the organic fraction of MSW and biosolids while generating energy and fertilizer. Higgins et al. (1981) states that approximately half of the total organic carbon (TOC) degraded by anaerobic microflora are digested to CH_4. Microorganisms play an important role in environmental systems for they are involved in the degradation of complex, hydrophobic, organic compounds (Konhauser, 2009).

High solids (HS) AD is carried out at a total solids (TS) content $\geq$ 15% (Semblante et al., 2015; Zhang et al., 2008). HS-AD has been considered a sustainable solution for managing the organic fraction of MSW because it: a) reduces the amount of high water content materials at incineration facilities; and b) diverts organic waste from landfills reducing greenhouse gas

emissions and improving leachate quality, while generating sustainable energy (Edelmann et al., 2005). In the U.S., HS-AD has only been implemented in limited situations because of operational, physical, chemical, and biological complexities (Amani et al., 2010).

Another type of AD system is liquid anaerobic digestion (L-AD). These systems have a TS content < 15% with liquid in them that is visible. Early literature on S/I ratios focused on L-AD systems under mesophilic temperature conditions of 30-37ºC (Gerardi, 2003). L-AD is not ideal for MSW feedstocks due to the substrates' low moisture content (Frigon & Guiot, 2010). Benefits a L-AD systems of high moisture content are that the water in them allows for immediate mass transfer of the breakdown products. In addition, L-AD systems require less inoculum and shorter retention times compared to HS-AD (Kothari et al., 2014; Li et al., 2011).

The literature review what was performed on L-AD systems found that higher S/I ratios produce greater CH_4 yields (Lopes et al., 2004). For example, Lopes et al. (2004) found that the increased S/I ratios correlated with decreased mass breakdown of chemical oxygen demand (COD) and total volatile solids (VS), as well as increased biogas production and CH_4 yield (Lopes et al., 2004). Heo et al. (2004) tested a S/I ratio of 0.2 based on VS with the digestion of food waste and biosolids at 35ºC, while varying mixture ratios and hydraulic retention times (HRTs). The authors observed that CH_4 yield improved with greater food waste substrate concentrations. Hobbs et al. (2017) investigated the effect of S/I ratio on L-AD of food waste at ratios of 0.30, 1.01, and 2.14 based on VS[1]. Mixtures with the highest S/I ratio resulted in greater CH_4 yield, but these reactors

[1]Authors reported results in COD/VS. For comparison purposes results were converted to VS/VS for this report using a conversion factor of 0.714 g VS/g COD.

experienced volatile fatty acid (VFA) and pH induced microbial inhibition. This work highlights the importance of knowing the initial properties of the substrates being digested such as COD, VFAs, and alkalinity to address concerns of microbial inhibition.

Therefore, the objective of this HS-AD research is to investigate the effect of S/I ratio on physical, chemical, and biological mechanisms for the digestion of food waste, yard waste, and municipal biosolids.

CHAPTER 2 LITERATURE REVIEW

In nature, AD is a very important process for the degradation of organic material. The process generally consists of liquefaction and hydrolysis of insoluble compounds and gasification of the intermediates (Naik et al., 2010). As a result, materials are converted into mainly the stable products of CH_4, CO_2, and digestate. AD does not require a high concentration of nutrients to complete the process and results in low biomass yields. The process most efficiently occurs under mesophilic (30-40°C) and thermophilic (50-60°C) temperatures (Adekunle & Okolie, 2015; Appels et al., 2008). When AD is completed with a TS ratio ≥ 15%, it is referred to as HS-AD (Molnar & Bartha, 1988). Figure 1 shows the essential HS-AD metabolic pathways that occur, microbial communities, and organic molecules involved. The HS-AD process uses a complex series of biochemical reactions where different types of microorganisms breakdown organic material in the absence of oxygen. Specifically, these processes are connected though symbiotic relationships and are termed hydrolysis, acetogenesis, acidogenesis, and methanogenesis (Khalid et al., 2011). Unfortunately, the processes can be easily disrupted by pH, VFA, and total ammonia nitrogen (TAN) induced microbial inhibition.

The S/I ratio is the amount of substrate TS or VS divided by the inoculum TS or VS. The S/I ratio influences the rate of gas production, CH_4 yield, and effective reactor volume (González-Fernández & García-Encina, 2009; Gunaseelan, 1995). In HS-AD, a balanced S/I ratio has been found to aid in helping to obtain the optimum reactor microbial metabolisms (Adekunle & Okolie,

2015; González-Fernández & García-Encina, 2009). In this ratio, the inoculum activity is important because it aids the digester startup (Raposo et al., 2009).

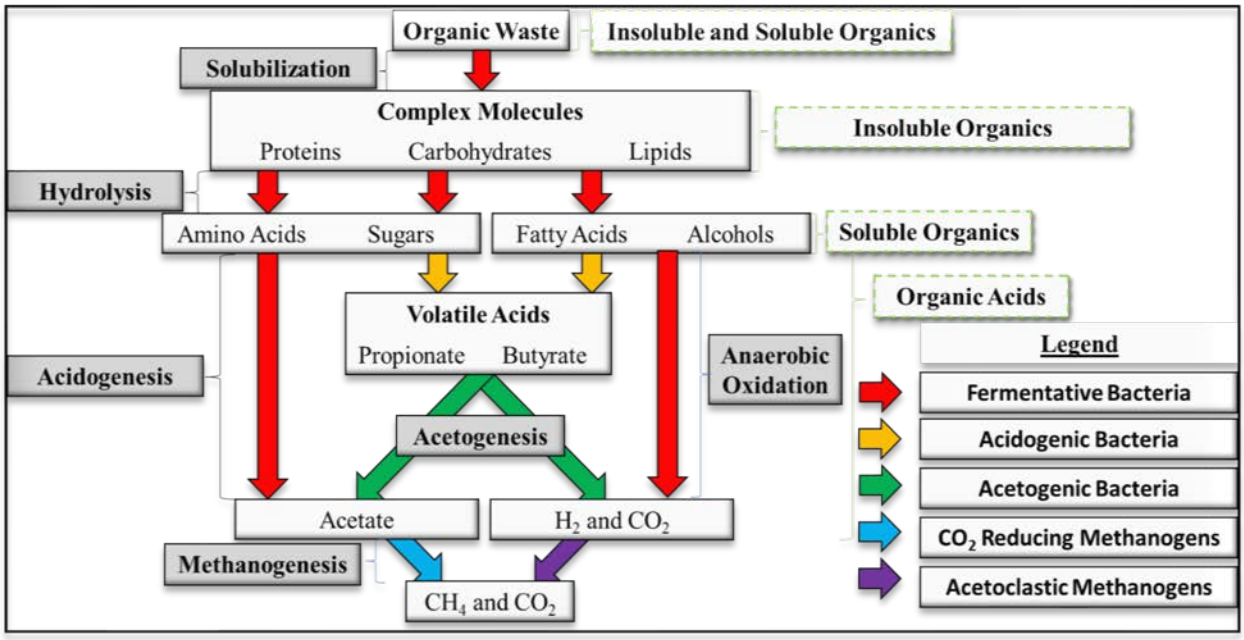

Figure 1 High Solids Anaerobic Digestion Metabolic Pathways.

A summary of the literature on how the S/I ratio effects mesophilic AD is shown in Table 1. The major conclusions from the literature are that HS-AD is more difficult to physically, chemically, and biologically balance to obtain the optimum microbial activity compared to L-AD, due to the lack of water in the system. After microorganisms break down materials into intermediary phases, the products should not be allowed to accumulate for at elevated concentrations some of the compounds will induce microbial inhibition. When a HS-AD system is balanced, it could produce more CH_4 volume and yield when compared to a L-AD system because of the greater concentration of material to be digested in the system. Properties that could induce microbial inhibition are excess TAN concentrations, increased VFAs in solution, and acidic pH values (Chen et al., 2008; Khanal, 2011). Kroeker et al. (1979) and McCarty (1964) state that microbial inhibition could occur at pH values > 7.4 when TAN values approach and exceed

concentrations of 1,700 mg TAN/L. The literature also notes that when TAN concentrations exceed 3,000 mg/L the ammonium ion becomes toxic and will cause microbial inhibition at any pH value (McCarty, 1964). Khanal (2011) found that VFA concentrations exceeding 10,000 mg/L could cause microbial inhibition. VFA concentrations are related to a system's pH because when there are excess VFAs in solution the system's pH has the potential to decrease and become acidic. Guan et al. (2015) states that pH values < 6.0 could cause microbial inhibition.

Table 1 Summary of Literature on S/I Ratio Effects on Mesophilic Anaerobic Digestion.

Topic	**Reference**
Effect of S/I ratio on L-AD of MSW	Boulanger et al. (2012)
Impact of S/I ratio in L-AD of swine slurry	González-Fernández and García-Encina (2009)
Effect of mixture ratio and HRT on L-AD of food waste and WAS with a constant S/I ratio	Heo et al. (2004)
Impact of S/I ratio on L-AD of food waste	Hobbs et al. (2017)
Influence of S/I ratio of AD of MSW	Lopes et al. (2004)
Effect of S/I ratio on the AD of vegetable waste	Lü et al. (2012)
Influence of S/I ratio on the AD of sunflower oil cake	Raposo et al. (2009)
Influence of S/I ratio on L-AD of bean curd refuse	Zhou et al. (2011)
Comparing HS-AD to L-AD of food waste and green waste using different S/I ratios	Chen et al. (2014)
Process performance of L-AD and HS-AD of municipal biosolids using difference S/I ratios	Liao et al. (2014)
HS-AD with constant S/I ratio feasibility study	Duan et al. (2012)

Additional research found that AD reactors with high S/I ratios could produce a wide range of CH_4 yields, because of microbial inhibition. González-Fernández and García-Encina (2009) investigated S/I ratio's impact on AD of swine slurry. Over 90 days, the same CH_4 yield was

generated using experimental S/I ratios of 0.7, 1.4, and 2.1 based on VS[2]. However, due to the accumulation of VFAs in the reactors, the rate of CH_4 production decreased as the S/I ratio increased, which may have been caused by insufficient alkalinity in the system. An S/I ratio of 0.7 based on VS was recommended for the digestion of swine slurry (González-Fernández & García-Encina, 2009). Boulanger et al. (2012) found that for L-AD of the organic fraction of MSW the greatest CH_4 yield was produced at an S/I ratio of 0.25 (based on VS) and microbial inhibition occurred when the S/I ratio was $\geq$ 8.33. At the higher S/I ratio, it was reported that the reactor reached a maximum accumulation of dissolved organic carbon and the hydrolysis process was compromised because of mass transfer limitations (Boulanger et al., 2012). Lü et al. (2012) studied L-AD with S/I ratios from 0.9 to 47.7 based on VS at 35ºC using paper mill wastewater anaerobic granular sludge inoculum. It was reported that at high S/I ratios, that the mixing of inoculum with the organic substrates prevented the initiation methanogenesis (Lü et al., 2012).

Another advantage of HS-AD systems is that the digesters use less water to complete the process and have low leachate production. In other words, HS-AD systems require a decreased digester size and a smaller footprint, which is beneficial when land area is limited (Kothari et al., 2014; Li et al., 2011). However, it turns out that water is necessary in AD systems to aid the chemical and biological processes. The water could dissolve substrates and help to transport them to microorganisms. Water also aids in the balance of acidogenic bacteria's VFA production and the conversion of acids by methanogenic bacteria (Liao et al., 2014). With less water in the HS-

[2]Authors reported results in COD/VS. For comparison purposes in this report results were converted to VS/VS using a conversion factor of 0.714 g VS/g COD.

AD system mass transfer may be reduced and incomplete mixing could cause microbial micro-niches to form where VFAs accumulate, pH is low, and microbial inhibition could occur (Karim et al., 2005).

The AD of materials at TS ≥ 15% (HS-AD) is helpful for breaking down many materials economically and efficiently. However, HS-AD may require mixing to overcome mass transfer limitations. Mechanical mixing or pumping leachate through the reactor solids can be useful in these types of systems digesting substrates to stoichiometrically balance the materials and transfer the breakdown products to the microorganisms. Some types of mixing methods include external leachate recirculation, internal mechanical mixing, and internal gas mixing (Appels et al., 2008).

Under mesophilic conditions, the S/I ratio inside AD reactors affects CH_4 production and yield. Liao et al. (2014) compared HS-AD (TS=15.7%) with L-AD reactors and found that they both achieved the same VS degradation rate. However, in the HS-AD system, lower CH_4 yield with increased volumetric biogas production rates and treatment capacity were observed, due to acidic pH induced microbial inhibition (Liao et al., 2014). Chen et al. (2014) compared the digestion of food waste and yard waste for HS-AD and L-AD (TS contents 5-25%), and found that CH_4 yields were higher for HS-AD with a higher volumetric productivity. Duan et al. (2012) found that HS-AD systems (TS = 15 and 20%) could support 4-6 times higher organic loading while obtaining similar CH_4 yields and VS reduction when compared to L-AD systems.

The biochemical reactions within HS-AD systems are complex and require specific microbial communities using symbiotic effects (Ali Shah et al., 2014; Amani et al., 2010). HS-AD systems operating at their optimum S/I ratio should produce greater VS reduction as well as CH_4 volume and yields. With greater CH_4 volume it will allow for increased energy revenues to be obtained. Previous studies have been performed on L-AD and HS-AD systems digesting single

and multiple substrates (Lopes et al., 2004; Lü et al., 2012). The studies found high S/I ratios resulted in restricted mass and energy transfer, which decreased degradation rates of the organic fraction of MSW. The studies also found that S/I ratios ≥ 1.0 could increase or decrease biogas generation and CH_4 yield depending on the microorganisms' activity (Boulanger et al., 2012; Liao et al., 2014).

CHAPTER 3 MATERIALS AND METHODS

3.1 Biochemical Methane Potential (BMP) Assays

Three sets of batch HS-AD experiments were carried out in parallel with nine BMP reactors. The S/I ratios for the different sets included: 1.0, 2.0, and 3.0 based TS. In addition, a set of five inoculum only blank BMPs was run in parallel to the experiments to determine how much CH_4 the reactors would produce without the addition of substrates and solid phase alkalinity sources. The chosen S/I ratios were based on a literature review of the optimum S/I ratios to digest the experimental substrates (Chen et al., 2014; Hobbs et al., 2017; Liao et al., 2014). The experiments performed for this thesis were based on defined methods as described by Angelidaki et al. (2009) and Chynoweth et al. (1993).

The BMP reactors consisted of 250-mL glass serum bottles sealed with rubber septums and metal crimp caps. Reactor contents were mixed by hand outside the reactors prior to the experiment to provide homogeneous mixture conditions. Following the placement of the S/I material into each BMP reactor the bottles were flushed with nitrogen gas to remove any free oxygen gas from the reactors. The BMPs were then placed in a thermostatically controlled room maintained at 35ºC. During the first 24-hours of the experiment, all excess gases produced were vented to the atmosphere. This was done to prevent any over pressurization of the bottles due to the rapid production of CO_2 at the start of a HS-AD reactor. At the end of the 24-hour period the BMP assays were again purged with nitrogen gas before being permanently sealed from the atmosphere

for the experimental duration. Table 2 shows the experimental set-ups for the different S/I ratios based on TS and VS.

Table 2 Experimental Set-up by Total and Volatile Solids Mass.

S/I Ratio	Inoculum (g)		Food Waste (g)		Yard Waste (g)		Municipal Biosolids (g)		Alkalinity (g $CaCO_3$/L)	Mixture %TS
	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	3	15.0
2.0	9.0	6.2	4.6	4.4	9.9	9.3	3.3	2.8	3	15.0
3.0	7.0	5.3	5.5	5.3	11.9	11.2	3.9	3.3	3	15.0
Inoculum Only (Blank)	18.8	14.1	0.0	0.0	0.0	0.0	0.0	0.0	0	19.0[3]

3.2 Feedstock and Inoculum

The following constituents were included in the BMP reactors: inoculum, food waste, yard waste, municipal biosolids, and alkalinity sources (see Table 2). The constituents were combined using a substrate to substrate (S/S) ratio to reflect the municipal solid waste composition in Hillsborough County Florida. This S/S ratio was 1.4:3.0:1.0 based on TS for food waste, yard waste, and municipal biosolids, respectively. The experiment study inoculum was prepared from dewatered anaerobically digested sewage sludge obtained from the Northeast Clearwater Treatment Facility (Clearwater, Florida). The Clearwater facility digests a mixture of primary sludge and WAS under mesophilic conditions with a solids retention time (SRT) of 21 days. At the facility the sludge was centrifuged to achieve a TS content of approximately 20%.

[3]On day 10 of the experiment 25.5-mL of DI water was added to all the inoculum only BMP reactors to adjust the TS% to 15.0.

Prior to the start of the experiments, the inoculum was acclimated to select microorganisms that could biodegrade the experimental substrates. This was accomplished by placing 750 grams (wet weight) of the inoculum per container into four 1-L reactors for 21 days with an S/I ratio of 0.5 based on TS at 35ºC. (Note: prior HS-AD experiments in the laboratory using the Northeast Clearwater dewatered anaerobically digested sewage sludge as an inoculum source for HS-AD of food waste, yard waste, and municipal biosolids were not successful without an experimental acclimation phase.) For the acclimation phase the substrates consisted of food waste, yard waste, and municipal biosolids with the addition of a solid phase alkalinity source at a concentration of 6 g $CaCO_3$/L. Specifically, the alkalinity was added as 2.3 g of $NaHCO_3$ (Arm & Hammer Baking Soda, Princeton, NJ) and 2.8 g of crushed oyster shells (95% $CaCO_3$) from a local feed store (Shells, Tampa, FL) both concentrations based on wet weight. After the acclimation period, the pure inoculum was added to the BMPs to yield total S/I ratios of 1.0, 2.0 and 3.0 based on TS.

The food waste was prepared as described by Ariunbaatar et al. (2014). The composition is shown in Table 3. In addition, the exact components can be seen in Table B 1 in Appendix B. The food waste was prepared by chopping the ingredients into small sizes by hand and then using a Hamilton Beach model 70725A series A5351CE food processor (Miami Lakes, FL) to cut the food an additional 30-60 seconds. To guarantee the resulting food waste had a particle size < 3 mm after it was mixed it was sieved through a 3x3-mm wire mesh. The food waste was prepared a maximum of two days before it was placed in the BMP reactors and was stored in a refrigerator prior to the experiment at 1.6 ºC.

Yard waste consisted of oak leaves, pine needles, grass clippings, and wood debris. The yard waste composition was developed based on discussions with operators from the city of Tampa yard waste facility. The yard waste fraction composition by percent wet mass is shown in Table 3.

In addition, the exact components can be seen in Table B 2 of Appendix B. The yard waste was processed by cutting it with scissors one-week prior to sample preparation. In typical full-scale HS-AD systems, yard waste is ground to < 40 mm (De Baere, 2010). However, to make the resulting yard waste homogeneous and allow it to be compared to previous research a 3x3-mm sieve was used to determine the maximum particle size (Hinds et al., 2016). Lastly, the yard waste was stored at room temperature prior to the start of the experiment.

Table 3 Waste Composition by Mass Fraction.

Substrate	Component	Wet Mass Fraction (%)
Food Waste	Fruits and Vegetables	72.8
	Meat	8.8
	Dairy Products	5.5
	Bread and Bakery	6.6
	Pasta and Rice	6.4
Yard Waste	Grass Clippings	25
	Oak Leaves	25
	Pine Needles	25
	Wood Debris	25

The municipal biosolids consisted of dewatered (via screw press) WAS from the Falkenburg Advanced Wastewater Treatment Plant (WWTP) in Tampa, FL. Before the WAS left the WWTP it was dewatered with the addition of a polymer. The municipal biosolids were gathered a maximum of one week before they were placed in the reactors and stored at room temperature prior to the experimental setup.

Preliminary studies in the USF Environmental Engineering laboratory and a literature review highlighted that a mixture of crushed oyster shells and $NaHCO_3$ as a solid phase alkalinity source improved CH_4 production and yields. These alkalinity sources were selected for this study because they are available in high volume and low cost in Hillsborough county. $NaHCO_3$ was identified to immediately release alkalinity and crushed oyster shells to release alkalinity at a slower rate (Waldbusser et al., 2011). The two sources were combined to provide a solid phase

alkalinity source of 3 g $CaCO_3$/L to the experimental S/I ratio mixtures. The wet weight concentration of crushed oyster shells was 67% of the total mass of alkalinity and the $NaHCO_3$ consisting of the rest (Brown & Li, 2013; Gerardi, 2003).

3.3 Analytical Methods

Biogas was collected three times weekly or approximately every other day (see Table B 3, 5, 7, and 9 for sampling dates). Samples were collected from the digestion bottle headspace using a 50-mL frictionless syringe with a metal luer lock tip (5157; Cadence Science Inc.) equipped with a 25-gauge needle (Z192406-100; BD PrecisionGlide) and previously described procedures (Jerger et al., 1982; Owen et al., 1979; Owens & Chynoweth, 1993). Specifically, the CH_4 concentration of the biogas was determined with a volume displacement method by dissolving the CO_2 portion of a 10-mL biogas sample into a 3 N NaOH barrier solution and measuring the resulting liquid displaced (ASTM, 2002; Manser et al., 2015). Blanks were used to compare the CH_4 production in the inoculum alone. The BMP reactors were shaken vigorously by hand for ten seconds prior to each biogas measurement to dislodge any gas bubbles trapped in the digestate. CH_4 volume and yield values were adjusted to standard temperature and pressure (STP: 273.2 K and 101.3 kPa).

Solid and liquid chemical analyses were performed weekly on the BMPs' digestate using one sacrificed BMP assay from each S/I ratio. For comparison purposes, the inoculum only blank was analyzed every other week. The analyses consisted of measurements of alkalinity, soluble chemical oxygen demand (sCOD), pH, TAN, TS, VS, and VFA. The liquid extract samples were prepared with 15 grams of wet sample diluted with 30-mL of deionized water, mixed vigorously for 3 minutes using a Scientific Industries Vortex Genie 2 (Bohemia, NY) and centrifuged at 4,500

rpm in an Eppendorf 5810 Centrifuge (Hauppauge, NY) for 10 minutes to obtain a representative liquid fraction as stated in EPA Method 9045D (Agency, 2007).

For all analyses, the contents of the digesters were taken from the BMP reactors and mixed thoroughly before grab and duplicate samples were collected to minimize any errors due to sample heterogeneity. *Standard Methods* (APHA et al., 2012) were used to measure the supernatant pH (4500-H+B) with an Oakton 2700 pH meter (Vernon Hills, IL), sCOD (5200B) with Lovibond measurement tubes (Sarasota, FL), and alkalinity as $CaCO_3$ (2302B). Hach kits (HACH, U.S. TNT 872) and a Hach DR2800 Spectrophotometer (Loveland, CO) were used to measure total VFA as acetic acid. A Timberline Instruments model TL-2800 Ammonia Analyzer (Boulder, CO) was employed to measure TAN in the liquid sample. Other than pH, the concentrations of the dissolved species were corrected to leachate concentrations based on the measured TS contents. TS and VS were measured according to *Standard Methods* (2540B and E) (APHA et al., 2012).

3.4 Data Analysis

The data analysis was completed comparing the means of total CH_4 production for each sample set. Specifically, CH_4 production was calculated by measuring the sampled biogas CH_4 concentration (%) and then multiplying it by the total sampled biogas volume (mL). Cumulative CH_4 production was determined by summing each day's individual CH_4 volume for each experiment set (mL). CH_4 yield was calculated by taking the cumulative sampled CH_4 production (mL) and dividing it all by the added %VS of the sample (g VS/g wet weight) multiplied by the total wet weight of the bottle (g). For this data analysis, the CH_4 yield from the inoculum only blanks were not subtracted from the yields of the experimental mixtures, which was not done because the S/I 2.0 and 3.0 mixtures produced less CH_4 than the blank. This allowed for a better comparison of the experiment mixtures.

3.5 Statistical Analysis

To determine the effect of S/I ratio on CH_4 production, the CH_4 data were statistically analyzed using a comparison method. Specifically, the analysis method consisted of a paired t test that compared the means of the replicate data. This test determined the probability that the measured values were not significantly different from each other at different points in time or the null hypothesis. The results from the t distribution test were compared against the critical values of a two tailed t distribution (significance level is $\alpha = 0.01$) to determine when the null hypothesis could be rejected (Washington et al., 2010). As samples were sacrificed throughout the experiment the degrees of freedom for the experimental sets changed. This fact was taken into consideration to determine the critical values to determine the t distribution significance confidence levels (see Appendix for 99% confidence (see Appendix B Table B 4, 6, 8 and 9 for the t distribution analysis and 99% confidence critical values).

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Cumulative CH_4 Production and Yield

Figure 2 and Figure 3 show the cumulative CH_4 production and yield curves, respectively, for the BMPs operated at varying S/I ratios, respectively. After the 21st day of the experiment, the S/I ratio of 1.0 based on TS produced a total CH_4 volume of 1,160 mL, which was the greatest among the experimental sets for that period. The inoculum only blank had the second greatest CH_4 production of 565 mL. Furthermore, the BMPs with S/I ratios of 1.0 based on TS and the blank (inoculum only) continued to have the greatest volumetric CH_4 productions until the conclusion of the experiment (day 47). Specifically, at the end of the experiment, Figure 2 shows the S/I ratio of 1.0 based on TS produced a total volume of 2,320-mL of CH_4. In contrast, the inoculum only blank produced a total CH_4 volume of 866-mL, while the S/I ratios of 2.0 and 3.0 based on TS produced total volumes of 824 and 159-mL of CH_4, respectively.

pH measurements can be used to indicate when there is acid induced microbial inhibition during HS-AD. This kind of inhibition greatly affects HS-AD microbial activity and causes reactors to produce negligible amounts of biogas and CH_4. Guan et al. (2015) stated that HS-AD microbial inhibition occurs in anaerobic digesters at pH values < 6.0. In addition, 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. Specifically, HS-AD systems sometimes experience acidic microbial inhibition when the microbial communities in the system are not balanced or when an easily degradable substrate

is introduced into the system, such as food waste, resulting in VFA accumulation. As shown in Figure 4 Figure 5, all the S/I mixture reactors studied experienced pH and VFA induced microbial inhibition at the beginning of the experiment (Chen et al., 2008; Guan et al., 2015; Khanal, 2011).

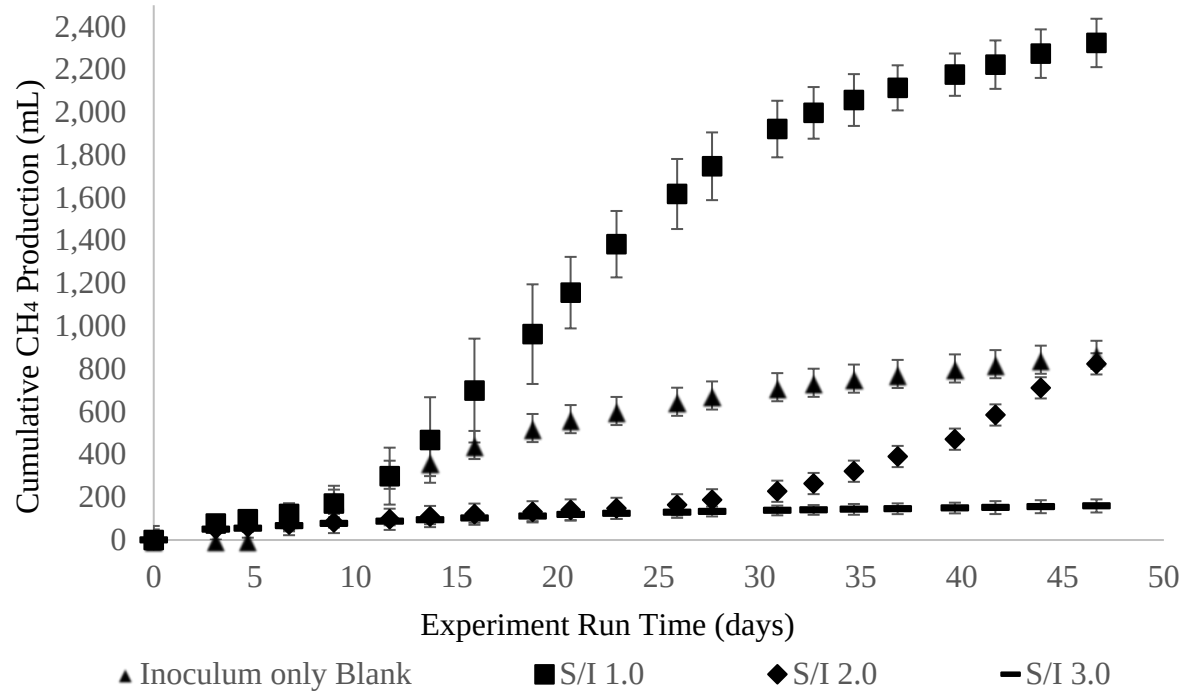

Figure 2 Cumulative Methane Production for Different Substrate to Inoculum Ratios Based on TS.
(error bars show ± one standard deviation from the mean value)

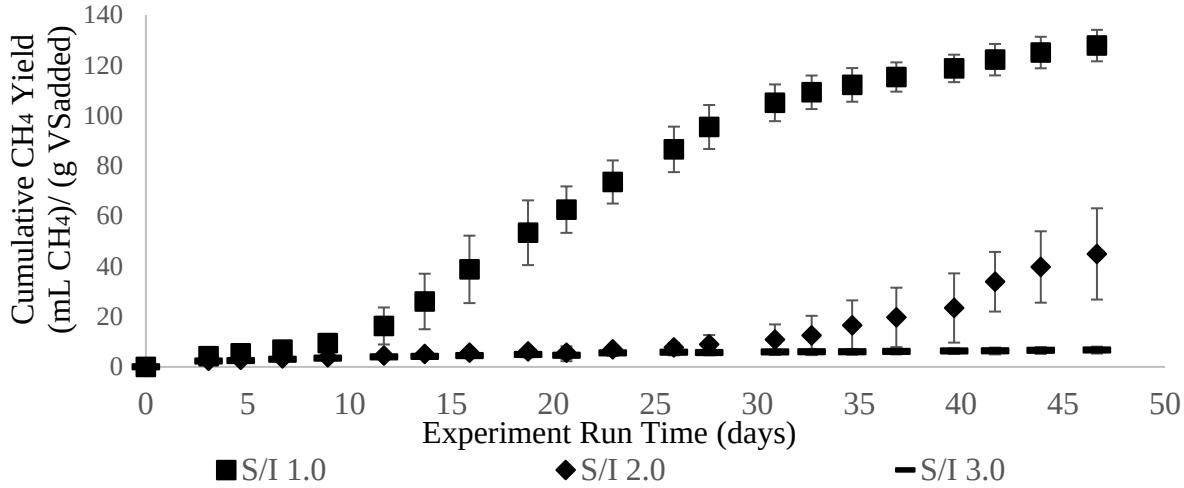

Figure 3 Cumulative Methane Yield for Different Substrate to Inoculum Ratios Based on TS.
(error bars show ± one standard deviation from the mean value)

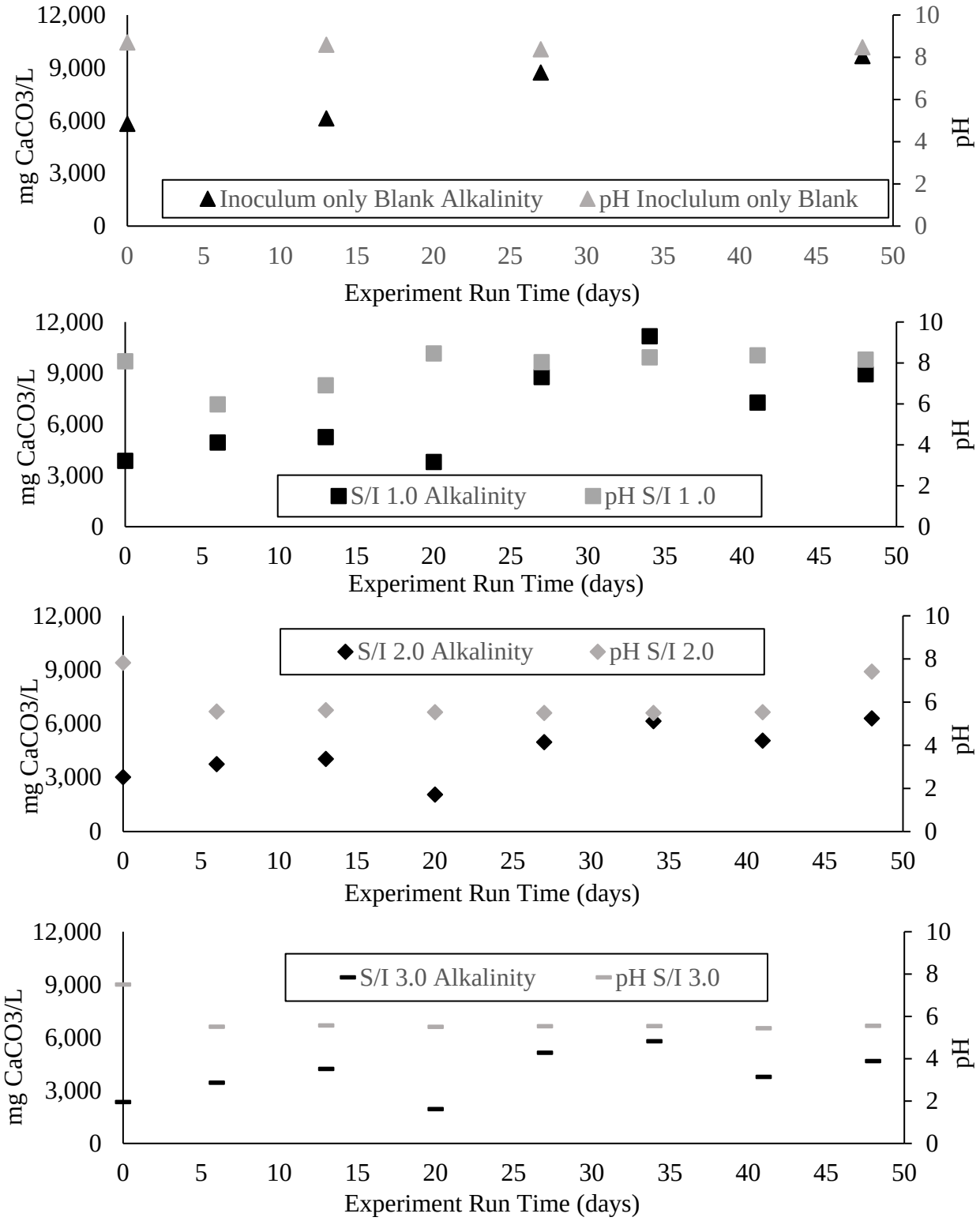

Figure 4 Different Mixture Alkalinity and pH Values versus Time for Different Substrate to Inoculum Ratios Based on TS.
(Methanogenic microbial inhibition occurs at pH < 6 (Guan et al., 2015))

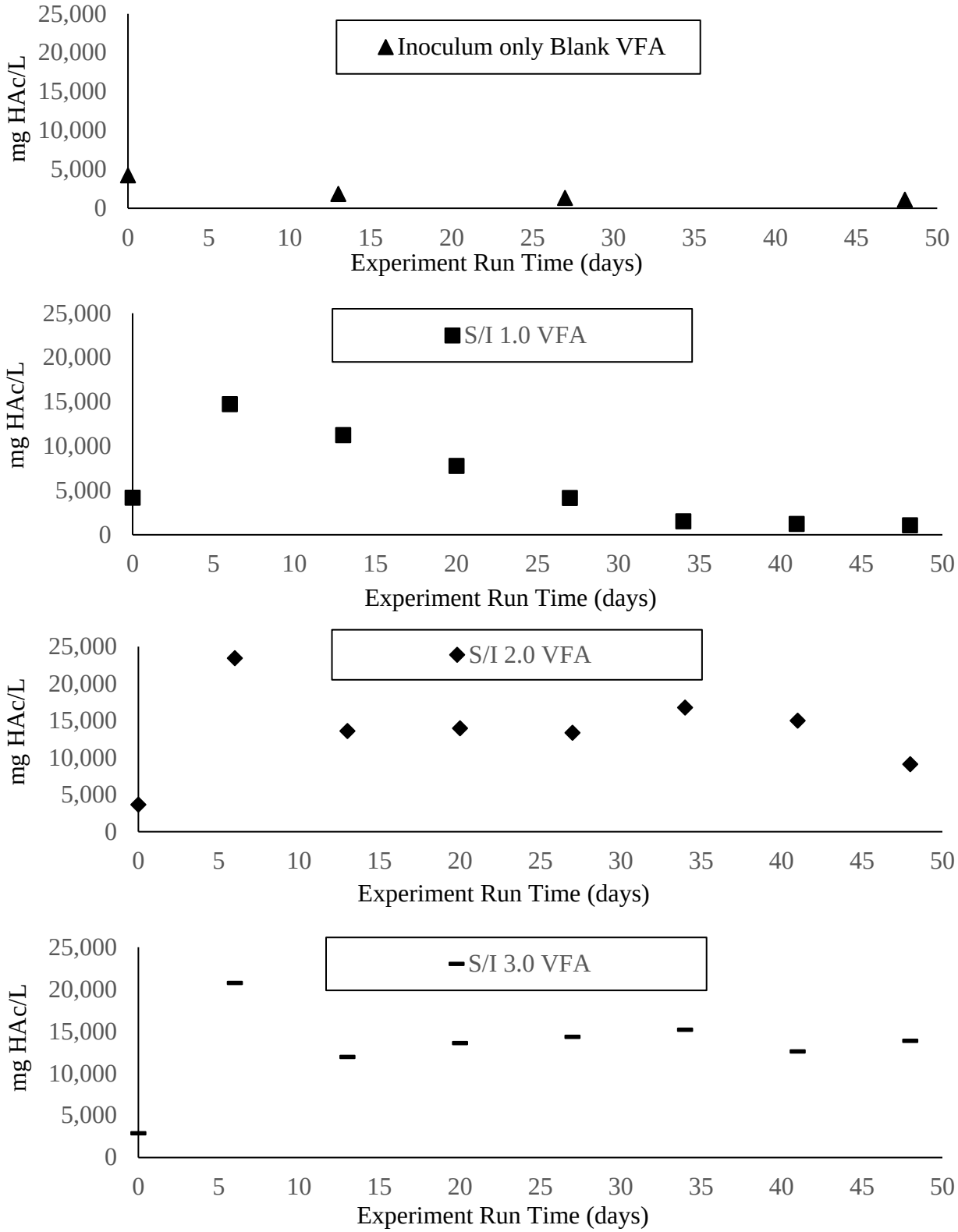

Figure 5 Different Mixture Volatile Fatty Acids versus Time for Different Substrate to Inoculum Ratios Based on TS.

In a balanced HS-AD system, all microorganisms will be functioning symbiotically. The primary metabolic pathways for these reactions are shown in Figure 1. From the results of the research completed for this thesis, the symbiotic relationships are shown in experimental results of the balanced systems that produced CH_4. Specifically, the results show that the alkalinity plotted with pH (Figure 4) and TAN concentrations (Figure 6) all increased with time, while the VFA concentrations initially increased and then decreased (Figure 5). Biochemically the alkalinity fluctuation can be explained by the fact that alkalinity is destroyed by VFA production and formed by methanogenic microorganisms while they are producing CH_4 (Demirel & Scherer, 2008). In addition, TAN is produced by the acidogenic microorganisms as they break down amino acids and proteins (Rajagopal et al., 2013). VFAs, sugars (products of the hydrolytic conversion of carbohydrates), and aromatics (secondary sources of COD) are fermented to acetate and dihydrogen by acetogenic microorganisms, which are consumed by methanogens to produce CH_4 (Ali Shah et al., 2014; Konhauser, 2009).

Figure 7 shows the weekly sacrificed reactor sCOD and VFA concentrations. The VFA concentrations were converted to COD units (mg COD/L) based on the theoretical COD of acetic acid for comparison purposes. The weekly chemical analysis measurements found that the VFAs converted into COD < mixture sCOD values. This indicates that soluble organic compounds other than VFAs, such as carbohydrates and proteins, are present in solution (Hinds et al., 2016). However, there was one occurrence when the VFA COD > mixture sCOD (day 7 S/I ratio of 2.0 based on TS), which may have occurred because the digestion process was not balanced at the start of the experiment and excess VFAs were being produced in the digestion process.

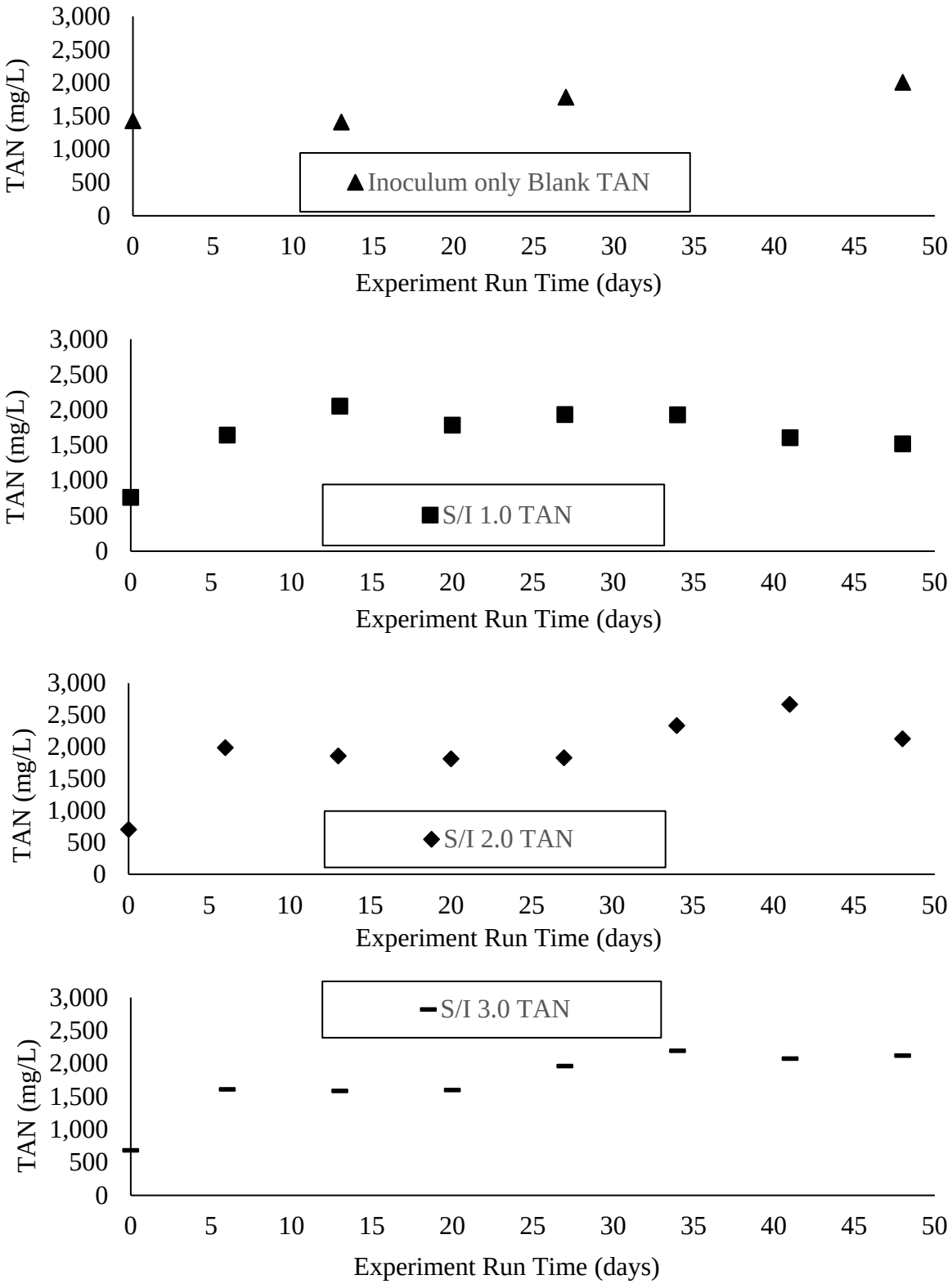

Figure 6 Different Mixture Total Ammonia Nitrogen versus Time for Different Substrate to Inoculum Ratios Based on TS.

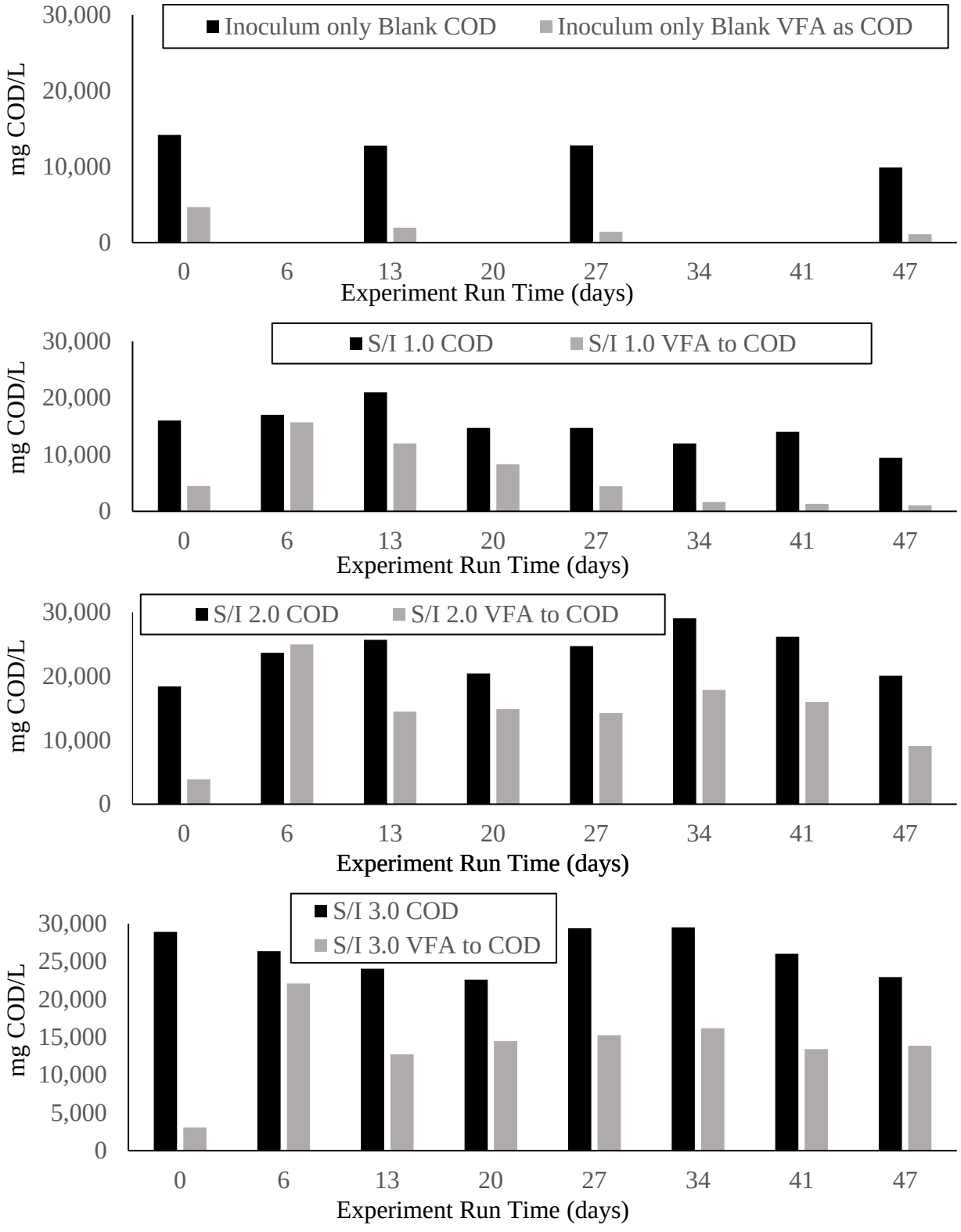

Figure 7 Mixture COD Values versus Time for Different Substrate to Inoculum Ratios Based on TS.

Figure 8 shows the VFA/alkalinity ratio versus time for the experimental BMPs. The Figure highlights that there is greater CH_4 production when the VFA/alkalinity ratio gets close to a value of 1.0. Brown and Li (2013) indicated that a VFA/alkalinity ratio < 0.4 should be maintained for optimum AD performance. However, the results from the current experiment showed that this VFA/alkalinity ratio may not be ideal for the digestion of food waste, yard waste, and municipal biosolids. This is because some of the reactors in this experiment performed well at VFA/alkalinity ratios > 0.4 (Figure 2 and 8).

Figure 8 also highlights when instability occurs within the system. Specifically, on day 6 and 20 of the experiment, the VFA/alkalinity ratios for the S/I ratios mixtures of 2.0 and 3.0 based on TS were found to be high (> 6.00) indicating instability (Lidholm & Ossiansson, 2008). In the case of day 6, the fluctuation occurred because the VFA concentrations in systems with S/I 2.0 and 3.0 significantly increased to > 20,000 mg acetic acid ($C_2H_4O_2$ or HAc/L) (Figure 5), while the alkalinity concentrations remained unchanged (Figure 4). In the case of day 20, the fluctuation occurred because there was a significant decrease in the systems' available alkalinity to approximately 2,000 mg $CaCO_3$/L in the both of the S/I 2.0 and 3.0's reactors (Figure 4).

In general, batch AD systems are run for approximately three weeks (Raposo et al., 2009; Zhou et al., 2011). This is done for efficiency because the greatest CH_4 production normally occurs during that period in balanced systems. However, unbalanced systems could remain in a lag phase where negligible amounts of CH_4 are being produced until the system is balanced (Adekunle & Okolie, 2015). This is what was observed for the S/I ratio of 2.0 based on TS prior to day 17 (Figure 2 and Figure 3).

The following is what was observed in the S/I ratio of 2.0 based on TS. The mixture began balancing itself as its TAN concentrations started to increase (around day 27) (Figure 6). Sterling

et al. (2001) noted that HS-AD system, pH and alkalinity increase with increasing NH_4^+ concentrations. As noted previously, oyster shells release alkalinity into solution after an extended period of time, which adds to the buffering capacity of the mixture (Waldbusser et al., 2011). Figure 4 shows that the alkalinity in the S/I 2.0 reactors begins to increase after day 20. Figure 2 highlights that around day 27 of the experiment the methanogens in the BMPs with S/I ratio of 2.0 based on TS were producing CH_4, which in turn increased the pH in the reactor as the VFA's were used (Appels et al., 2011). As a consequence, the effects of VFA and pH induced microbial inhibition were reduced (Guan et al., 2015; Khanal, 2011). The VFA readings in the reactor decreased on day 42, which indicates that they may have been used by the methanogens. On the last day of the experiment the VFA/alkalinity ratio for the S/I ratio of 2.0 based on TS mixture was approaching a balanced value (Brown & Li, 2013). In contrast, during the experimental time frame (49 days), the S/I ratio of 3.0 (based on TS) mixture was never able to overcome pH and VFA induced microbial inhibition to produce a significant amount of CH_4.

The statistical analysis used a t test to indicate that there was no significant difference between values at different points in time (Table B 3, 6, 8, and 10). The tests showed that the means of the inoculum only blank and S/I ratio of 1.0 based on TS mixtures were not significantly different from each other prior to day 19 of the experiment, implying that at day 19 of the experiment the means were statistically different from each other. These results confirm that the S/I ratio 1.0 based on TS on day 47 of the experiment produced the highest cumulative CH_4 production and yield. In addition, a t test between the inoculum only blank and the S/I 2.0 mixture showed that the means were not significantly different from each other. A t test between the S/I ratio of 2.0 and 1.0 based on TS revealed that the means were not significantly different from each other on the last day of the experiment. The statistical analysis showed that the S/I ratios of 3.0

and 2.0 based on TS were not significantly different from each other prior to day 17 of the experiment. Lastly, the t tests showed that there were no points where S/I 3.0 and the inoculum only blank mixtures as well as S/I ratio of 3.0 and 1.0 based on TS mixtures were found to not be significantly different from each other.

As stated above, Figure 3 shows the cumulative CH_4 yield. The CH_4 yields for the experimental mixtures show the same trends as the CH_4 production curves (Figure 2). The final yield for the experimental mixtures was 126, 45.6, and 6.75-mL CH_4/ (g VS_{added}) for the S/I ratio of 1.0, 2.0, and 3.0 based on TS mixtures, respectively. This finding is in good agreement with Lopes et al. (2004) who studied HS-AD with a S/I ratio of 5.7 with bovine rumen fluid inoculum, and Hobbs et al. (2017) who studied the CH_4 production for a range of relevant food waste S/I ratios.

At the end of week three of the experiment the CH_4 percentage values were found to be 67.3, 73.8, 31.0, and 27.0, for the inoculum only blank and S/I ratios of 1.0, 2.0, and 3.0 based on TS, respectively (values can be seen in B Table B 3, 5, 7 and 9). In addition, at the end of the experiment the CH_4 percentages were found to be 63.7, 66.5, 62.7, and 32.7 for the inoculum only blank, and S/I ratios of 1.0, 2.0, and 3.0 based on TS, respectively. This finding is in good agreement with Chen et al. (2014) who studied HS-AD for food waste and green waste, Duan et al. (2012) for HS-AD of municipal biosolids, and Heo et al. (2004) for the digestion of food waste and municipal biosolids. At the end of the experiment, the S/I ratio of 3.0 based on TS mixtures were not comparable to the results in these studies because of microbial inhibition factors still present.

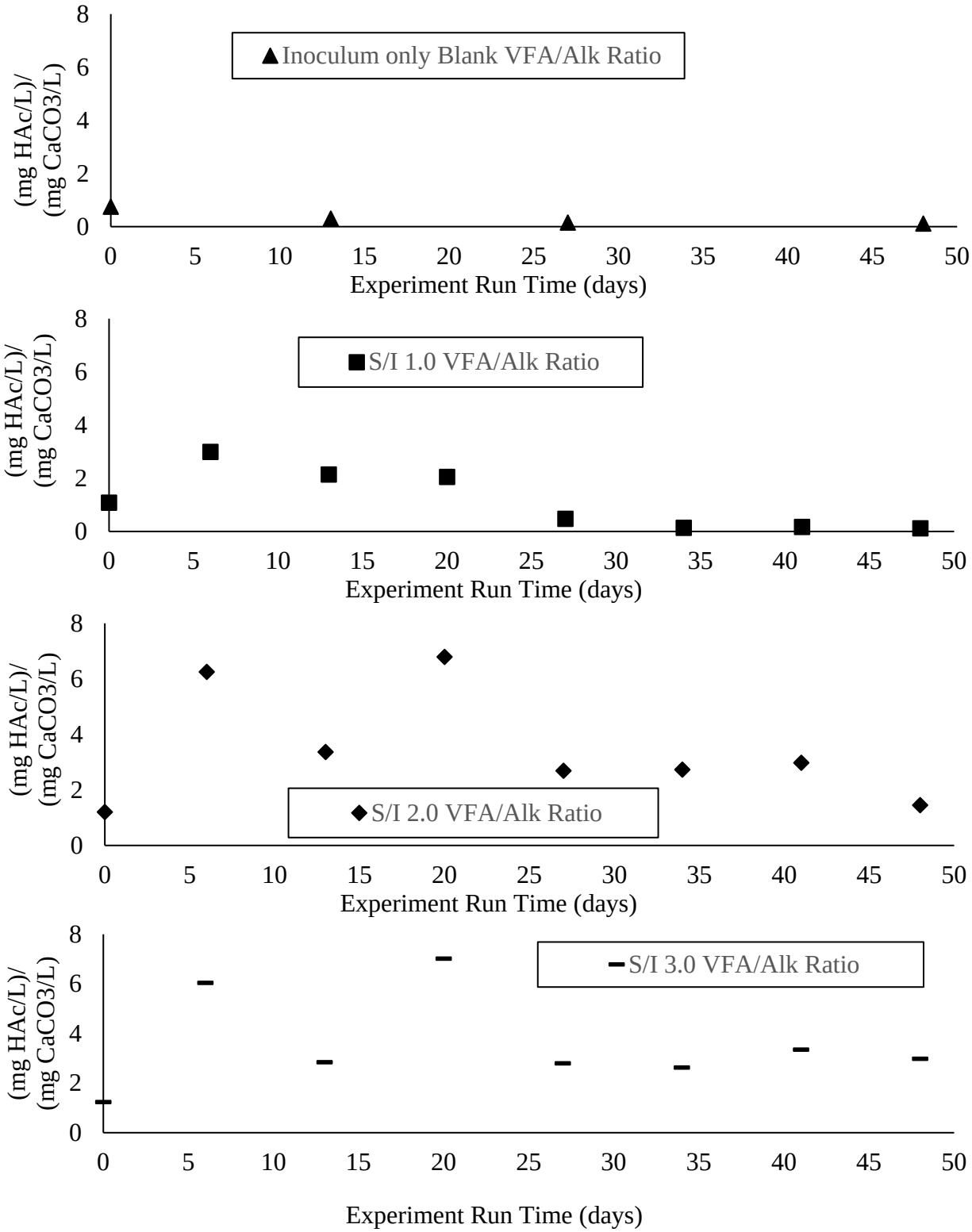

Figure 8 Volatile Fatty Acids to Alkalinity Ratio versus Time for Different Substrate to Inoculum Ratios Based on TS.
(Optimal anaerobic digestion performance ratio < 0.4 (Brown & Li, 2013))

4.2 Total and Volatile Solids Reduction

Figure 9 highlights the different experimental mixture TS and VS reductions with respect to time. The general trends shown for the BMP reactors are that TS and VS percentages decrease as the experiment progresses. This is because the organic materials are degraded to CH_4 and CO_2 in the HS-AD process (Abbassi-Guendouz et al., 2012; Adekunle & Okolie, 2015). The variability of the TS and VS readings can be explained by the fact that the measurements only represent the readings from the sacrificed reactor(s) for each chemical analysis (small sample size). In summary, all of these processes occurred as expected during this experiment (Amani et al., 2010; Rajagopal et al., 2013).

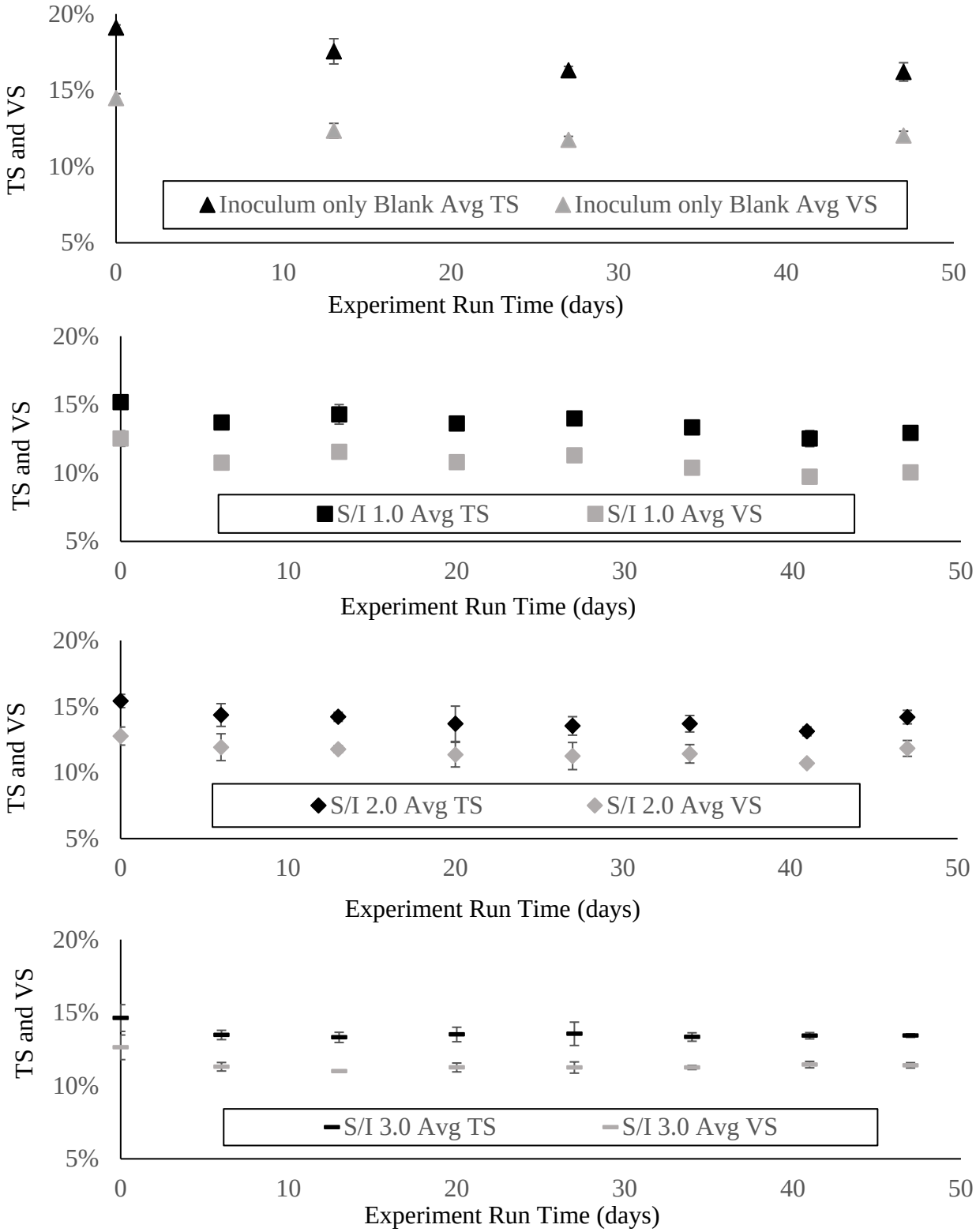

Figure 9 Different Mixture TS and VS Reduction versus Time for Different Substrate to Inoculum Ratios.
(error bars show one standard deviation from the mean value)

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

The objective of this HS-AD research was to investigate the effect of S/I ratio on physical, chemical, and biological mechanisms during HS-AD of food waste, yard waste, and municipal biosolids. The results indicate that the optimal ratio for the digestion of food waste, yard waste, and municipal biosolids was 1.0 based on TS. This ratio produced the greatest cumulative CH_4 volume of 2,320-mL and yield of 126-mL CH_4/ (g VS_{added}) while reducing TS and VS. The statistical analysis found the results of the S/I 1.0 mixture were significantly ($P < 0.05$) different from the inoculum only blank at the end of the experiment.

Within the area of U.S. MSW management, it is recommended that based on these results it might be financially feasible to construct more HS-AD facilities to process the organic fraction of the MSW waste stream. This will help to better manage the waste stream, decreasing its VS content, while producing CH_4 for energy generation and digestate that can be used for fertilizer, soil conditioning, and/or composting. In conclusion, this research highlights that a S/I ratio of 1.0 based on TS should be used to optimize the digestion process when processing the organic fraction of MSW, which includes food waste, yard waste, and municipal biosolids.

Future research could include the investigation of greater S/I ratios based on TS over longer lengths of time. This topic is of interest because it is important to understand what will occur if HS-AD digesters are given longer time frames to operate. In addition, research for the HS-AD of food waste, yard waste, and municipal biosolids could focus on different TS contents, S/S ratios, and the addition of different types of substrates. This investigation would help researchers to

understand other parameters that may influence the optimum S/I ratio based on TS or the greatest CH_4 yield and volume. Lastly, an important area for future research includes determining the effects different concentrations of solid phase alkalinity sources that may influence volatile solids reduction, CH_4 volume, and CH_4 yield.

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APPENDIX A: ABBREVIATIONS

Table A 1 List of Abbreviations.

Abbreviation	**Definition**
Avg	average
BMP	biochemical methane production reactor
CH_4	methane
C/N	carbon to nitrogen
COD	chemical oxygen demand
Conc	concentration
Cum	cumulative
DOF	degrees of freedom
HRT	hydraulic residence time
HS-AD	high solids anaerobic digestion
L-AD	liquid anaerobic digestion
MSW	municipal solid waste
rpm	revolutions per minute
sCOD	soluble chemical oxygen demand
S/I	substrate-to-inoculum
Sig	significantly
SRT	solid residence time
S/S	substrate-to-substrate
Stdv	standard deviation
TAN	total ammonia nitrogen
TS	total solids
VFA	volatile fatty acids
VS	volatile solids
WAS	waste activated sludge
WWTP	wastewater treatment plants
99% Conf	99% confident the means are not significantly different from each other

APPENDIX B: SUPPLEMENTAL MATERIALS

Table B 1 Food Waste Components.

Material	Mixture Composition (% wet weight)	Material Details
Vegetables	36.3	Spring mix green vegetables, russet potatoes with peel, yellow onion
Fruit	36.4	Naval orange peel, McIntosh apple center, del monte banana peel
Dairy products	5.5	Great value shredded mild cheddar cheese
Bread and bakery	6.6	L'oven fresh white bread
Meat	8.8	Bremer gravy & Salisbury streaks (beef), john soules chicken fajitas, Gorton's frozen grilled tilapia fish
Pasta & Rice	6.4	Barilla sausage & tomato rotini (pasta), 1-minute fragrant Thai jasmine rice

Table B 2 Yard Waste Components.

Material	Mixture Composition (% wet weight)	Material Details
Leaves	25	Sand live oak (Quercus geminata)
Pine needles	25	Long leaf pine (Pinus palustris)
Grass	25	Bermuda Grass from Kaytee (Chilton, WI)
Wood debris	25	Florida Select Natural Eucalyptus Mulch from Scotts (Palmdale, FL)

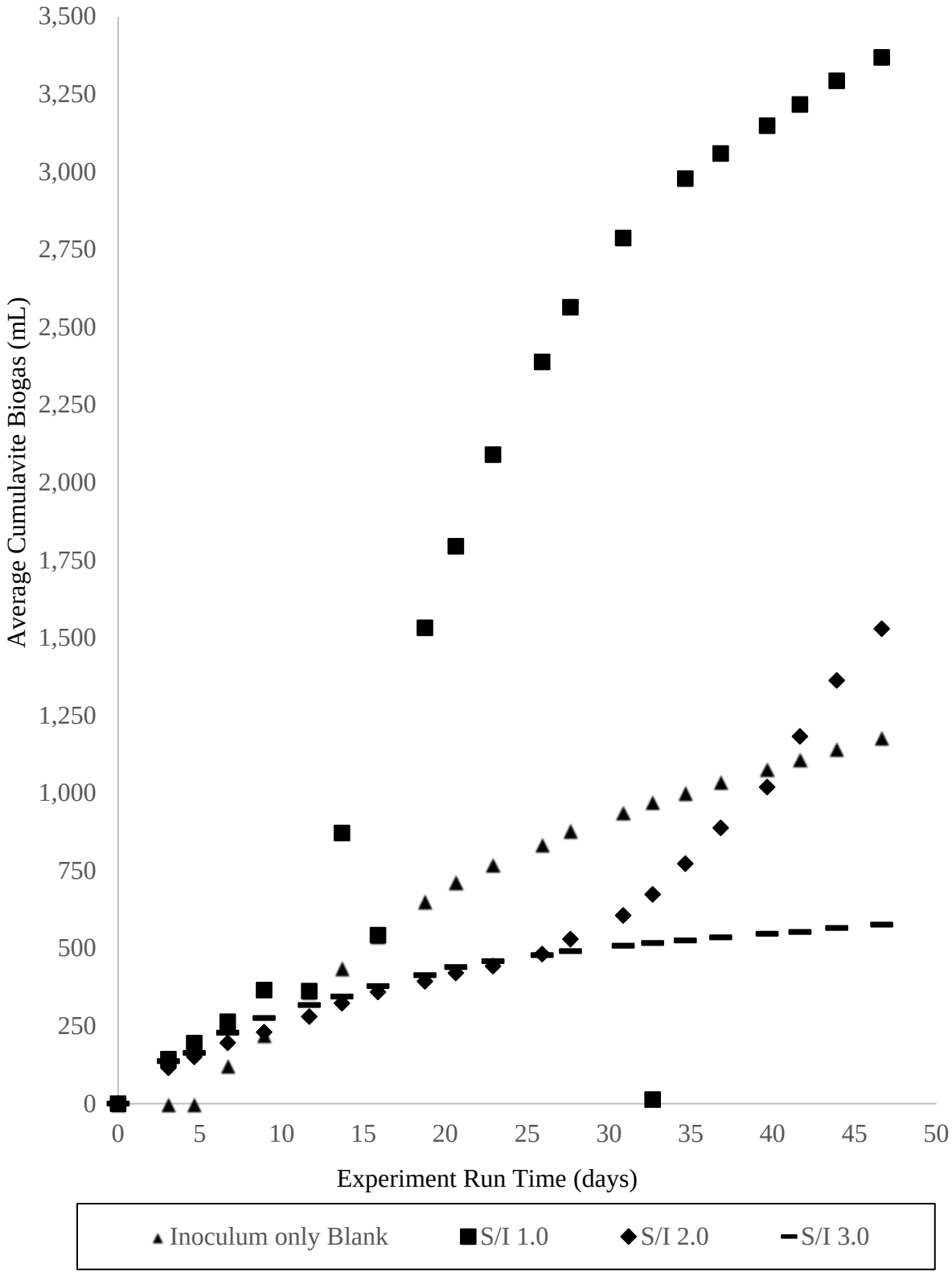

Figure B 1 Cumulative Biogas or Different Substrate to Inoculum Ratios.

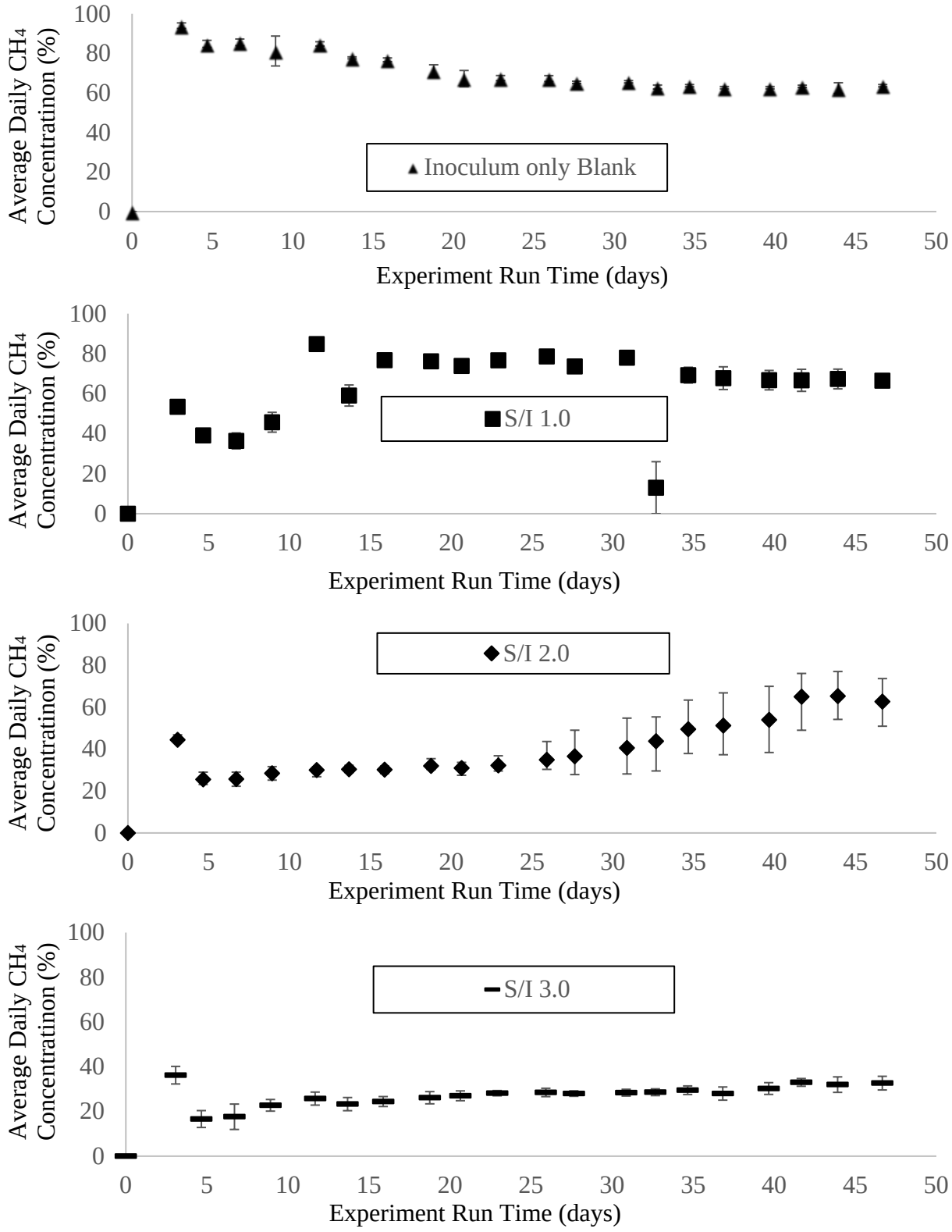

Figure B 2 CH_4 Content for Different Substrate to Inoculum Ratios over Time.
(error bars show ± one standard deviation from the mean value)

Table B 3 Inoculum only Blank Experimental Data.

Date	Sample Size	Avg Daily Biogas (mL)	Avg Cum Biogas (mL)	Avg Daily CH_4 Conc (%)	Stdv	Avg Cum CH_4 (mL)	Stdv	CH_4 Yield (CH_4 mL/ (g VS added))	Stdv
0	5	0.0	0.0	0.0	0.0	0.0	0.0		
3	5	0.0	0.0	94.0	1.4	0.0	0.0	0.0	0.0
5	5	0.0	0.0	84.8	1.8	0.0	0.0	0.0	0.0
7	5	124.1	124.1	85.6	1.7	106.4	13.8	7.6	1.0
9	5	100.3	224.4	81.2	7.6	81.4	9.7	13.4	1.6
12	5	138.1	362.5	84.8	1.1	117.2	11.4	21.7	2.0
14	5	76.5	439.0	77.8	0.5	59.5	15.7	25.7	3.1
16	5	103.7	542.7	76.8	1.0	79.7	24.5	31.4	4.5
19	5	111.0	653.7	71.3	3.0	79.2	8.7	37.0	4.9
21	5	62.0	715.8	67.3	4.1	41.7	4.2	40.0	5.1
23	4	56.3	772.1	67.5	1.3	38.0	12.8	42.7	6.0
26	4	64.3	836.3	67.3	1.5	43.2	7.5	45.8	6.5
28	4	44.9	881.2	65.3	0.6	29.3	1.5	50.8	3.7
31	4	59.1	940.3	65.7	0.6	38.8	0.9	53.6	3.7
33	4	32.8	973.1	63.0	1.0	20.7	0.8	55.0	3.7
35	4	30.4	1003.6	63.7	0.6	19.4	1.1	56.4	3.7
37	3	34.9	1038.4	62.7	0.6	21.8	1.0	58.0	3.7
40	3	41.1	1079.5	62.7	0.6	25.7	1.2	59.8	3.8
42	3	31.6	1111.1	63.3	0.6	20.0	0.9	61.2	3.8
44	3	33.4	1144.5	62.4	2.7	20.8	1.6	62.7	3.9
47	3	35.8	1180.3	63.7	0.6	22.8	1.6	64.3	4.0

Table B 4 Inoculum only Blank Statistical Analysis.

Date	Sample Size	t Test (S/I 1.0 Compared to Inoculum only Blank)	DOF	99% Conf	t Test (S/I 2.0 Compared to Inoculum only Blank)	DOF	99% Conf	t Test (S/I 3.0 Compared to Inoculum only Blank)	DOF	99% Conf
0	5									
3	5	5.69	8	3.355	16.34	8	3.355	5.65	8	3.36
5	5	6.70	8	3.355	21.12	8	3.355	5.94	8	3.36
7	5	0.84	11	3.106	-12.66	5	4.032	-3.77	11	3.11
9	5	-0.46	9	3.250	-38.73	4	4.604	-10.61	7	3.50
12	5	-0.08	8	3.355	-58.95	4	4.604	-20.30	6	3.71
14	5	0.90	8	3.355	-74.53	4	4.604	-23.48	5	4.03
16	5	1.79	7	3.499	-92.19	4	4.604	-26.80	5	4.03
19	5	3.22	7	3.499	-87.68	4	4.604	-29.70	5	4.03
21	5	6.04	9	3.250	-68.85	4	4.604	-29.75	5	4.03
23	4	7.78	8	3.355	-42.72	3	5.841	-29.02	3	5.84
26	4	9.19	8	3.355	-19.03	4	4.604	-30.67	3	5.48
28	4	10.49	6	3.707	-9.52	8	3.355	-36.52	4	4.60
31	4	13.60	5	4.032	-5.53	5	4.032	-37.69	4	4.60
33	4	15.58	6	3.707	-4.16	5	4.032	-38.93	4	4.60
35	4	16.03	6	4.604	-3.00	5	4.032	-36.18	4	4.60
37	3	16.61	5	4.032	-1.98	3	5.841	-32.85	3	5.84
40	3	18.18	5	4.032	-1.46	3	5.841	-34.04	3	5.48
42	3	16.19	4	4.604	-1.21	3	5.841	-29.02	3	5.48
44	3	15.45	3	5.841	-0.53	2	9.925	-27.69	3	5.48
47	3	15.78	3	5.841	-0.13	2	9.925	-28.24	3	5.48

Table B 5 S/I 1.0 Experimental Data.

Date	Sample Size	Avg Daily Biogas (mL)	Avg Cum Biogas (mL)	Avg Daily CH_4 Conc (%)	Stdv	Avg Cum CH_4 (mL)	Stdv	CH_4 Yield (CH_4 mL/ (g VS added))	Stdv
0	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
3	9	143.3	143.3	53.4	2.6	76.7	24.1	4.2	1.3
5	9	51.6	194.9	39.1	3.3	97.2	26.0	5.3	1.4
7	9	68.9	263.8	36.4	4.0	124.2	33.5	6.8	1.8
9	8	101.7	365.5	45.7	5.0	170.9	64.9	9.3	3.5
12	8	138.1	362.5	84.8	1.1	294.6	133.1	16.1	7.3
14	8	282.4	871.4	59.1	5.3	470.8	199.9	25.7	10.9
16	7	103.7	542.7	76.8	1.0	701.9	242.8	38.3	13.3
19	7	345.3	1532.2	76.1	2.9	965.7	232.9	52.7	12.7
21	7	262.4	1794.5	73.8	1.7	1132.0	167.2	61.8	9.1
23	6	295.3	2089.9	76.7	1.2	1330.9	155.3	72.7	8.5
26	6	298.4	2388.3	78.6	1.0	1565.5	163.8	85.5	8.9
28	6	176.2	2564.5	73.6	3.0	1726.8	158.3	94.3	8.6
31	5	222.8	2787.4	78.0	1.9	1900.9	132.2	103.8	7.2
33	5	13.0	13.0	13.0	13.0	1976.5	120.7	107.9	6.6
35	5	84.9	2978.6	69.3	3.9	2029.7	121.1	110.8	6.6
37	4	80.9	3059.5	67.8	5.7	2086.7	105.4	113.9	5.8
40	4	89.3	3148.8	66.8	4.9	2148.3	98.9	117.3	5.4
42	4	68.5	3217.3	66.7	5.5	2211.3	113.3	120.7	6.2
44	3	75.9	3293.3	67.3	4.9	2262.7	113.5	123.5	6.2
47	3	75.3	3368.6	66.5	2.6	2312.8	113.2	126.2	6.2

Table B 6 S/I 1.0 Statistical Analysis.

Date	Sample Size	t Test (S/I 2.0 Compared to S/I 1.0)	D O F	99% Conf	t Test (S/I 3.0 Compared to S/I 1.0)	D O F	99% Conf
0	9						
3	9	-9.49	9	3.25	-3.55	9	3.25
5	9	-15.22	9	3.25	-5.49	9	3.25
7	9	-21.87	8	3.36	-6.21	9	3.25
9	8	-37.04	7	3.50	-10.24	7	3.50
12	8	-65.15	7	3.50	-22.46	7	3.50
14	8	-119.37	7	3.50	-36.98	7	3.50
16	7	-180.40	6	3.71	-51.20	6	3.71
19	7	-203.71	6	3.71	-67.22	6	3.71
21	7	-180.38	6	3.71	-75.74	6	3.71
23	6	-129.59	5	4.03	-85.27	5	4.03
26	6	-64.17	6	3.71	-98.77	5	4.03
28	6	-34.02	7	3.50	-121.48	5	4.03
31	5	-20.43	8	3.36	-123.69	4	4.60
33	5	-16.22	7	3.50	-128.92	4	4.60
35	5	-12.74	6	3.71	-120.16	4	4.60
37	4	-9.55	4	4.60	-110.78	3	5.48
40	4	-8.10	4	4.60	-114.15	3	5.48
42	4	-9.04	4	4.60	-96.79	3	5.48
44	3	-6.24	3	5.48	-85.34	2	9.93
47	3	-4.69	2	9.93	-86.54	2	9.93

Table B 7 S/I 2.0 Experimental Data.

Date	Sample Size	Avg Daily Biogas (mL)	Avg Cum Biogas (mL)	Avg Daily CH_4 Conc (%)	Stdv	Avg Cum CH_4 (mL)	Stdv	CH_4 Yield (CH_4 mL/ (g VS added))	Stdv
0	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	9	115.6	115.6	44.4	2.4	51.4	5.6	2.4	0.3
5	9	34.9	150.5	25.6	3.5	60.4	5.1	2.8	0.2
7	9	45.5	196.0	25.8	3.3	72.4	4.9	3.4	0.2
9	8	34.3	230.4	28.5	3.2	82.2	4.8	3.9	0.2
12	8	50.0	280.4	30.0	1.5	97.1	6.2	4.6	0.3
14	8	43.1	323.4	30.4	1.9	111.1	6.0	5.2	0.3
16	7	36.1	359.5	30.2	1.7	122.0	6.0	5.7	0.3
19	7	34.3	393.8	32.0	3.5	133.1	7.6	6.3	0.4
21	7	26.9	420.7	31.0	2.7	142.5	10.5	5.7	2.6
23	6	21.9	442.6	32.3	4.6	150.1	16.5	7.0	0.8
26	6	39.3	481.9	34.9	8.7	166.7	39.2	7.8	1.8
28	6	47.7	529.6	36.6	12.4	194.0	79.4	9.1	3.7
31	5	76.2	605.8	40.6	14.2	234.0	131.1	11.0	6.2
33	5	68.1	673.9	43.8	11.6	270.1	168.9	12.7	7.9
35	5	98.8	772.7	49.5	13.9	356.4	215.3	16.7	10.1
37	4	115.2	887.9	51.3	15.6	425.2	255.3	20.0	12.0
40	4	131.2	1019.1	54.0	16.0	506.1	297.4	23.8	14.0
42	4	163.4	1182.5	65.0	11.1	731.8	256.3	34.4	12.0
44	3	180.2	1362.8	65.3	11.7	858.6	306.8	40.3	14.4
47	3	166.4	1529.1	62.7	11.0	970.8	392.1	45.6	18.4

Table B 8 S/I 2.0 Statistical Analysis.

Date	Sample Size	t Test (S/I 3.0 Compared to S/I 2.0)	DOF	99% Conf	Stdv
0	9				0.00
3	9	-0.18	10	3.17	2.40
5	9	-0.75	10	3.17	3.47
7	9	-0.65	9	3.25	3.28
9	8	-0.56	8	3.36	3.16
12	8	-0.99	9	3.25	1.51
14	8	-1.56	8	3.36	1.93
16	7	-1.58	7	3.50	1.73
19	7	-1.59	7	3.50	3.46
21	7	-1.58	8	3.36	2.68
23	6	-1.62	9	3.25	4.58
26	6	-2.32	9	3.25	8.73
28	6	-4.12	6	3.71	12.44
31	5	-6.23	4	4.60	14.21
33	5	-8.57	4	4.60	11.58
35	5	-11.20	4	4.60	13.92
37	4	-13.76	3	5.48	15.61
40	4	-18.13	3	5.48	15.98
42	4	-20.24	3	5.48	11.14
44	3	-22.39	2	9.93	11.72
47	3	-26.56	2	9.93	11.02

Table B 9 S/I 3.0 Experimental Data and Statistical Analysis.

Day	Sample Size	Avg Daily Biogas (mL)	Avg Cum Biogas (mL)	Avg Daily CH_4 Conc (%)	Stdv	Avg Cum CH_4 (mL)	Stdv	CH_4 Yield (CH_4 mL/ (g VS added))	Stdv
0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	9.0	136.8	136.8	36.2	3.9	50.1	15.9	2.3	0.7
5	9.0	26.5	163.3	16.6	3.8	54.6	16.5	2.5	0.7
7	9.0	64.9	228.2	17.6	5.7	65.7	19.1	3.0	0.9
9	8.0	47.2	275.4	22.8	2.6	76.3	18.4	3.5	0.8
12	8.0	41.8	317.2	25.8	2.9	87.0	18.8	3.9	0.9
14	8.0	27.2	344.4	23.3	2.9	91.0	20.2	4.1	0.9
16	7.0	33.6	378.0	24.4	2.2	99.4	21.8	4.5	1.0
19	7.0	35.3	413.3	26.1	2.7	108.7	23.7	4.9	1.1
21	7.0	26.0	439.3	27.0	2.2	117.7	25.6	4.6	2.3
23	6.0	19.2	458.5	28.2	1.2	123.1	25.6	5.6	1.2
26	6.0	19.5	478.0	28.5	1.9	128.7	26.1	5.8	1.2
28	6.0	12.6	490.6	28.0	1.2	125.8	23.0	5.7	1.0
31	5.0	17.4	508.0	28.4	1.5	130.8	22.8	5.9	1.0
33	5.0	8.7	516.7	28.6	1.5	133.3	22.8	6.0	1.0
35	5.0	8.6	525.3	29.5	1.9	133.1	25.2	6.0	1.1
37	4.0	10.0	535.3	28.0	2.9	136.0	25.1	6.2	1.1
40	4.0	11.3	546.6	30.3	2.6	139.4	25.1	6.3	1.1
42	4.0	5.9	552.5	33.0	1.7	141.1	30.3	6.4	1.4
44	3.0	13.0	565.5	32.0	3.5	145.4	30.4	6.6	1.4
47	3.0	10.6	576.1	32.7	3.1	149.0	30.6	6.7	1.4