1 Semi-continuous mesophilic anaerobic digester performance
2 under variations in solids retention time and feeding frequency

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Abstract

The goal of this research was to understand the effect of solids retention time (SRT) and feeding frequency on the performance of anaerobic digesters used to recover bioenergy from swine waste. Semi-continuous mesophilic anaerobic digesters were operated at varying SRTs and feeding frequencies. Performance metrics included biogas and methane production rates, biomass robustness and functionality and removals of volatile solids, soluble chemical oxygen demand, the fecal-indicator bacteria *E. coli*, and the human pathogen *Salmonella*. Biochemical methane formation potential and specific methanogenic activity assays were used to demonstrate biomass robustness and functionality. Results indicated that anaerobic digesters fed weekly had higher average methane yields (0.20 vs. 0.18 m³ CH₄/kg VS_{added}), specific methanogenic activities (40 vs. 35 ml/day), and fecal indicator bacteria destruction (99.9% vs. 99.4%) than those fed every-other day. *Salmonella*, soluble COD, and VS destruction did not change with varied feeding frequency; however, higher removals were observed with longer SRT.

Key Words: Anaerobic Digester Operations; Bioenergy Recovery; International Sustainable Development; Greenhouse Gas, Methanogenic Activity; Pathogen Removal; Swine Waste

1.0 Introduction

As household-scale anaerobic digestion is being actively promoted in developing countries, research is needed to better understand the performance of these systems and their potential impact on environmental health. Household scale anaerobic digestion has been promoted for several decades to provide renewable energy production in rural areas of China, India, Sub-Saharan Africa and the Americas (Perez et al., 2014). The most widely used household systems are fixed dome, floating drum, and plastic tubular digesters. These are

considered to be semi-continuous reactors because substrate addition occurs on an interval that is intermittent. They are used to treat agricultural residues (e.g. animal manure, crop residuals) and sometimes human wastewater. Benefits of household anaerobic digestion can include access to improved sanitation, decreased demand for woody biomass for heating and cooking, and production of an effluent that can be used as a soil amendment (Rowse, 2012). Globally, an estimated 35 million household-scale (3 to 10 m³) fixed-dome digesters are currently in use (Bruun et al., 2014), while China plans to install up to 80 million digesters by 2020 (NDC, 2007). Since many household-scale as well as large-scale anaerobic digesters are operated in a semi-continuous manner, more research is needed to understand how traditional operating parameters affect the performance of these systems.

A large number of prior studies on anaerobic digestion performance have mainly been focused on optimizing biochemical methane production and volatile solids (VS) destruction through manipulations in operating strategies in anaerobic digesters operated as continuously mixed flow reactors (CMFRs) (e.g., Lee et al., 2011; Chen et al., 2012). However limited knowledge exists pertaining to the performance of semi-continuous and/or unmixed systems. Lee et al. (2011) showed that removal efficiencies for chemical oxygen demand (COD) and VS in bench-scale CMFR mesophilic anaerobic digesters decreased with a reduction in solids retention time (SRT) from 20 to 4 days. That study also reported that a reduction in SRT caused significant shifts in the microbial population as bacteria and archaea present became less diverse. This observation infers that the physiochemical characteristics of the biomass change with variations in SRT in CMFRs. This is hypothesized to influence the biochemical performance of the system when coupled with longer or shorter feeding frequencies such as those found in semi-continuous systems. A comparison between continuous influent additions and semi-continuous

additions in anaerobic bioreactors was conducted by Li et al. (2014a) using chicken manure and corn stover. They compared the mesophilic anaerobic co-digestion in semi-continuous and continuous systems and found that 25% more methane was produced in the semi-continuous configuration, 281 ml/g-VS_{added} compared to 223 ml/g-VS_{added} when the influent was added constantly. Their results indicate a biochemical advantage to feeding the system less often without changing the organic loading rate. Other studies have evaluated continuous against semi-continuous mesophilic anaerobic digestion under varying organic loading rates. These studies concluded that biochemical methane production was slightly better in the semi-continuous systems, and also increased in both types as the organic loading rate increased up to 6 kg/m³/day, after which methanogenesis inhibition was likely due to volatile fatty acid accumulation that occurred (Escudero et al., 2014; Li et al., 2014b). Wang et al. (2014) presented a feeding strategy that alternated the substrate composition (i.e. food waste, chicken manure) of a semi-continuous mixed mesophilic anaerobic digester, and found that biogas production was improved when food waste was fed more often than manure. This finding has a useful application to anaerobic digester use at the household scale because of the blend of domestic and agricultural wastes that are commonly found there; however the effect of SRT or the length of time between feedings (i.e., feeding frequency) on system performance was not investigated, as was true for the other publications reviewed.

The performance of an anaerobic system can also be measured in terms of pathogen removal instead of biochemical methane production. As demonstrated by recent investigations (Chen et al. 2012; Huong et al., 2014; Manser et al., 2015), the survival of indicator bacteria and human pathogens during mesophilic anaerobic digestion is common. The US Environmental Protection Agency recommends that sludge digested anaerobically under mesophilic

temperatures should be kept out of human and animal contact for at least one year after digestion (USEPA, 2000). Further investigation is needed to find operational strategies for household scale anaerobic systems that enhance pathogen removal because the biosolids are typically promoted for immediate land application. Recalling that changes to the SRT of continuous anaerobic systems may cause shifts in the microbial populations (Lee et al., 2011) through different methane production values, it is important to investigate if this finding holds true for other microorganisms, such as *E. coli* or *Salmonella*, in semi-continuous systems because of the threat to public health that mesophilic sludge can present. Furthermore, the yet undefined influence of varying feeding frequencies on the semi-continuous system could be used to enhance pathogen removal and warrants investigation.

The objective of this research was to investigate how variations in SRT and feeding frequency both influence biochemical processes of mesophilic anaerobic digesters operated semi-continuously in an unmixed reactor. This configuration was selected to mimic how millions of anaerobic household digesters are operated in the field. Performance metrics used in this study include: the biochemical methane formation potential assay (BMP), the specific methanogenic activity assay (SMA), biogas production and methane content, VS removal, soluble chemical oxygen demand (sCOD) removal, *E. coli* removal, and *Salmonella* removal. To our knowledge this is the first study to also investigate the fate of indicator bacteria and human pathogens during semi-continuous anaerobic digestion under different feeding frequencies under conditions that would be encountered in the field at millions of existing or planned household systems.

2.0 Methods

2.1 Anaerobic Digester Set-up

A pilot-scale (21 L) semi-continuous anaerobic digester that was operated for more than one year with an influent swine waste feed and an SRT of 21 days prior to the beginning of this experiment. The pilot-scale digester was initially inoculated with seed sludge from an anaerobic digester treating food waste in the laboratory of Dr. Ann Wilkie in the Department of Soil and Water Science at the University of Florida in Gainesville, FL (Kinyua et al., 2014). The inoculum biomass for the small-scale experimental digesters used in this study was harvested from the pilot-scale system. Influent for the experimental systems consisted of 280 grams of fresh manure (Twenty-Four Rivers Family Farm, Plant City, Florida) and 1,500 ml of local groundwater (University of South Florida Botanical Gardens).

As described by Manser et al. (2015), 900-ml of the inoculum biomass from the pilot-scale system was transferred into six unique one-liter glass bottles to represent bench-scale anaerobic digesters. These were labeled as E15, E30, E45, W15, W30, and W45). The label identification represents the feeding frequency (E – every other day; W – weekly) and the average SRT (15, 30 or 45 days). The six experimental digesters were consistently fed the same swine manure slurry, as described previously, throughout the start-up, operational and experimental phases. However, it should be noted that due to daily variations in the manure source some variations in the feed composition were observed during the study. The detailed values reported in Tables 1 (influent) and 2 (effluent) are averages of at least 10 weeks of measurements. Urea was also added to simulate the presence of urine and to maintain the total nitrogen in the system to 1,000 mg/L. Each digester was operated at 35°C with no mixing, which is similar to a previous bench-scale experiment optimizing digestion of swine manure (Kinyua et al. 2014). The top of each reactor was fitted with a three piece air-lock (Northern Brewer #7010) to maintain anaerobic conditions while allowing biogas to escape; the lock was removed quickly

only to perform sampling, feeding, and wasting. The exception to this was during biogas production measurements when the biogas was routed to a wet-tip gas meter, as described in Section 2.3.

2.2 Analytical Methods

Anaerobic digester performance was measured by collecting 50-ml grab samples during the feeding (influent) and wasting (effluent) exchange steps, with the exception of digester E45 as the influent/effluent volume was limited to 40 ml for this system. Samples were collected every other Monday for 10 months. The grab samples were divided, with one sample used to measure total VS (ASTM 2540E). The other sample was centrifuged at 10,000 rpm (10 rcf) for 10 minutes. The supernatant was analyzed colorimetrically for the following: sCOD by oxidization by potassium dichromate (Hach TNT825), volatile fatty acid (VFA) by diol reaction and reduction with Fe^{3+} (Hach TNT872) and reported as mg HOAc/L, and ammonia by reaction with ions of hypochlorite and salicylate in sodium nitroprusside to form indophenol blue (Hach TNT832).

2.3 Biogas Quality and Quantity

The volume (ml) of biogas produced was measured at 35°C using a Wet Tip gas meter (Wayne, PA) over 21 days for each digester and then normalized by the amount of substrate (VS) added to the digester. The methane fraction of the biogas was measured by dissolving the carbon dioxide portion of a 10-ml of biogas sample into 1% v/v potassium hydroxide solution and measuring the displaced liquid volume (ASTM D1827). The methane produced for each digester was determined from the average methane concentration of the biogas and the amount of biogas produced during the twenty-one day measurement period. The calculated volume was then corrected to STP conditions by a factor of 1.12, assuming constant pressure.

2.4 Indicator Organism Assays

The densities of *E. coli* and *Salmonella* were measured by collecting 50-ml grab samples (40-ml from digester E45) during random feeding (influent) and wasting (effluent) maintenance steps. Samples from the influent and effluent were collected on three separate occasions at least ten days apart. Samples for *E. coli* analysis were immediately processed according to the IDEXX Colilert system (Westbrook, ME) manufacturer instructions. *Salmonella* samples were maintained at 4°C during transportation to EMSL Analytical Laboratories in Orlando, FL, where the samples were processed according to *Standard Method 9260B*.

2.5 BMP and SMA Assays

After eight months of operation BMP and SMA assays were conducted. The BMP assay was used to assess the robustness of the biomass from each digester. The assay was performed according to the method of Lisboa and Lansing (2013), with the exception that no additional nutrients were added because swine manure has been shown to contain sufficient nutrients for the test (Garcia et al., 2010). Triplicate 150-ml glass septum sealed bottles were used for the assay. For each bottle, 40 ml of biomass (approximately 30 g/L) was extracted from each digester and centrifuged at 10,000 rpm (10 rcf) for 10 minutes. The supernatant was discarded and the centrate was suspended in a phosphate buffered saline solution in sufficient volume to reach 40 ml. This suspension was added to a bottle containing 60 ml of sterilized substrate (4 g/L glucose), capped and placed on a shaking table (85 rpm) at 35°C for 14 days. The volume of biogas produced was measured daily by using a gas-tight 50-ml glass syringe. The methane content of the biogas was measured three times during the 14 days as described previously. The SMA assay was used to assess the functionality of the biomass. The experimental set-up and procedure for the SMA assay was identical to the BMP assay with one exception, the quantity

and quality of the biogas generated was only measured for the first 72 hours (3 days versus 14) of the experiment. This method was similar to that presented by Jimenez et al. (2015).

2.7 Statistical Methods

The one-way normal analysis of means (ANOM) procedure in Minitab 16 was used to compare anaerobic digester operation parameters and pathogen removal within a 95% confidence interval. To complete the analysis, groupings were created for the respective parameters based upon: average SRT, feeding frequency and individual digesters. For example, statistical differences resulting from variations to feeding frequency were detected by grouping digesters E15, E30 and E45 and comparing the results with the group W15, W30, and W45.

3.0 Results and Discussion

3.1 Digester Performance

The six anaerobic digesters in this study were monitored for the quantity of biogas produced (Figure 1) and methane content (Table 2) during the experiment. After multiplying the quantity by the methane content, the value was corrected to STP and normalized by the organic loading rate to obtain a methane yield (Figure 1). Methane yields ranged from 0.15 to 0.23 m³ CH₄/kg-VS_{added}, which were similar to values reported by Kinyua et al. (2014) while semi-continuously digesting swine manure at 35°C in reactors fed three times per week at SRTs ranging from 15 to 42 days. The best performances in that study were observed at SRTs of 21 and 28 days, which supports our observations that an SRT of 30 days was more productive than 15 or 45 days. Digester W30 had the highest methane yield (0.23 m³ CH₄/kg-VS_{added}), while digester E45 had the lowest (0.15 m³ CH₄/kg-VS_{added}). When grouped by SRT, the 15 and 30 day digesters had a higher methane yield than the 45 day groups. When grouped by feeding frequency, the digesters fed weekly produced 10% more biogas than those fed every other day

(0.20 compared with 0.18 m³ CH₄/kg-VS_{added}). Methane contents for the six digesters are shown in Table 2. The average value of 60% +/- 1.2% falls into the range reported in a review of continuous anaerobic digestion (Amani et. al. 2010). Methane content of the biogas increased with increasing SRT; however no difference in methane content was observed with variation in the feeding frequency. Our results suggest that a semi-continuous anaerobic digester that is fed with a longer interval between feedings is capable of producing more methane. If a longer feeding frequency is combined with an SRT of 30 days this effect is enhanced. Therefore this research suggests that household scale digesters should be fed less frequently. Note that the ability of farmers to operate digesters at longer feeding frequencies might be limited by such factors as the frequency of cleaning livestock pens, availability of substrate storage tanks, veterinary health considerations and labor availability.

VS removal among the six digesters was not significantly different (Table 2), with the exception of E15, which had the lowest removal of the group (38%) compared with the average (44%) and best (47%) of all six digesters. The ANOM statistical procedure indicated that digesters with a higher SRT (E45 and W45) had higher VS removal compared to the average VS removal of the other digesters (45% compared to 41%). A higher VS removal associated with higher SRT was expected because a longer retention time provides more opportunity for volatile compounds to be degraded by the microbial community. In a similar experiment investigating the effect of SRT on the bioavailability of organic carbon in anaerobically digested swine waste, VS removals improved slightly (50 to 52%) when the SRT of the digester was increased from 14 to 28 days (Kinyua et al, 2014). No effect on VS removal was observed between the digesters fed every other day or weekly. Considering that the digesters fed weekly produced more biogas than those fed every other day, it is interesting that the VS removal in the weekly fed systems

was not better. The average effluent sCOD concentration in the six digester effluents was 2,160 mg/L, which corresponds to an average removal of 59% (from Table 1, influent sCOD was 5,290 mg/L). The highest sCOD removal was observed in digesters E45 (62%) and W45 (61%) compared to the lowest removal in digester E15 (56%). Similar to VS removal, removal of sCOD improved with a longer SRT; however, variations in the feeding frequency did not affect sCOD removal (59% for groups E and W). Kinyua et al. (2014) also observed better sCOD removal with a longer SRT, 71% removal at a 14 day SRT up to 75% removal at 42 days in a semi-continuous operating scheme. The one-way normal ANOM procedure verified that there were no significant differences in the sCOD removal among the combinations of SRT or feeding frequency. In terms of practical implementation at the household scale, recovering resources from the sCOD and VS should be optimized by designing for a digester volume that can support an SRT of 30 to 45 days.

3.2 Biochemical Methane Formation Potential Assay

The BMP assay was used to measure the biomass robustness of the six digesters. Six different methane production trends were observed over the fourteen day experiment (Figure 1). Digester E45 had the greatest methane production (320 ml CH₄), while digesters E30 and W15 produced at least 33% less methane (both < 215 ml CH₄). Digester E30 and W15 also stopped producing biogas after eight days in the limited substrate environment compared to digester E45. This reduction is demonstrated by the slope of the BMP curve for each digester on days 10 through 14, 10.4 ml CH₄/day in the gas productive digester E45 compared to 2.2 ml CH₄/day in digester W15. Because the amount of available food is fixed, the biomasses that were able to produce biogas consistently during the test period can be considered to be more robust because they were able to adapt and thrive in the new environment more readily than the other biomasses

(Elbeshbishy et al., 2012). Molecular qPCR studies carried out by St. Pierre et al. (2013) showed that an increase in SRT from 21 to 30 days resulted in a more diverse methanogenic population. Our observations also suggest that anaerobic digesters with a longer time between feeding (i.e., longer feeding frequency) produce methane faster than those fed more often, implying the presence of a robust and diverse microbial population. This finding provides support for operating a household digester with a longer interval between substrate additions to select for a biomass that is capable of withstanding process changes and interruptions.

3.3 Specific Methanogenic Activity Assay

The SMA assay was used to observe the functionality of the biomass, which was defined in this study as the ability of the biomass to adapt or transition to a new process environment without sacrificing biogas production (Sorenson and Ahring, 1993). The SMA assay used the amount of biogas (or methane) produced during the first three days of the BMP assay and is represented by the slope of a line from day zero to day three in Figure 2. Figure 3A presents the results of that analysis and shows that digester W30 had the highest SMA (42 ml CH₄/day) and that digester E45 had the lowest (33 ml CH₄/day) in the limited substrate environment. This result is interesting because the biomass from digester E45 was the most robust in terms of surviving in the limited substrate environment used in the assay, but it was the slowest to acclimate to the limited substrate environment. This implies that a combination of shorter intervals between feedings and a longer SRT will promote a more reliable anaerobic digestion biomass that is resistant to system shock. Reliability is important at the house-hold scale because resources to re-establish a functioning anaerobic biomass may be limited. Figure 3B shows that the digesters that were fed less frequently had significantly higher SMA assay scores (40 ml CH₄/day) when compared to the more frequently fed systems (35 ml CH₄/day). One reason for

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4 261 this observation is because the longer cycled biomass is accustomed to periods of starvation so
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6 262 the balance of microbial activity favors the production of storage polymers instead of immediate
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9 263 biogas production (Hwang et al., 2010). This makes it possible for the biomass from these
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16 266 stability can be improved by feeding the digester less frequently.
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19 267 3.4 *E. coli* and *Salmonella* Removal

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21 268 It is important to understand how indicator bacteria and human pathogens interact with an
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24 269 anaerobic microbial population that has adapted to a variable feeding environment because these
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26 270 microorganisms, and other human pathogens present in livestock waste, have been shown to
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29 271 survive the mesophilic anaerobic digestion process (Johnson, et al., 1998; Chen et al., 2012;
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31 272 Manser et al., 2015). The removal of *E. coli* was measured in the six digesters. The fecal
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34 273 indicator bacteria *E. coli* was selected because it sometimes can be pathogenic to humans and
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36 274 allows for comparison with previously published research. Measured concentrations of *E. coli*
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38 275 (MPN/g biosolids) in the influent and effluent of the six digesters are shown in Figure 4A. A
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41 276 removal of $\geq 2\text{-log}_{10}$ reduction in the *E. coli* was observed in all digesters, digester W30 nearly
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43 277 achieved a 3-log_{10} reduction (99.8%) and digester E45 barely reached the 2-log_{10} (99.1%) level.
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46 278 Similar results were observed by Smith et al. (2005), who observed a 2-log_{10} reduction in *E. coli*
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48 279 after 20 days at 30°C during the mesophilic digestion of municipal sludge. As shown in Table 2,
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51 280 the concentrations of total ammonia nitrogen did not exceed 1,000 mg/L ($\text{NH}_3\text{-N}$), which when
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53 281 coupled with the observed temperature (35°C) and pH of the systems (average of 7.26) does not
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55 282 allow for the formation of free ammonia and limits the level of *E. coli* removal that can be
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58 283 achieved. Vinnerås et al. (2008) reported an inactivation threshold concentration of ammonia for
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indicator bacteria to be approximately 40 mM free ammonia (e.g. 2,100 mg/L NH₃-N and pH 8.9 at 24 °C). The volatile fatty acid concentrations reported in Table 2 also remained below lethal levels for many microorganisms, including fermentative bacteria such as *E. coli* (3,600 mg/L as acetate; Wolin, 1969) and methanogenic bacteria (10,000 mg/L as acetate, Amani et al., 2010). The performance results in Table 2 are useful because they show that the experimental digesters were functioning well below inhibitory levels for methanogenesis despite the lack of disinfection occurring within them. This exemplifies a common trade-off that must be made when mesophilic anaerobic digesters are used at the household scale, especially when fed with substrates containing human pathogens.

Using a 95% confidence interval, the one-way normal ANOM procedure showed that when grouped by feeding frequency, there was greater removal of *E. coli* in the digesters fed weekly (99.7%) compared to the digesters fed every-other-day (99.4%). This result is shown in Figure 4B and was verified by single factor ANOVA (95% confidence interval) where the F_{value} (6.1) exceeded the F_{critical} of 4.5 ($\alpha = 0.05$, $p = 0.02$). *E. coli* serves as an indicator bacteria for many other human pathogens that survive the digestion process. Based upon this finding, it may be possible to operate household digesters with a longer interval between feedings to encourage pathogen destruction.

The different *E.coli* removals could be due to the effect of the indigenous microbial community structure of each digester, which was adapted to thrive in the anaerobic environment though mechanisms of predation and competition. Stolpovsky et al. (2011) modeled microbial population shifts of two competing species exposed to periodic feeding and used the model to infer that microorganisms with an effective dormancy-reactivation strategy developed through long periods between feeding cycles may have a competitive advantage over microorganisms

that have not acclimated to the starvation period. That is, organisms that can switch rapidly in response to fluctuations in external conditions may outcompete fast-growing organisms. The higher nutrient (N and P) level of the swine manure influent may also increase the activity of native bacterial predators such as ciliated protozoa (Garcia et al., 2010). Protozoa have been shown to prey upon *E. coli* in activated sludge systems (Curds and Fey, 1969); a higher number of protozoa also have been correlated with higher biogas production during anaerobic wastewater treatment (Pyria et al., 2007). Our results show that digesters with higher biogas production had better *E. coli* removal. Digester W30 produced more biogas (715-ml) and had significantly higher *E. coli* removal (99.9%) compared to digester E45 (472-ml, 99.3%). Referring to Figures 3B and 4B, it can be seen that a longer interval between feedings promoted a higher SMA assay score and better removal of *E. coli*. Our observation supports the results of Choi et al. (2009) who found that the increased activity of the native microbial population (in our case measured by SMA assay) is capable of outcompeting other microorganisms like *E. coli* in anaerobic wastewater treatment systems. This finding is important for the operation and performance of household anaerobic digesters because effluents may be used to fertilize food crops.

Measured concentrations of *Salmonella* (MPN/4g biosolids) in the influent and effluent of the six digesters are shown in Figure 5A. The range of influent *Salmonella* concentrations was highly variable; however, consistent removal was achieved by the six digesters with an average removal of 86%. Although not significant, removal was better when the SRT and feeding frequency of the digesters were increased simultaneously. These results are reasonable, as 0.5 to 4-log₁₀ *Salmonella* destruction during mesophilic anaerobic digestion has been observed previously (USEPA, 2013); and can be partially explained by the non-lethal concentrations of ammonia and VFAs observed in the digesters during the experiment (Table 2). Huong et al.

(2014) studied biogas plants on swine farms in Vietnam and found that of the 96 farms surveyed, almost 80% discharged effluent containing pathogens, such as *Salmonella*, and/or fecal indicator bacteria. Certain strains of *Salmonella*, such as *S. Typhimurium*, have been shown to be more resistant to environmental stresses and survive for longer periods of time when compared to *E. coli* (Franz et al. 2005). Understanding the fate of *Salmonella* during mesophilic anaerobic digestion is important because it is a human pathogen that can be derived from the handling of agricultural wastes. Our results demonstrate the difficulty in predicting the removal of pathogens, such as *Salmonella* from the effluent of anaerobic digestion systems. This issue could be exacerbated when other human pathogens (i.e. roundworms, viruses, protozoa) are considered because their removals may not correlate with *E. coli*.

4.0 Conclusions

This study demonstrated that semi-continuous mesophilic anaerobic digesters operated with longer feeding frequencies will have higher average methane yields, better specific methanogenic activities, and improved fecal indicator bacteria destruction than those fed on a shorter interval. These results have direct implications on how household- and large-scale biological waste-to-energy systems can be operated to improve resource recovery and protect public health. Furthermore, based upon the range of SRTs tested in our study the digesters operated at the highest SRT resulted in the better performance when coupled with a longer feeding frequency.

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Table 1 – Summary of the swine manure slurry influent key parameters; data shown are the averages of weekly measurements collected for 10 months. Standard deviations are shown in parentheses.

Parameter (units)	Swine Manure Slurry Influent Parameter (standard deviation)
pH	8.4 (0.2)
Total Solids (g/L)	68 (19.6)
Volatile Solids (g/L)	46 (13.7)
Soluble Chemical Oxygen Demand (mg /L)	5,290 (460)
Ammonia Nitrogen (mg/L NH ₃ -N)	434 (133)
Volatile Fatty Acids (mg/L HOAc)	1,130 (330)

Table 2 – Summary of the experimental digester performance data, standard deviations are listed in parentheses.

Parameter (units)	E15	E30	E45	W15	W30	W45
pH	7.18 (0.1)	7.29 (0.08)	7.31 (0.09)	7.21 (0.1)	7.25 (0.1)	7.30 (0.1)
Volatile Solids Removal (%)	38 (4)	46 (3)	47 (5)	43 (4)	44 (3)	44 (4)
Ammonia (mg/L NH ₃ -N)	1,000 (165)	970 (122)	1,000 (101)	950 (126)	910 (133)	960 (82)
Soluble Chemical Oxygen Demand (mg /L)	2,260 (434)	2,190 (392)	2,010 (280)	2,200 (530)	2,240 (374)	2,080 (254)
Volatile Fatty Acid (mg HOAc/L)	430 (50)	415 (49)	360 (42)	440 (67)	470 (60)	390 (44)
Methane Concentration (%)	59 (2)	60 (3)	61 (2)	57 (3)	59 (4)	60 (2)

Figureg

Figure 1 – The yields of the biogas and methane produced by the unmixed semi-continuous mesophilic anaerobic digesters in this study, corrected to STP.

Figure 2 – The biochemical methane formation potential of the six experimental digesters in this study.

Figures 3A-C – The specific methanogenic activity of the six experimental digesters are shown in (A), the same parameter is shown in (B) for digesters grouped by feeding frequency and digesters grouped by solids retention time are shown in (C).

Figures 4A-C – The *E. coli* concentrations of the influent and six experimental digesters are shown in (A), the same parameter is shown in (B) for digesters grouped by feeding frequency and digesters grouped by solids retention time are shown in (C).

Figure 5A-C –The *Salmonella* concentrations of the influent and six experimental digesters are shown in (A), the same parameter is shown in (B) for digesters grouped by feeding frequency and digesters grouped by solids retention time are shown in (C).

Swine Waste

Semi-continuous
Mesophilic Anaerobic
Digestion

Resource Recovery

E. Coli Removal