Co-digestion of Different Wastes for Enhanced Methane Production

THESIS

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By

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Abstract

In this study, co-digestion of different organic wastes were conducted in collaboration with Quasar energy group in order to determine the feasibility of methane production from certain waste streams such as expired creamer, hand sanitizer and algae waste. To fulfill the purpose, three specific objectives were to: (1) determine the methane potentials of four different food wastes (expired creamer, expired beer, food processing waste (FPW), and fat, oil, and grease (FOG)), (2) identify the suitable mixing ratios of dairy manure with creamer and sludge cake with hand sanitizer in semi-continuous anaerobic digestion, and (3) investigate the efficacy of the algae waste in co-digestion with corn stover. The algae wastes were collected after lipid extraction.

Batch reactors were used to determine the methane potentials of four different food wastes. All the batches were filled with 800 g digested dairy manure as seed and 200 g mixture of wastes. The co-digestions of dairy manure with different levels of food wastes were also tested in order to obtain the best mixtures. The digestions of 100% creamer, 100% beer, 100% FPW, 100% FOG as well as 100% dairy manure were also tested. The percentage was based on the VS content of each component. Beer and FOG showed higher methane yield than the FPW and creamer in the co-digestion with dairy manure.
Laboratory 4-L reactors were discharged and fed daily for the semi-continuous co-digestions. Constant mixing was achieved by magnetic stirrer. Three levels of hand sanitizer were tested (0.08%, 0.17%, and 0.35%) at the organic loading rate (OLR) of 4 g VS/L/d and hydraulic retention time (HRT) of 15 days. Three levels (37.0%, 54.3%, and 68.7%) creamer was co-digested with dairy manure. A very small amount of hand sanitizer could improve the methane yield significantly in the co-digestion with sludge cake. The highest methane production (2.16 L/L/day) was obtained with 0.17% hand sanitizer addition, while 0.35% hand sanitizer addition caused upset of the digestion at the HRT of 15 days. Higher methane production rate was obtained with increased creamer addition up to 69%.

The solid-state co-digestions of algae waste and corn stover were conducted in 500 mL flasks. At the TS of 23%, the addition of algae waste deceased when the C/N ratios of the mixtures (including seed) from 23 to 15. The highest methane yield (166.7 mL/g VSadded) was obtained at the C/N ratio of 23. The addition of algae waste failed to improve the methane yield in the co-digestion with corn stover. The possible reason could be the seed already provided sufficient nitrogen source.
Dedication

This document is dedicated to my family.
Acknowledgments

I wish to thank my advisor Dr. Yebo Li for his instructions. Without his support, I would not finish my master study within two years. I also appreciate the encouragement and intellectual suggestions from Dr. Jay Martin and Dr. Fred Michel.

My lab mates Caixia Wan, Guiming Fu, Quancheng Zhou, and Arnold Lubguban were very helpful during the past year. It was always a pleasure to discuss the research with them.

I would also like to thank Mike Klingman for his technical support and Mary Wicks for her time on the thesis. I am very grateful to Candy McBride for her administrative guidance.

Quasar energy group generously sponsored my research and provided equipments. I appreciate the help from the interns such as Max Wilker, Liz Szado, and Scotty Evans.

The deepest gratitude goes to my family for their priceless love and support. Special thanks to my fiancé for his understanding and care.
Vita

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Fields of Study

Major Field: Food, Agricultural and Biological Engineering
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Chapter 1: Introduction

In 2009, 250 million tons of total municipal solid waste (MSW) were generated in the US, of which 12.7% were food scraps. In addition, about 135 million tons of the total wastes were disposed by landfill without energy recovery. Every ton of landfilled MSW produces 0.2 m$^3$ leachate (Ozkaya, 2005), and in total, landfilled MSW accounts for 8% of the global anthropogenic CH$_4$ emissions (US EPA 1994). In addition, CH$_4$ is about 20 times more potent as a greenhouse gas than CO$_2$. Therefore, in order to control future CH$_4$ emissions, efficient waste treatment approach for MSW is urgently needed.

Currently, composting, combustion and anaerobic digestion (AD) are three alternative MSW processing methods. The major difference between composting and AD is that the former requires the presence of oxygen, while AD by definition proceeds without oxygen. Although AD costs more on both equipment and operation than the composting, it is more beneficial in terms of energy production (Mata-Alvarez et al., 2000). Combustion also converts wastes into energy, but combustion is more costly due to the relatively high moisture content of MSW. Furthermore, combustion ash is difficult to separate and utilize, but digestate from AD can be used as a fertilizer (Chang, 2004). Finally, AD offers a promising commercial solution to treat the wastes by decomposing most of the influent organic waste (approximately 50-80%) into a collectable stream of
biogas that normally consists of 50-70% methane and 30-50% carbon dioxide. The biogas can be used to generate heat and/or power.

The AD process can be divided into four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 1). During the first stage, the complex polymers such as proteins, polysaccharides and lipids were broken down into amino acids, monomeric sugars, long chain fatty acids (LCFA) and alcohols. Then in the next stage, formate, methanol, H₂, CO₂ and volatile fatty acids (VFA), including acetate, propionate, butyrate, valeric acid, iso-butyrate and iso-valeric acid, are generated. In the third stage, the VFA can be converted into H₂ and acetate by hydrogen-producing acetogenic bacteria (Weiland, 2010). In the last stage, methane is produced by two groups of methonogens (hydrogen-utilizing methanogen and acetate-utilizing methanogen) from H₂ and CO₂ or acetate (Montero et al., 2009). Hydrogen may be a limiting substrate because the accumulated hydrogen can inhibit the hydrogen-producing acetogenic bacteria (Bagi et al., 2007). Ammonia is produced during the acetogenesis of nitrogenous matter mostly in the form of proteins (Kayhanian, 1999).

Four major types of anaerobic digesters are currently in use: covered lagoon, complete mix digesters, plug flow, and fixed film digesters (Singh and Prerna, 2009). The covered lagoon digester is usually used to treat dilute manure with a total solid (TS) of 1-2% under the ambient temperature with a retention time over 30 days. Although it is simple and low cost, the efficiency is relatively low. The complete mix digester can treat wastes with a wide range of TS, but it requires additional mechanical mixing. The plug flow digesters do not need mixing and can treat wastes with the solid content of 10-14%.
Figure 1 The biochemical stages of the AD process (Based on Gujer and Zehnder, 1983)
Co-digestion of two or more wastes has the following advantages:

- Produce relatively higher methane yield. For example, the energy production potential could be improved by 200% from the continuous co-digestion of food waste with manure than the digestion of manure alone (El-Mashad and Zhang, 2007). The microbial synergism effect makes a major contribution to the higher methane yield (Carucci et al., 2005).

- Provide balance nutrients. The C/N ratio of the major wastes such as manure is commonly as low as 8-15. The addition of the substrates with higher C/N such as food waste can provide more balanced nutrients (Montusiewicz et al, 2008).

- Increase the volume of available feedstocks. The volume of the wastes from one resource may be limited. For example, the amount of manure from one farm may be not sufficient for large-scale digester. The addition of other wastes can raise the capacity of the digester.

- Improve the economics of AD facility. The addition of other wastes will make the digester more profitable especially considering the tipping fee of processing waste.

- Dilute toxic substance. Toxic substance in one feedstock may be diluted in co-digestion (Gomez et al., 2006).

However, the industrial application of the co-digestion requires more knowledge on the selection of feedstocks and suitable mixing ratios.
Chapter 2: Literature Review

The general assumption is that the methane yield varies solely with the proportion of substrates used in the co-digestion if all other factors, such as mixing and temperature, are kept the same. The criteria for evaluating the co-digestion process were VS reduction and methane yield (Callaghan et al., 1999). The possible sources of ‘disconnection’ between methane yield and mass balance included (1) the anaerobic biodegradability of organics other than cellulose and hemicelluloses, (2) non-methanogenic biodegradability and (3) carbon converted to biomass (McCarty et al., 1991).

2.1 Batch processes

Co-digestion of manure/sludge with processing wastes

Co-digestion of brewery waste, fish offal, yogurt waste, fruit and vegetable waste, and chicken manure with cattle manure were studied by Callaghan et al. (1999). VS reduction and methane yield were used to evaluate the process (Table 1). The highest methane yield was obtained from the co-digestion of fish offal and cattle manure. Although chicken manure inhibits the anaerobic digestion process, the VS reduction of co-digestion of chicken manure with cattle slurry reached 81%.
Table 1 Methane yields and VS removal rates of batch mode co-digestion

<table>
<thead>
<tr>
<th>Feedstocks</th>
<th>T (°C)</th>
<th>Methane Yield (L/g VS\textsubscript{added})</th>
<th>VS Removal Rate (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM\textsuperscript{a} and Energy Crops\textsuperscript{b}</td>
<td>35</td>
<td>0.14-0.35</td>
<td>-</td>
<td>Kaparaju et al.,2002</td>
</tr>
<tr>
<td>DCM and CBP\textsuperscript{c}</td>
<td>35</td>
<td>0.18-0.39</td>
<td>-</td>
<td>Kaparaju et al.,2002</td>
</tr>
<tr>
<td>CM and Straw</td>
<td>55</td>
<td>0.29-0.34</td>
<td>-</td>
<td>Hashimoto, 1983</td>
</tr>
<tr>
<td>CM and FVW\textsuperscript{d}</td>
<td>35</td>
<td>0.23\textsuperscript{f}</td>
<td>52</td>
<td>Callaghan et al., 1999</td>
</tr>
<tr>
<td>CM and YW sludge\textsuperscript{e}</td>
<td>35</td>
<td>0.27\textsuperscript{f}</td>
<td>45</td>
<td>Callaghan et al., 1999</td>
</tr>
<tr>
<td>CM and Brewery waste</td>
<td>35</td>
<td>0.31\textsuperscript{f}</td>
<td>34</td>
<td>Callaghan et al., 1999</td>
</tr>
<tr>
<td>CM and Fish Offal</td>
<td>35</td>
<td>0.37\textsuperscript{f}</td>
<td>47</td>
<td>Callaghan et al., 1999</td>
</tr>
<tr>
<td>CM and Chicken Manure</td>
<td>35</td>
<td>0.12-0.15\textsuperscript{h}</td>
<td>49-81</td>
<td>Callaghan et al., 1999</td>
</tr>
<tr>
<td>PPW\textsuperscript{g} and Pig Slurry</td>
<td>35</td>
<td>0.41</td>
<td>72</td>
<td>Monou et al., 2008</td>
</tr>
<tr>
<td>PPW and Abattoir Wastewater</td>
<td>35</td>
<td>0.25</td>
<td>60</td>
<td>Monou et al., 2008</td>
</tr>
<tr>
<td>FOG\textsuperscript{h}, TWAS\textsuperscript{i} and PS\textsuperscript{j}</td>
<td>35</td>
<td>0.63</td>
<td>53</td>
<td>Kabouris et al., 2009</td>
</tr>
</tbody>
</table>

\textsuperscript{a}DCM: digested cow manure; b. Energy Crops include clover, grasshay and oats; c. CBP: confectionery by-products, including chocolate, blackberry and confectionery raw materials; d FVW: fruit and vegetable wastes; e. YW sludge: yogurt waste; f. L/g VS\textsubscript{removal}; g. PPW: potato processing wastewater; h. FOG: fat, oil and grease; i. TWAS: thickened waste activated sludge; j. PS: primary sludge.
A simple and rapid method was applied by Monou et al. (2008) to determine the methane potential of potato wastewater, pig slurry, abattoir water, co-digestion of potato wastewater and pig slurry (50:50 based on wet weight) and co-digestion of equal wet weight of abattoir water and potato processing water using brewery waste as the seed. Equal amount of sludge was withdrawn and filled each day for the first five days in order to exclude the effect of the seed. Although the introduction of potato wastewater into abattoir water didn’t change the VS deduction rate (60%), the methane yield decreased from 0.37 L/g VS$_{\text{added}}$ to 0.25 L/g VS$_{\text{added}}$. This indicated the imbalance between the acidogenesis and methanogenesis. The highest methane yield was 0.41 L/g VS$_{\text{loaded}}$ from the co-digestion of potato wastewater and pig slurry, compared to 0.37 L/g VS$_{\text{loaded}}$ for potato wastewater and 0.25 L/g VS$_{\text{loaded}}$ for pig slurry (Table 1).

Kaparajiet al. (2002) examined the methane potential of industrial confectionery by-products using digested cattle manure as seed under 35°C (Table 1). The methane potential for chocolate, blackberry and confectionery raw materials were 0.37, 0.39 and 0.32 L/g VS$_{\text{added}}$, respectively.

Fat, oil, and grease (FOG) is a suitable feedstock for co-digestion for its high carbon content and methane potential. By batch anaerobic ultimate biodegradability test, the dewatered FOG was reported to have a methane yield of 0.993 L/g VS$_{\text{added}}$ (1.404 L CH4/g VS$_{\text{destroyed}}$) (Kabouris et al., 2009). Kabouris et al. (2008) examined the biodegradability of the co-digestion of primary sludge (21%, based on the VS, same in the following) and thickened waste activated sludge (31%) with polymer-dewatered FOG.
(48%). The methane yield was improved by 116% with addition of FOG at a loading rate of 3 g VS/L (Table 1).

**Co-digestion of manure with cellulosic materials**

Hashimoto (1983) studied the co-digestion of cattle manure with hard winter-wheat straw in both batch (Table 1) and continuous reactors (Table 2). The ultimate methane yield decreased linearly from 0.38 L/g VS$_{\text{added}}$ to 0.28 L/g VS$_{\text{added}}$ as the straw percentage increased from 0% to 100%.

Kaparajiet al. (2002) examined the methane potential from the co-digestion of digested cattle manure with energy crops (Table 1). The methane potentials for clover, grass hay and oats ranged from 0.14 L/g VS$_{\text{added}}$ to 0.35 L/g VS$_{\text{added}}$ under 35°C.

### 2.2 Continuous processes

**Co-digestion of manure/sludge with processing wastes**

Five mixing ratios (100:0, 75:25, 50:50, 25:75 and 0:100 based on the wet weight) of sludge and brewery waste were compared in the terms of methane yield and composition of digested sludge in terms of total nitrogen, phosphorus, potassium (N, P, K) and heavy metals (Cd, Cr, Pb, Ni, Cu, Zn and Hg) with a retention time of 20 days and loading rates of 0.9-1.5 g/L/day (Pecharaply et al., 2007). The methane yield increased from 0.40 L/g VS$_{\text{removed}}$ to 0.51-0.65 L/g VS (Table 2) with the introduction of brewery sludge. The alkalinity was observed in the range of 1000 - 5000 mg/L and pH ranged from 6.5 to 7.6 when the process was stable.

Kabouris et al. (2009) tested the co-digestion of 21% primary sludge, 31% thickened waste activated sludge, and 48% FOG in a two-phase process. While the acid
phase digester was kept at the mesophilic temperature, the methane phase was conducted under either mesophilic or thermophilic condition. The performance is shown in the Table 2.

El-Mashad and Zhang (2007) studied the co-digestion of dairy manure and food waste in the continuous digester (Table 2). The methane yield of 0.504 L/g VS_{added} was achieved when the manure and food waste ratio was 52:48 (based on the VS) at the organic loading rate (OLR) of 4g VS/L/d and HRT of 20 days at 35±2 °C. Four mixing ratio of food waste from dining center and dairy manure (0:1, 1:1, 3:1, and 6:1) and three HRT were examined in the two-phase co-digestion (Table 2). The optimal methane production rate (3.97 L/L/day) was obtained when the mixing ratio of food waste and manure was 6:1 and HRT for the acidification and methanogenesis was 1 and 12 days, respectively (Li et al, 2010).

Co-digestion of fruit and vegetable waste and chicken manure with digested cattle manure respectively was studied in the continuous process (Table 2). When the OLR increased from 3.19 to 4.75 g VS/L/d, the methane yield for fruit and vegetable waste increased from 0.23 to 0.45 L CH_{4}/ g VS_{added} while the methane yield of chicken manure decreased from 0.13 to 0.04 L/ g VS (Callaghan et al., 2002).
### Table 2 Methane yields and VS removal rate from the continuous co-digestion

<table>
<thead>
<tr>
<th>Feedstocks</th>
<th>T (°C)</th>
<th>Methane Yield (L/g VS&lt;sub&gt;added&lt;/sub&gt;)</th>
<th>VS Removal Rate (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM&lt;sup&gt;a&lt;/sup&gt; and Food</td>
<td>35</td>
<td>0.22-0.41</td>
<td>46-62</td>
<td>El-Mashad and Zhang, 2007; Neves, 2009; Li et al., 2010</td>
</tr>
<tr>
<td>Waste</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM and OFMSW&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35</td>
<td>0.34-0.46</td>
<td>54-74</td>
<td>Hartman and Ahring, 2005</td>
</tr>
<tr>
<td>CM&lt;sup&gt;c&lt;/sup&gt; and Straw</td>
<td>35</td>
<td>0.26-0.29</td>
<td>-</td>
<td>Hashimoto, 1983</td>
</tr>
<tr>
<td>CM and Straw</td>
<td>55</td>
<td>0.41-0.44</td>
<td>-</td>
<td>Hashimoto, 1983</td>
</tr>
<tr>
<td>CM and FVW&lt;sup&gt;d&lt;/sup&gt;</td>
<td>35</td>
<td>0.25-0.45</td>
<td>30-50</td>
<td>Callaghan et al., 2002</td>
</tr>
<tr>
<td>CM and Chicken Manure</td>
<td>35</td>
<td>0.04-0.11</td>
<td>28-50</td>
<td>Callaghan et al., 2002</td>
</tr>
<tr>
<td>CM and Energy Crops&lt;sup&gt;e&lt;/sup&gt;</td>
<td>35</td>
<td>0.21</td>
<td>-</td>
<td>Kaparaju et al., 2002</td>
</tr>
<tr>
<td>CM and CBP&lt;sup&gt;f&lt;/sup&gt;</td>
<td>35</td>
<td>0.28</td>
<td>-</td>
<td>Kaparaju et al., 2002</td>
</tr>
<tr>
<td>Swine Manure and Wheat Straw</td>
<td>55</td>
<td>0.271</td>
<td>-</td>
<td>Wang et al., 2009</td>
</tr>
<tr>
<td>FOG&lt;sup&gt;g&lt;/sup&gt;, TWAS&lt;sup&gt;h&lt;/sup&gt; and PS&lt;sup&gt;i&lt;/sup&gt;</td>
<td>35</td>
<td>0.47</td>
<td>45</td>
<td>Kabouris et al., 2009</td>
</tr>
<tr>
<td>FOG, TWAS and PS</td>
<td>52</td>
<td>0.55</td>
<td>51</td>
<td>Kabouris et al., 2009</td>
</tr>
<tr>
<td>Sewage Sludge and Brewery</td>
<td>36</td>
<td>0.51-0.65&lt;sup&gt;j&lt;/sup&gt;</td>
<td>16-19</td>
<td>Pecharaply et al., 2007</td>
</tr>
</tbody>
</table>

a. DM: dairy manure; b. OFMSW: organic fraction of municipal solid waste; c. CM: cow manure; d. FVW: fruit and vegetable waste; e. Energy Crops include clover, grass hay and oats; f. CBP: confectionery by-products, including chocolate, blackberry and confectionery raw materials; g. FOG: fat, oil and grease; h. TWAS: thickened waste activated sludge; i. PS: primary sludge; j. L/g VS<sub>removal</sub>. 

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Kaparajiet al. (2002) examined the methane potential of industrial confectionery by-products and the co-digestion with manure in the farm scale digester (Table 2). The addition of confectionery by-products into the farm digester fed with cattle manure increased the methane yield by 27%.

**Co-digestion of manure / sludge with cellulosic materials**

Energy crops such as grass hay, oats and clovers were studied in the farm scale digester under 35°C by Kaparaju et al. (2002). The co-digestion of crop residue and manure did not improve the methane yield (Table 2). Lehtomaki et al., (2007) studied the co-digestion of manure and with different energy crops such as sugar beet tops, grass silage and oat straw were digested in CSTR under the mesophilic condition. The highest methane yield (0.268 L/ g VS\_added) was obtained from co-digestion of manure with grass silage (7:3 based on VS) when OLR and HRT were 2g/L/d and 20 days, respectively. The highest methane yields from co-digestion of manure with sugar beet tops and straw were 0.229 and 0.213 L/ g VS\_added, respectively.

The co-digestion of wheat straw and swine manure was conducted in lab-scale continuous stirred reactors by Wang et al. (2009) with a HRT of 15 days. A methane yield of 0.271 L/g VS\_added was obtained (Table 2). No increase in biogas production was observed when the wheat straw was pretreated with wet explosion.

After 42 days co-digestion of 50% (VS/VS) OFMSW with 50% cow manure, Hartman and Ahring (2005) switched the co-digestion into the single digestion of OFMSW. The digestion of the OFMSW achieved methane yield of 0.46 L/ g VS\_added and
VS reduction rate of 73%, while the co-digestion had methane yield of 0.34-0.46 L/ g VS\textsubscript{added} and the VS removal rate was 54-74% (Table 2).

2.3 Effect of C/N Ratio, ammonia, VFA and LCFA

Possible inhibitive substrates include free ammonia (NH\textsubscript{3}) or ammonium ions (NH\textsubscript{4}\textsuperscript{+}), hydrogen sulfide, heavy metals (Co, Cu, Fe, Ni and Zn), alternative electron acceptors (NO\textsubscript{3}\textsuperscript{-} or SO\textsubscript{4}\textsuperscript{2-}), alkalinity cations (Ca, Mg, K and Na), benzene ring compounds, LCFA and excess VFA especially propionate (Gerardi, 2003).

2.3.1 C/N ratio

The carbon to nitrogen (C/N) ratio was found to be very important in the methane production. On one hand, higher carbon content means higher potential to produce CH\textsubscript{4}. On the other hand, low nitrogen could be the limiting factor because the microbes need a considerate amount of nitrogen to maintain their growth. For example, the C/N ratios (26, 95 and 18) of the grass, oat straw and sugar beet tops was found to be the major affected the methane yields when they were co-digested with manure. The highest methane yield of 0.268 L/ g VS\textsubscript{added} was obtained from the co-digestion of manure with grass because the C/N ratio of the grass (26) is more appropriate (Lehtomaki et al., 2007). Kayhanian and Hardy (1994) reported that the optimal C: N ratios were between 25 and 30 for a mixture of organic fractions of MSW. However, Mshandete et al. (2004) observed that the highest methane yield of 0.62 L CH\textsubscript{4}/g VS\textsubscript{added} was obtained with C/N ratio of 16 when fish waste and sisal pulp was co-digested.

Two methods of computing C/N ratios were discussed by Kayhanian and Tchobanoglous (1992). The first one is based on the total dry carbon and nitrogen, while
the second one is the ratio of the total biodegradable organic carbon and total nitrogen. The biodegradable fraction was calculated by extracting the lignin content. By comparing the relationship of the two C/N ratios and the VS removal rates of paper and yard waste, the second method was proved to be a better solution.

2.3.2 Ammonia

Most ammonia from the co-digestion of food wastes is derived from proteins. Kayhanian (1999) summarized two theories of mechanism in the ammonia inhibition. One was that the ammonium iron could inhibit the methane-synthesizing enzyme. The other one was that the hydrophobic ammonia molecule can diffuse into the cells and cause proton imbalance.

The free ammonia can be calculated by the following equation:

\[
\text{NH}_3 \text{ conc.} = \text{Total ammonia nitrogen conc.} \times \frac{\text{Ka} \times [H]}{[H] + 1},
\]

where Ka is the temperature dependent dissociation constant (0.564*10^{-9} under 25°C, 1.097*10^{-9} at 35°C, and 3.77*10^{-9} at 55°C); [H] is hydrogen iron concentration= 10^{\text{pH}}. Braun et al. (1981) found that the free ammonia amount of above 150 mg/L could inhibit the unadapted digestion. However, Callaghan et al. (1999) proved that the starting inhibition amount of free ammonia should be 250 mg/L. Decrease of pH from 8 to 7.4 and reduction of temperature reduced the amount of free ammonia (Braun et al., 1981).

Ammonium ion could have a more significant effect on the process than free ammonia (Lay et al., 1997). The ammonical nitrogen concentration of 1.5g/L- 2.5g/L was considered inhibitive for unadapted culture under mesophilic condition. Ammoniacal nitrogen critically affected the production of methane at a concentration of 5g N/L when
it is added gradually. When ammoniacal nitrogen concentration exceeded 4g N/L, the yield of methane was reduced by 25%. (Angelidaki and Ahring, 1993). The high level of ammoniacal nitrogen in the wastes could also cause long lag time (Callaghan et al., 1999).

2.3.3 LCFA and VFA

LCFA has potential to increase the methane yield, but it could also cause inhibition. Under thermophilic conditions, glyceridetrioleate (GTO as a model lipid) was reported to be inhibitive at concentration higher than 2.0 g/L by Angelidaki et al. (1990). Under mesophilic conditions, pulse addition of 18 g COD/L oily waste from canned fish processing industry into the co-digestion system of manure with food waste led to the accumulation of 375 g COD LCFA/kg TS, resulting no biogas production (Neves et al., 2009). The soluble calcium salt could reduce the inhibitory effect of LCFA if added at the beginning (Hanaki et al., 1981). Once the inoculums were adapted, the process had a better tolerance to LCFA (Angelidaki et al., 1990).

VFA such as propionate and butyrate are important intermediates in the AD process (Hanaki et al., 1981). Propionate was reported inhibitive when the concentration exceeded 1,000 ppm in biowaste digestion by Gallert et al. (2003), but the propionate concentration of 1,793 ppm did not result in upset of digester containing food waste (Nayono et al., 2009).

2.4 Conclusion

In conclusion, anaerobic digestion is a promising waste treatment process, because it can not only reduce the waste volume but also capture energy. Co-digestions of manure or sludge with other wastes, such as grease and food-processing waste, could
increase the profitability of AD with the collection of tipping fees and increased biogas production. However, due to the various properties of different wastes, lab or pilot scale co-digestion experiments are necessary to determine the suitable mixing ratios.
Chapter 3: Anaerobic Co-digestion of Dairy Manure with Four Food Wastes

Co-digestions of manure with four different food wastes in the batch reactors under mesophilic conditions were studied in this chapter. The purpose, which is to obtain the optimal methane yield from a mixture of different waste combinations, was achieved through the two following objectives: determination of the methane potential of different food wastes, and study of the effect of different mixing ratio of manure and food wastes on the biogas production.

Currently, no other research has been reported on the study of the co-digestions of dairy manure with expired creamer and beer. All the wastes used in this study were collected from Ohio farms and food industries. The results of this study are served as important baseline data for the future design and operation of anaerobic digester by Quasar energy group.

3.1 Materials and methods

3.1.1 Feedstock and inoculums

The four food-derived feedstocks tested in this study include FPW, FOG, beer and creamer. The beer and creamer were expired food waste from a beverage manufacturer in Ohio. FPW and FOG were provided by two companies that treat food wastes from the restaurants. The dairy manure was collected from a local farm. The seed was digested.
dairy manure from an on-farm digester. The composition of feedstocks was analyzed after arrival, and samples were stored in a refrigerator at 0-4°C.

The characteristics of the seed, dairy manure and food wastes were shown in Table 3. Food wastes generally have higher COD content (122-732 g/L) than dairy manure (93 g/L). Creamer has the highest COD content of 732.2 g/L. The alkalinity, pH, and ammoniacal-nitrogen of food wastes were <0.1 g/L, 4.2-4.8, and <1 g/L, respectively. In comparison, FPW has relatively high alkalinity (0.85 g/L), neutral pH (7) and high ammoniacal-nitrogen content (3.1 g N/L). FPW has the lowest C/N ratio (8.7) while creamer has the highest (146.2). C/N ratio of FOG (18.5) is close to that of the manure (12.7).
Table 3 Characteristics of the substrates used in the batch tests

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Seed</th>
<th>Manure</th>
<th>Beer</th>
<th>FOG</th>
<th>FPW</th>
<th>Creamer</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS, %</td>
<td>6.6(0.4)</td>
<td>13.0(1.0)</td>
<td>3.5(0.2)</td>
<td>15.6(0.3)</td>
<td>12.3(0.3)</td>
<td>44.4(0.9)</td>
</tr>
<tr>
<td>VS/TS, %</td>
<td>70.8(0.3)</td>
<td>83.5(2.2)</td>
<td>95.4(0.2)</td>
<td>81.8(1.3)</td>
<td>90.0(0.5)</td>
<td>98.4(0.0)</td>
</tr>
<tr>
<td>COD, g/L</td>
<td>38.5(1.9)</td>
<td>93.0(15.1)</td>
<td>122.3(0.2)</td>
<td>233.6(11.6)</td>
<td>235.7(1.7)</td>
<td>732.2(8.9)</td>
</tr>
<tr>
<td>pH</td>
<td>8.4(0.1)</td>
<td>7.0(0.2)</td>
<td>4.4(0.0)</td>
<td>4.2(0.0)</td>
<td>7.0(0.1)</td>
<td>4.8(0.1)</td>
</tr>
<tr>
<td>NH₄-N, g/L</td>
<td>1.4(0.1)</td>
<td>1.9(0.0)</td>
<td>0.1(0.0)</td>
<td>0.4(0.0)</td>
<td>3.1(0.1)</td>
<td>-</td>
</tr>
<tr>
<td>Alkalinity, g/L</td>
<td>1.1(0.2)</td>
<td>1.37(0.0)</td>
<td>-</td>
<td>-</td>
<td>0.9(0.1)</td>
<td>0.0(0.0)</td>
</tr>
<tr>
<td>C, %</td>
<td>2.27(0.04)</td>
<td>5.52(0.15)</td>
<td>2.74(0.06)</td>
<td>9.10(0.22)</td>
<td>8.86(0.19)</td>
<td>24.50(0.65)</td>
</tr>
<tr>
<td>N, %</td>
<td>0.27(0.00)</td>
<td>0.44(0.01)</td>
<td>0.09(0.00)</td>
<td>0.49(0.02)</td>
<td>1.02(0.04)</td>
<td>0.17(0.01)</td>
</tr>
<tr>
<td>C/N</td>
<td>8.5(0.1)</td>
<td>12.7(0.2)</td>
<td>3.0(0.7)</td>
<td>18.5(0.6)</td>
<td>8.7(0.1)</td>
<td>146.2(0.4)</td>
</tr>
</tbody>
</table>

*The C% and N% were based on the wet weight. Data in the parentheses are standard deviation.
3.1.2 AD tests

The batch reactor (2 L) was connected to a Tedlar gasbag, which collected the biogas produced (Fig. 2). The working volume of the reactor was 1 L.

![Diagram of a laboratory batch reactor](image)

Figure 2 Schematic of the laboratory batch reactor: 1-2-Litre glass reactor, 2- rubber stopper with a metal tube through it, 3-valve, 4- biogas collection Tedlar bag.

All the reactors were kept in a walk-in incubation room with a controlled temperature of 35 ±2 °C. For each test, 800 g seed manure was used as inoculum and mixed with 200g feedstocks. The VS loading rate for all the batch tests were 15g/L.
Beer was exception due to its low VS. The total solid content was adjusted to 6% by diluting with DI water. There were twelve formula as following: dairy manure (control), 100% creamer, 100% beer, 100% FPW, 100% FOG, 29% creamer and 71% manure, 74% creamer and 26% manure, 24% beer and 76% manure, 50% beer and 50% manure, 25% FPW and 75% manure, 50% FPW and 50% manure, and 40% FOG and 60% manure. The percentage was based on the VS content of each component. All the tests were run for up to 35 days and duplicated tests were conducted for each condition. The C/N ratio was 8.8 for the control and 7.8-8.1, 8.0-8.1, 7.6-7.8 for the co-digestions containing FPW, beer and FOG, respectively. The mixtures containing 29%, 49%, 74%, and 100% creamer had the C/N ratios of 9.1, 9.8, 10.6, and 11.4, respectively.

**3.1.3 Analytical methods**

Content in the anaerobic digester was analyzed before and after digestion for the following parameters: TS, VS, pH, alkalinity, VFA and ammoniacal-nitrogen.

Total solids (TS) and total volatile solids (VS) were measured according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). TS content was determined via drying at 105 °C to constant weight and VS was analyzed by combustion of dry mass in a muffle oven at 505 °C.
Table 4 Experimental design for the batch tests

<table>
<thead>
<tr>
<th>Tested Mixture</th>
<th>Food Waste (% of VS)</th>
<th>Dairy Manure (% of VS)</th>
<th>VS$_{added}$ (g)</th>
<th>Inoculum (% of VS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>FPW</td>
<td>25 75 15 71</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 50 15 71</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 - 15 71</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Creamer</td>
<td>29 71 15 71</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>74 26 15 71</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 - 15 71</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td>24 76 14 72</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 50 10 78</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 - 7 85</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>FOG</td>
<td>40 60 15 71</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 - 15 71</td>
<td>15</td>
<td>71</td>
<td></td>
</tr>
</tbody>
</table>

pH was measured using glass pH probe (Mettler Toledo DG 115-SC, USA). The total VFA and alkalinity were measured by titration (Mettler Toledo DL 22 Food and Beverage Analyzer, USA). Alkalinity was measured at the end point of pH 4.4.

Total carbon and total nitrogen were analyzed with a carbon, nitrogen and sulfur analyzer (VARIO MAS CNS, Elementar, German).

Gas composition was determined using a GC (HP6890, Agilent Technologies, Wilmington, DE) equipped with a 30 m×0.53 mm×10 μm alumina/KCl deactivation column and a thermal conductivity detector (TCD). Helium was used as the carrier gas at
a flow rate of 5.2 ml /min. The temperatures of the column, injector, and detector were 40, 150 and 200 °C, respectively.

The biogas yield was expressed as the ratio of volume of biogas produced and the amount of VS removed from the feedstock mixture.

All the digestion tests were conducted in duplicates and the average values were reported. Biogas yield and VS removal rate were analyzed using analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons to determine which variables were significantly different. Significance will be assessed at alpha = 0.05.

3.2 Results and discussion

3.2.1 Biogas production

The daily methane production rates from the control and digestions of 24%, 50%, and 100% beer are shown in Fig. 3A. Double peaks were observed from the co-digestions containing beer. The first peak was formed by the readily digested small-molecular components such as alcohol while the second one was due to the degradation of the complex substrates such as polysaccharides. The digestion of 100% beer produced methane much faster than the others two digestion tests. On the 5th day, the digestion of 100% beer reached its first peak value of 0.80 L/day while the digestion of 24% and 50% beer both reached their first peak values on the 7th day. The peak value from the digestion of 50% beer (0.81 L/day) was 42.1% higher than that of 24% beer (0.57 L/day). The digestion of 100% beer reached its second peak (0.73 L/d) on the 13th day. One day later, the digestion of 50% beer had the second peak (0.74 L/d), which was 54.2% higher than the second peak value from the digestion of 24% beer obtained on the 22nd day. As the
percentage of beer decreased, the second peak of methane production rate delayed. The reason could be the microbe grew faster when more beer was presented. After 25 days, gas production halted. Although the digestion of 50% beer produced higher peak daily methane production rate than the digestion of 100% beer, the total biogas production from the former (10.39L) was 1.01L less than the latter (11.40L). The digestion of 50% beer produced 26.9% more total methane than the digestion of 24% beer (Fig. 3B). It was possible that the 100% beer caused inhibition for its high alcohol content therefore the total methane production was similar to that from 50% beer. The trends of the methane contents were similar for digestions of 100% and 50% beer. They reached highest methane content (73.6-73.7%) in 9-10 days. Afterward, the methane contents decreased to about 54%. The methane content from the digestion of 24% beer was less stable than the other two digestions containing beer. The peak value of methane content was 10% less than the other two digestions on the 10th day (Fig. 3C).
Figure 3 Daily methane production rate (A), accumulative methane production (B), methane content (C) from the co-digestion of beer

Continued
Daily methane production rate, accumulated methane and methane content from the digestions containing FOG are shown in Fig. 4. The digestion of 100% FOG produced the peak methane production rate (0.87 L/day) on the 11th day while peak methane production rate (0.52 L/day) from 40% FOG was produced on 9-15th day. After 25 days, methane production was less than 0.15 L/day (Fig. 4A). At the end, the digestion of 100% FOG (14.01 L) produced 46.5% more methane than 40% FOG (9.56 L) (Fig. 4B). The methane content from both digestions was very close. Digestion of 100% FOG
had slightly higher peak methane content (71.0%) on the 11th day, compared to 68.6% from the digestion of 40% FOG on the 15th day (Fig. 4C).
Figure 4 Daily methane production rate (A), accumulative methane production (B), methane content (C) from the co-digestion of FOG.
Daily methane production rate, accumulated methane and methane content from the digestions containing FPW are shown in Fig. 5. The digestion of 25% FPW reached the peak methane rate on the 9th day, which was 4 days faster than the digestion of 50% FPW and 5 days faster than 100% FPW. Some component in the FPW was responsible for the lag time. The peak methane rate from the digestion of 50% FPW was 6.5% higher than peak methane rate of 100% FPW and 32.0% higher than that of 25% FPW. Not much methane was produced after 25 days for digestions of 24% and 50% FPW. The digestion of 100% FPW did not produced much methane during the first 10 days then very little after 30 days instead of 25 days (Fig. 5A). Although slower methane production, the digestion of 100% FPW produced the highest accumulative methane
(10.27 L), which was 16.3% more than 50% FPW and 29.5% more than 25% FPW (Fig 5 B). On the 13th day, highest methane content (72.6%) was produced from the digestion of 25% FPW. Two days later, 3.3% less peak methane content was produced by 50% FPW. On 16th day, 69.7% peak methane content appeared from the 100% FPW (Fig. 5C).
Figure 5 Daily methane production rate (A), accumulative methane production (B), methane content (C) from the co-digestion of FPW
The highest daily methane rates from digestions of three levels of creamer were in the narrow range from 0.66 g/L to 0.69 g/L. The digestion of 29% creamer produced its highest methane rate on 12\textsuperscript{th} day, which was 2 days earlier than 74% creamer and 5 days before 100% creamer (Fig. 6A). The highest accumulative methane production of 10.07 L was obtained from the digestion of 74% creamer, which was 10.5\% more than the digestion of 100\% creamer and 42.4\% more than that of 29\% creamer (Fig. 6B). It was possible that the high fat content in the 100\% creamer produced excess amount of LCFA, which caused inhibition. The digestion of 100\% creamer obtained the highest methane
content (71.0%) on 15\textsuperscript{th} day, compared to 68.5% from 29\% creamer on 16\textsuperscript{th} day and 69.9 \% from 74\% creamer on 14\textsuperscript{th} day (Fig.6C).
Figure 6 Daily methane production rate (A), accumulative methane production (B), methane content (C) from the co-digestion of creamer.

Continued
The addition of food waste improved the methane production both in methane volume and in methane content. In the respect of methane volume produced, the highest methane production was 14.01 L from digestion of 100% FOG and the lowest was 7.55 L from 29% creamer. Compared to control (1.78 L), the addition of food waste improved the methane production by 4.2-7.9 times. The methane production increased with the increase in the percentage of the food waste due to the increased carbon content. As an exception, the methane production from 100% creamer is lower than that from 74% creamer due to the inhibition caused by the accumulated VFA or LCFA.
For the methane production rate, digestions containing beer produced methane faster than the other three feedstocks. Digestions containing beer reached peak values of 0.57-0.81 L/day between 5th to 7th days. In comparison, the digestions containing FPW, FOG, and creamer reached the peak values during 9-17 days. The main composition of beer was alcohol, extractives, and very little amount of protein other than water. The alcohol could be converted into methane or acetate directly through two independent metabolic pathways (Speece, 1996). Besides, the extractives could also be utilized by the microbes rapidly. The other food wastes, on the other hand, mostly consisted of lipid, starch, complex carbohydrates or protein, had to be hydrolyzed first, which could take a relatively long time.

The methane percentage of the biogas from the digestions containing four food wastes reached its peak value of 63.5-73.7% between 9th and 16th day, compared to the highest methane content of 59.9% on the 14th day from the control (dairy manure). The methane content had similar trend: initial increase reaching a peak value and then followed by decay. This was in accordance with the co-digestion of 75% brewery waste and 25% sewage sludge, where the methane content increased first then decreased (Babel et al., 2009). Noticeably, the methane contents had a trend to be stable at the end of digestion. If time extended, the methane content will become stable. The methane content was in the range between 50-75%. The range is very typical in the digestions containing highly degradable feedstock such as food waste. For example, the methane content from the co-digestion of sewage sludge and cheese whey was reported to be 63-76% by
Carrieri et al. (1993). A range of 58.5-65.3% of methane content was obtained from the co-digestion of food waste and dairy manure (El-Mashad and Zhang, 2007).

### 3.2.2 Methane yield and VS removal

By comparing the methane yield based on the total VS$_{\text{added}}$ in the reactors (including inoculum), the single digestions of FOG and beer had the highest methane yields (0.28 L/g VS$_{\text{added}}$) (Table 5). With the increase of the food waste percentage, the methane yield based on total VS added also increased. For example, the methane yield from the digestion of 100% FPW (0.21 L/g VS$_{\text{added}}$) was 16.7% higher than that of 50% FPW and 31.3% higher than that of 25% FPW. Creamer was the exception. The digestion of 74% creamer had 45% higher methane yield than 29% creamer and 16.7% higher than 100% creamer. Creamer contained a very considerate amount of fat. In the degradation of fat, LCFA was possible to inhibit the AD process (Angelidaki et al., 1990). It was possible that the amount of LCFA inhibited the digestion of 100% creamer. Noticeable, the methane yield from 100% beer was only 6.7% higher than 50% beer. In comparison, the methane yield from 50% beer was 41.1% higher than 24% beer. The alcohol content in the 100% beer was possible to cause inhibition.
Table 5 Methane yields and VS removal rates for the batch tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Methane yield</th>
<th>VS removal rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unit</td>
<td>L/g VS&lt;sub&gt;added&lt;/sub&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>0.05(0.00)</td>
<td>0.15(0.03)</td>
</tr>
<tr>
<td>25%</td>
<td>0.16(0.03)</td>
<td>0.71(0.07)</td>
</tr>
<tr>
<td>50%</td>
<td>0.18(0.02)</td>
<td>0.74(0.04)</td>
</tr>
<tr>
<td>100%</td>
<td>0.21(0.00)</td>
<td>0.60(0.00)</td>
</tr>
<tr>
<td>FOG</td>
<td>40%</td>
<td>0.19(0.01)</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>0.28(0.03)</td>
</tr>
<tr>
<td>Beer</td>
<td>24%</td>
<td>0.17(0.04)</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>0.24(0.05)</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>0.28(0.03)</td>
</tr>
<tr>
<td>Creamer</td>
<td>29%</td>
<td>0.15(0.03)</td>
</tr>
<tr>
<td></td>
<td>74%</td>
<td>0.21(0.02)</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>0.18(0.02)</td>
</tr>
</tbody>
</table>

The methane production resulted from the partial VS reduction. The increase percentage of food waste not only increased the methane yield based on VS added but also resulted in the increase of the VS removal rate. The highest VS removal rate was 49.85% when 100% FOG was digested, which was 27.00% higher than that of the 40% FOG (p<0.05). As for 100% creamer, although the methane yield was lower than 74% creamer, the VS removal rate was significantly higher (p<0.5). This indicated the imbalance between acidogenesis and methanogenesis. Other researchers had similar observations. For example, Callaghan et al. (1999) found out that the co-digestion of 15% chicken manure with cattle slurry produced very high VS deduction but very low gas production.
In terms of methane yield calculated based on the destroyed VS amount, the control had the methane yield of 0.15 L/g VS<sub>r</sub>, which is comparable with the methane yield of 0.16 L/g VS<sub>r</sub> in the study by Callaghan et al. (1999). The co-digestion of manure with food waste improved the methane yield by 1.8-5.2 times, compared to the single digestion of dairy manure (Table 5). Unlike the methane yield based on the VS added, the methane yield from 40% FOG and 50% FPW was higher than other levels of FOG and FPW, respectively. This indicated that those two digestions had more balanced acidogenesis and methanogenesis. The VS removed was utilized to produce methane more efficiently than the other levels. The data based on the VS removed showed the advantage of the co-digestion of dairy manure with food wastes compared to digestion of food waste only. The different percentages of creamer and FOG resulted in significantly different methane yields (p<0.07). This is in accordance with the data in the paper by Lansing et al. (2010), in which the different combinations of cooking grease (0%, 2.5%, 5%, and 10%) caused significant changes in biogas production. The levels of FPW did not affect the methane yield significantly probably because of the similar C/N ratios, pH, and TS of the FPW and dairy manure (Table 3).

Subtracted the methane production from the control, the methane potentials from the FPW, FOG, beer, and creamer were 0.56, 0.80, 1.42, and 0.48 L/g VS<sub>added</sub> (data not shown). The methane potential from beer was significantly higher than the values from the other three feedstocks (p<0.05). The high methane potential of beer may be caused by small molecular alcohols that can be used by the microbes easily. The higher methane
potential from the FOG than from FPW and creamer may be because of the relatively high lipid content.

3.2.3 The analysis of the effluent

The pH of all the batches at the start of the experiments were in the range from 7.6 to 8.2 which were not significantly affected by the addition of acidic food wastes (Fig. 7). This shows the buffering ability of the dairy manure. Angelidaki and Ahring (1992) had the similar finding. The methanogens grow if pH is above 6.2 (Gerardi, 2003); therefore the maintenance of the pH between 6 and 8 is crucial for efficient methane production.

From the co-digestion of manure with creamer and beer, the pH remains constant throughout the digestion process (Fig. 7). However, an obvious increase from 7.8 to 8.2 in pH was observed at the end of the co-digestion of manure with 100% FPW. This result is in accordance with the fact that the nitrogen content (Table 3) of FPW (1.02%) is higher than that of FOG (0.49%), creamer (0.17%) and beer (0.09%). The nitrogeinous matter in the feedstock mostly exists in the form of protein. Degradation of protein produces ammonia, which may result in an increase of the pH (Kayhanian, 1999).
In general, the addition of the food waste diluted the VFA concentration at the beginning of the tests (Fig. 8). During the AD, the VFA was simultaneously utilized to produce methane while it is generated. At the end of the digestion, the concentration from all the batch tests ranged from 1960 to 2925 mg/L. That indicated that the VFA generated from the feedstocks added in the batch tests were almost completely utilized. Except for the digestion of 100% creamer, the total VFA concentration increased after the AD. For the substrates containing high C/N ratio such as creamer (146), the increase of the VFA from the digestion of 100% creamer is related to the rapid degradation of the fat (Cirne et al., 2007).
3.3 Conclusion

The introduction of food wastes enhanced the dairy manure digestion system by boosting both methane production and VS removal. The methane yields of digestions containing food waste were 1.8-5.2 times of that of the control (100% dairy manure). The highest methane yield of 1.02 L/ g VS was obtained from the digestion of 100% beer waste. The VS removal rates varied between 21.5% and 49.9%. Similar VFA concentrations obtained at the end of the digestion indicated that the digestion process was not inhibited by the accumulation of VFA.
Beer produced methane faster than the other three feedstocks because the alcohol was converted into methane faster by the microbes than the other components such as lipid, carbohydrates and protein.

Beer and FOG had higher methane potential than FPW and creamer. The suitable mixing ratio for beer was between 50% and 100% since the digestion of 100% beer had similar methane production with 50% beer. The most suitable mixing ratio was around 74% for the creamer and 100% for the FPW. The best mixtures were 100% FOG and around 50% beer among the mixtures tested.
Chapter 4: Co-digestion in the Continuous Reactors

In this chapter, the co-digestion of dairy manure with 37.0%, 54.3%, and 68.7% creamer waste and sludge cake with 0.08%, 0.17%, and 0.35% hand sanitizer were studied in six 4-litre semi-continuous reactors under mesophilic condition. The purpose of this chapter was: (1) to evaluate the effect of different mixing ratios of wastes on the methane yield in the co-digestions, (2) to study the AD process by analyzing the methane production rate, methane content, pH and VFA concentration during the digestion, and (3) to compare the co-digestion of dairy manure with creamer waste and the co-digestion of sludge cake with hand sanitizer.

The introduction of wastes such as expired creamer and hand sanitizer into the AD system is unique and innovative. The data serves as important baseline data for the operation of full-scale digesters.

4.1 Materials and methods

4.1.1 Waste collection and characteristic
Table 6 Characteristics of the wastes used in the semi-continuous reactors

<table>
<thead>
<tr>
<th>Name</th>
<th>Digested Manure</th>
<th>Dairy Manure</th>
<th>Creamer Waste</th>
<th>Seed Sludge</th>
<th>Sludge Cake</th>
<th>Hand Sanitizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS, %</td>
<td>5.7 (0.4)</td>
<td>5.9 (0.0)</td>
<td>44.4 (0.9)</td>
<td>7.9 (0.1)</td>
<td>31.1 (0.8)</td>
<td>0.4 (0.0)</td>
</tr>
<tr>
<td>VS/TS, %</td>
<td>57.1 (0.1)</td>
<td>73.2 (0.9)</td>
<td>98.4 (0.0)</td>
<td>57.1 (0.2)</td>
<td>78.7 (0.3)</td>
<td>100.0 (0.0)</td>
</tr>
<tr>
<td>C, %</td>
<td>2.94 (0.33)</td>
<td>3.57 (0.15)</td>
<td>24.50 (0.65)</td>
<td>2.99 (0.02)</td>
<td>14.28 (0.65)</td>
<td>30.87 (2.33)</td>
</tr>
<tr>
<td>N, %</td>
<td>0.35 (0.02)</td>
<td>0.43 (0.01)</td>
<td>0.17 (0.01)</td>
<td>0.54 (0.01)</td>
<td>1.35 (0.02)</td>
<td>0.03 (0.00)</td>
</tr>
<tr>
<td>C/N</td>
<td>8.4 (1.2)</td>
<td>8.3 (0.3)</td>
<td>146.2 (0.4)</td>
<td>5.5 (0.1)</td>
<td>10.6 (0.3)</td>
<td>1029.0 (17.2)</td>
</tr>
<tr>
<td>pH</td>
<td>8.0 (0.0)</td>
<td>7.6 (0.1)</td>
<td>4.8 (0.1)</td>
<td>8.3 (0.0)</td>
<td>6.8 (0.2)</td>
<td>6.1 (0.0)</td>
</tr>
<tr>
<td>COD, g/L</td>
<td>63.8 (1.3)</td>
<td>- (8.9)</td>
<td>732.2 (1.8)</td>
<td>79.8 (3.8)</td>
<td>94.8 (4.2)</td>
<td>1461.7 (4.2)</td>
</tr>
</tbody>
</table>

*The C% and N% were based on the wet weight. Data in the parentheses are standard deviation.
The dairy manure was collected from a dairy farm in Wooster, OH. The creamer was expired food waste from a food company in Ohio. The sludge cake was dewatered municipal waste from Akron’s wastewater treatment plant. The hand sanitizer was collected from a company that manufactured hygiene products in Ohio. All the wastes were analyzed after arrival and stored under 0-4°C.

The characteristics of the wastes and seeds are shown in Table 6. The hand sanitizer had very low TS (0.44%) but very high C/N ratio (1029) as a result of high carbon content (30.87%). The sludge cake had relative low C/N ratio (10.6). Sludge cake and hand sanitizer had similar pH (6.1 and 6.8). Creamer waste had higher TS and C/N ratio than dairy manure but lower pH.

4.1.2 Anaerobic digestion tests

Six identical laboratory bench-scale anaerobic digesters (4L) were operated in the incubation room. The temperature was maintained at 35±2°C. The schematic and picture of the digester are shown in Fig. 9 and 10. The working volume was maintained at 2L. Magnetic stirrer running at 1000 rpm provided the mixing mechanism. The digesters were manually sampled and fed daily. In order to take representative samples, a mixer was turned on for 30 seconds before sampling and after feeding. Amount of 133 mL of effluent was taken from each digester daily. As a result the hydraulic retention time (HRT) of the reactor was 15 days. Two effluent ports are located at the liquid surface and the bottom of digester respectively. The bottom port is used for withdrawing sample and the other one is used for feeding.
Three digesters were inoculated with 2 L active seed sludge from the *Quasar energy group*’s anaerobic digester in Akron. The feed was with the mixture of sludge cake and hand sanitizer. Three different concentrations of the sanitizer were added: 0.08%, 0.17%, and 0.35%, resulting in the C/N ratios of 11.9, 13.3, and 15.1, respectively. The other three digesters were inoculated with 2 L digested seed manure from an on-farm anaerobic digester. Co-digestion of dairy manure with 37.0%, 54.3%, and 68.7% creamer was tested. The C/N ratios for the three mixtures of dairy manure and creamer were 10.3, 12.3, and 15.4. All the percentages were based on the volatile solid (VS).
Figure 9 Schematic of the laboratory semi-continuous bench-scale reactor system: 1-digester, 2-mixer, 3-valve, 4-feeding port, 5-sampling port, 6-liquid level, 7-stir bar, 8-magnetic stirrer (set at 1000 rpm), and 9-biogas collection Tedlar bag.
Figure 10 Laboratory 4-litre semi-continuous mixing reactors
The digester headspace was flushed with nitrogen gas at 1.5 L/min for 5 minutes to ensure the anaerobic conditions, then the digesters were allowed to sit for a couple of days before sampling and feeding. The organic loading rate (OLR) for all the digesters was 4 g VS/L/day.

4.1.3 Chemical analysis

The wastes were analyzed for the following parameters: TS, VS, total carbon, total nitrogen, pH, and COD. The volume of biogas was analyzed daily. The methane content, pH and total VFA concentration were analyzed every two or three days. When digestions were in steady state, the effluent was also analyzed for TS and VS.

The volume of the biogas in the Tedlar gasbag was measured using a wet-drum volumetric gas meter (Ritter, Bochum, Germany). Gas composition was determined using a GC (HP6890, Agilent Technologies, Wilmington, DE) equipped with a 30 m×0.53 mm×10 µm alumina/KCl deactivation column and a thermal conductivity detector (TCD). Helium was used as the carrier gas at a flow rate of 5.2 ml/min. The temperatures of the column, injector, and detector were 40, 150 and 200 °C, respectively.

Total solids (TS) and volatile solids (VS) were measured according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). TS content was determined via drying at 105 °C to constant and VS was analyzed by combustion of dry mass in a muffle oven at 505 °C.

pH was measured using a glass pH probe (Mettler Toledo DG 115-SC, USA). The total VFA was measured by titration (Mettler Toledo DL 22 Food and Beverage Analyzer, USA). Total carbon and total nitrogen were analyzed with a carbon, nitrogen
and sulfur analyzer (VARIO MAS CNS, Elementar, German). The COD content was analyzed following the standard HACH method and using the HACH COD digestion vials, DRB digital reactor block and DR 2800 portable spectrophotometer.

The methane yield, VS removal rate, pH, total VFA, and methane content during the last week of the operation were analyzed using analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons to determine which variables were significantly different. Significance was assessed at alpha = 0.05.

4.2 Results and discussion

4.2.1 Co-digestion of sludge with the hand sanitizer

The average biogas production rate, biogas yield, methane production rate, methane yield, VS removal rate, TS, VS, pH and total VFA during the final week of digestions of sludge and the HS are shown in Table 7. All the values in the table were averaged over four measurement points. The digestion of 0.17% HS produced the highest biogas yield (0.80 L/ g VS$_{added}$), which was 23.8% higher than the digestion of 0.08% HS.

The methane yield (0.54 L/g VS$_{added}$) of digestion with 0.17% HS was 27.8% higher than the digestion with 0.08% HS (0.39 L/g VS$_{added}$). The difference in the methane yield was significant (p< 0.05). The VS removal rates between these two digestions were similar (42.45%-42.80%). A combination of low methane yield, low methane content, and high VFA concentration from the digestion of 0.35% HS indicated the digester failed. The accumulation of VFA (12918.5 mg/L) in the upset digester was a result of the imbalance between the acidogenesis and the methanogenesis.
Table 7 Measured parameters for biogas and digester effluent from semi-continuous co-digestion of hand sanitizer and sludge cake

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Hand sanitizer content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>Biogas production rate</td>
<td>L/L/d</td>
<td>2.45(0.12)</td>
</tr>
<tr>
<td>Biogas yield</td>
<td>L/g VS&lt;sub&gt;r&lt;/sub&gt;</td>
<td>1.40(0.07)</td>
</tr>
<tr>
<td></td>
<td>L/g VS&lt;sub&gt;added&lt;/sub&gt;</td>
<td>0.61(0.03)</td>
</tr>
<tr>
<td>Methane content</td>
<td>%</td>
<td>62.80(3.41)</td>
</tr>
<tr>
<td>Methane production rate</td>
<td>L/L/d</td>
<td>1.54(0.11)</td>
</tr>
<tr>
<td>Methane yield</td>
<td>L/g VS&lt;sub&gt;r&lt;/sub&gt;</td>
<td>0.88(0.06)</td>
</tr>
<tr>
<td></td>
<td>L/g VS&lt;sub&gt;added&lt;/sub&gt;</td>
<td>0.39(0.03)</td>
</tr>
<tr>
<td>Total VFA</td>
<td>mg/L</td>
<td>3715.2(440.6)</td>
</tr>
<tr>
<td>pH</td>
<td>--</td>
<td>7.83(0.09)</td>
</tr>
<tr>
<td>TS</td>
<td>%</td>
<td>5.72(0.12)</td>
</tr>
<tr>
<td>VS/TS</td>
<td>%</td>
<td>62.89(0.56)</td>
</tr>
<tr>
<td>VS reduction</td>
<td>%</td>
<td>42.80(1.14)</td>
</tr>
</tbody>
</table>
The hand sanitizer has potential to increase the methane yield with a very small amount addition due to its high C/N ratio (1029). The methane yield from the sludge cake was reported to be 0.45 L/g VS\text{added} (Lay et al., 1997), which was 20% smaller than the methane yield of 0.17% hand sanitizer in this study. The methane yield from the co-digestion of sludge and the hand sanitizer was comparable with the value from the co-digestions containing sludge in the literature. When 70% sludge (50% primary sludge and 50% waste activated sludge) and 30% sludge from greaser traps was co-digested, a methane yield of 0.34 L/g VS\text{added} and VS removal rate of 58% were obtained at pilot-scale with the HRT of 13 days at 35°C (Davidsson et al., 2008). A biogas yield of 0.6 L/g VS\text{added} was obtained from the co-digestion of sewage sludge and potato processing waste (Murto et al., 2004). A methane yield 0.62 L/g VS, was obtained in the temperature phased anaerobic digestion treating primary and activated sludge (Riau et al., 2009).

As is shown in Fig. 4.3, the biogas production was started on the very first day in three digesters containing the hand sanitizer. During the first 5-6 days, the biogas production rates from the three digesters were similar. The highest biogas production rate (2.0 L/L/day) was observed in the digester containing 0.35% hand sanitizer on the 6\textsuperscript{th} day. The digester containing 0.08% hand sanitizer reached its peak value of 3.2 L/L/d on the 7\textsuperscript{th} day. For the digester containing 0.17% hand sanitizer, a peak value of 4.6 L/L/d was obtained for the biogas production rate. After reaching the peak values, the biogas production rates from the three digesters decreased dramatically and then remained stable after 15 days (1 HRT). During the final week, the digestion of 0.17% hand sanitizer had the highest biogas production rate (3.20 L/L/d), which was 30.6% higher than that
containing 0.08% HS. Due to upset, the digester containing 0.35% hand sanitizer only produced 0.22 L/L/d biogas (Fig. 11A). The upset may be caused by the anti-microbial substance in the sanitizer.

The methane contents from the three digesters were very similar during the first three days. On the 4\textsuperscript{th} day, the digestion of 0.35% hand sanitizer produced its peak value 34.3%. After that, the methane content decreased slowly. The methane contents in the other two digestions were very close during the first 15 days (1 HRT). Both of digestions containing 0.17% and 0.08% HS reached the peak value, 70.1% and 70.7%, respectively, on the 7\textsuperscript{th} day. During the final week, the methane content from the digestion of 0.17% hand sanitizer was 67.4%, which was 4.6% more than the digestion with 0.08% hand sanitizer (Fig. 11B).
Figure 11 Daily biogas production rate (A) and methane content (B) from co-digestion of sludge cake with hand sanitizer.
During the co-digestions of sludge cake and the hand sanitizer, the VFA concentration increased rapidly in the first 6 days. The peak VFA values ranged from 7570 to 9870 mg/L in the digestions of 0.17% and 0.08% hand sanitizer were appeared on the 7-8th day, which indicated slower growth of methanogens than acetogens. After the 8th day, the VFA from the digestion of 0.17% hand sanitizer dropped to 3340-6336 mg/L while the VFA from the digestions of 0.08% dropped to between 3348 and 6047 mg/L. During the final week, VFA from digestions of 0.17% and 0.08% HS decreased to 3340-3550 mg/L. For the digestion of 0.35% hand sanitizer, the VFA concentration exceeded 12,000 mg/L on the 6th day and stayed above 11,000 mg/L during the remaining days (Fig 12A).

The pH value from the digestion of 0.35% hand sanitizer fell below 6 after 6 days, which was caused by the high VFA. In comparison, the pH value from the digestions containing 0.08% and 0.17% hand sanitizer ranged from 7.8-8.2 during the whole process (Fig. 12B). The short increase of VFA in the digestions of 0.17% and 0.08% HS did not cause any obvious change in the pH, which showed the buffering capacity of the digestion system. The optimal pH was reported to be 6.8 in the digestion of sludge cake. The methane production rate decreased when the pH was lower than 6.6 and larger than 7.8. Moreover, the digestion was upset when the pH was lower than 6.1 and larger than 8.3 (Lay et al., 1997).
Figure 12 The VFA concentration (A) and pH (B) from co-digestion of sludge cake with hand sanitizer.
4.2.2 Co-digestion of dairy manure with creamer

As is shown in Table 8, the highest methane yield (0.47 L/ g VS$\text{added}$) and VS removal rate (69.5%), was obtained with the co-digestion of manure with 68.7% creamer. The methane yield of digestion with 68.7% creamer addition was 6.8% higher than that with 54.3% creamer addition and 42.4% higher than that with 37.0% creamer addition. The methane yield of co-digestion with 37.0% creamer addition was significantly lower than that with 54.3% and 68.7% creamer addition (p<0.05). Meanwhile, the VS removal rate of co-digestion with 69.5% creamer addition was 7.8% and 15.5% higher than that with 54.3% and 37.0% creamer addition, respectively. The VS removal rates were significantly affected by the creamer addition in range from 37.0% to 68.7% (p<0.05).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>37.0</th>
<th>54.3</th>
<th>68.7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biogas production rate</strong></td>
<td>L/L/d</td>
<td>2.37(0.06)</td>
<td>2.68(0.13)</td>
<td>3.07(0.02)</td>
</tr>
<tr>
<td><strong>Biogas yield</strong></td>
<td>L/g VS&lt;sub&gt;r&lt;/sub&gt;</td>
<td>1.06(0.03)</td>
<td>1.07(0.05)</td>
<td>1.06(0.01)</td>
</tr>
<tr>
<td></td>
<td>L/g VS&lt;sub&gt;added&lt;/sub&gt;</td>
<td>0.59(0.02)</td>
<td>0.67(0.03)</td>
<td>0.77(0.01)</td>
</tr>
<tr>
<td><strong>Methane content</strong></td>
<td>%</td>
<td>55.38(1.11)</td>
<td>64.30(1.07)</td>
<td>60.27(3.39)</td>
</tr>
<tr>
<td><strong>Methane production rate</strong></td>
<td>L/L/d</td>
<td>1.30(0.06)</td>
<td>1.74(0.09)</td>
<td>1.86(0.09)</td>
</tr>
<tr>
<td><strong>Methane yield</strong></td>
<td>L/g VS&lt;sub&gt;r&lt;/sub&gt;</td>
<td>0.58(0.03)</td>
<td>0.70(0.04)</td>
<td>0.64(0.03)</td>
</tr>
<tr>
<td></td>
<td>L/g VS&lt;sub&gt;fed&lt;/sub&gt;</td>
<td>0.33(0.02)</td>
<td>0.44(0.02)</td>
<td>0.47(0.02)</td>
</tr>
<tr>
<td><strong>Total VFA</strong></td>
<td>mg/L</td>
<td>4266.2(1066.3)</td>
<td>4139.6(909.6)</td>
<td>2536.6(316.4)</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>--</td>
<td>8.06(0.07)</td>
<td>8.08(0.08)</td>
<td>7.83(0.01)</td>
</tr>
<tr>
<td><strong>TS</strong></td>
<td>%</td>
<td>4.43(0.16)</td>
<td>3.49(0.25)</td>
<td>2.80(0.06)</td>
</tr>
<tr>
<td><strong>VS</strong></td>
<td>%</td>
<td>64.69(0.96)</td>
<td>66.68(3.14)</td>
<td>68.20(1.21)</td>
</tr>
<tr>
<td><strong>VS reduction</strong></td>
<td>%</td>
<td>54.00(2.63)</td>
<td>61.78(1.62)</td>
<td>69.53(1.04)</td>
</tr>
</tbody>
</table>

Table 8 Measured parameters for biogas and digester effluent from the semi-continuous co-digestion of creamer and dairy manure
El-Mashad and Zhang (2007) studied the co-digestion of dairy manure with food waste. At the OLR of 4 g VS/L/day, the methane yield was 0.319-0.329 L/g VS\textsubscript{added} and methane content was 63-65% while the VS removal rate ranged from 54.7 to 61.5%. In the co-digestion of onion juice and wastewater sludge, the highest methane yield obtained was 0.37 L/g VS\textsubscript{added} and the biogas yield was 0.62 L/g VS\textsubscript{added} (Romano and Zhang, 2008). At the HRT of 18 days and C/N ratio of 22.2, the methane content from the digestion of cheese whey was 58% (Backus et al., 1988). The highest methane yield in this study was 27%-47% higher than the values in the literature, while methane content was close. The highest VS removal rate in this study was 8% higher than the literature.

During the co-digestion of dairy manure with creamer, the biogas production rates during the first couple days were much higher than that of the co-digestions of sludge with hand sanitizer. During the first 15 days (1 HRT), the biogas production rates of the digestions with three creamer addition rates were close to each other. Afterward, the biogas production rates were relatively stable. Some of the random decrease and increase in the biogas and methane production may be caused by error occurred in operations such as leaking or sampling. During the final week, the highest biogas production rate of 3.07 L/L/d was obtained from the digestion with 68.7% creamer addition, followed by 2.68 L/L/d from the digestion with 54.3% creamer addition and 2.37 L/L/d from the digestion with 37.0% creamer addition (Fig. 13A).

As is shown in Fig 13B, the methane contents increased rapidly during the first few days and then become stable until the end of the digestions. The methane contents from the digestions of 68.7% and 54.3% were very close during the whole process, if the
randomly decrease in digestion of 54.3% creamer during 23-25 days was ignored.

Although the digestion of 54.3% creamer produced a slightly higher average methane percentage (64.3%) than 68.7% (60.3%) during the final week, the difference was not significant (p>0.05). The methane content from 37.0% creamer (55.4%) was significantly lower than those of the 54.3% and 68.7% creamer (p<0.05).
Figure 13 Daily biogas production rate (A) and methane content (B) from co-digestion of dairy manure with creamer waste
The VFA from the co-digestions of dairy manure and creamer started at about 8000-9000 mg/L and then decreased to the 2537 - 4266 mg/L at the end of digestion (Figure 14A). The consumption may be caused by (1) the washout of the original VFA contained in the inoculums and (2) the adaptability of the microbes. The decrease in the VFA was also confirmed by Wang et al. (2009) in the continuous co-digestion of dairy manure and straw. Similar VFA range (6000-10000 mg/L) was also observed in the continuous digestion process of cheese whey when the HRT was 12 days (Backus et al., 1988). For the digestion of 68.7% creamer, an obvious decrease was observed during 19-21 days, which was in accordance with the fact that the methane production rate dramatically went down during 21-24 days. After that decrease, the VFA concentration went up, so was the methane production rate. Although the VFA decreased during the digestion, the pH was stable and changed subtly. This shows the buffering capacity of the dairy manure. During the final week, the average VFA concentration (4266 mg/L) from the digestion with 37% creamer addition was significantly higher than that of with 68.7% creamer addition (2537 mg/L) (p<0.05). In comparison, the pH value (8.06) from the digestion with 37% creamer addition was also significant higher than that with 68.7% creamer addition (7.83) (p<0.05). This is in accordance with the finding by Braun et al. (1981). When the pH decreased from 8 to 7.4, the VFA decreased from 316 to 20 mg/L. The decreased pH could lower the inhibition of ammonia.
Figure 14 The VFA concentration (A) and pH (B) from co-digestion of dairy manure and creamer
At the beginning, all co-digestions had a similar pH value around 8 (Figure 14B). Although creamer has very low pH value (4.8), the pH value of the co-digestion system stayed neutral after the addition of considerable amount of creamer. This indicated the high alkalinity of the dairy manure. The pH value from the digestion of 68.7% creamer matched well with the pH values (7.66 to 7.73) obtained in the co-digestion of dairy manure and food waste (El-Mashad and Zhang, 2007)

4.3 Conclusion

Increased biogas and methane production was obtained from the co-digestion of both dairy manure with waste creamer and sludge cake with hand sanitizer.

The highest methane yield of 0.54 L/g VS$_\text{added}$ and VS removal rate of 42.5% was obtained with 0.17% hand sanitizer addition when sludge cake was digested with hand sanitizer. Besides, 0.34% hand sanitizer addition caused failure of the digestion process. This study showed that, a little amount of hand sanitizer could improve the methane yield greatly but too much would cause upset. The suitable percentage of sanitizer in the mixture was between 0.17% and 0.35% when the HRT was 15 days.

When dairy manure was co-digested with waste creamer, the highest methane yield of 0.47 L/g VS$_\text{added}$ and VS removal rate of 69.5% was obtained with 68.7% creamer addition. Therefore, the mixing of 68.7% creamer and 31.3% dairy manure was suggested in co-digestion. When the percentage of the creamer increased, the digester could either produce more methane or upset.
Chapter 5: Solid-state Anaerobic Co-digestion of Corn Stover and Algae Waste

After lipid extraction, algae biomass residue has relatively low C/N ratio, which makes it a promising nitrogen source in AD. This chapter examined the feasibility of the solid-state anaerobic digestion of algae waste with corn stover, which acted as a carbon source. The effects of C/N ratio and TS content on the methane yield and VS reduction were studied in this chapter.

5.1 Materials and methods

5.1.1 Characteristics of substrates

The seed sludge was digested wastewater obtained from an on-farm digester in Ohio. The corn stover was collected from OARDC farm and then dried and ground through 5mm sieve. The algae biomass residue was collected from the lipid extraction process. The seed sludge and the algae biomass residue were stored in 0°C refrigerator while the corn stover was stored in tight bags at room temperature.

The characteristics of the wastes including the seed sludge are shown in Table 9. The corn stover and algae biomass residue had comparable TS but very different C/N ratio. The carbon contents of corn stover and algae biomass residue were very close, but the algae biomass contained very high nitrogen content (7.2%) compared to corn stover.
In the result, the C/N ratio of the algae wastes was only 6.0 while the corn stover had 72.7. The pH of the algae biomass residue was as high as 9.8.

Table 9 Characteristics of the wastes used in the solid-state co-digestion

<table>
<thead>
<tr>
<th>Feedstocks</th>
<th>TS, %</th>
<th>VS/TS, %</th>
<th>C, %</th>
<th>N, %</th>
<th>C/N</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn stover</td>
<td>94.7(0.2)</td>
<td>88.0(0.8)</td>
<td>40.9(0.0)</td>
<td>0.6(0.0)</td>
<td>72.7(1.0)</td>
<td>-</td>
</tr>
<tr>
<td>Algae waste</td>
<td>93.1(0.0)</td>
<td>68.8(0.6)</td>
<td>40.3(0.3)</td>
<td>6.7(0.1)</td>
<td>6.0(0.1)</td>
<td>9.8(0.2)</td>
</tr>
<tr>
<td>Seed sludge</td>
<td>7.0(0.1)</td>
<td>74.3(0.3)</td>
<td>3.1(0.1)</td>
<td>0.4(0.0)</td>
<td>7.6(0.2)</td>
<td>8.2(0.1)</td>
</tr>
</tbody>
</table>

*The C% and N% were based on the wet weight. Data in the parentheses are standard deviation.

5.1.2 Solid-state AD experiments

The 500 mL flasks sealed with rubber stoppers were placed in the incubation room under 35±2°C. The flasks were connected to Tedlar gasbags using metal tubes. The working volume was 250 mL. Certain amounts of inoculum, algae waste and corn stover were mixed to obtain the desired C/N ratios and TS contents. The experimental design and the composition of the mixtures in the batch tests are shown in Table 10. The digestions of different mixtures were conducted for up to 33 days. Samples were taken from each reactor before and after digestion for TS, VS, pH and total VFA analysis.
During the digestion, the gas volume and composition were measured every other day. All the tests were conducted in duplicates.

<table>
<thead>
<tr>
<th>Table 10 Experimental design for the solid-state co-digestions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition (% based on the VS)</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>TS=23</td>
</tr>
<tr>
<td>TS=23</td>
</tr>
<tr>
<td>TS=23</td>
</tr>
<tr>
<td>TS=23</td>
</tr>
<tr>
<td>C/N=20</td>
</tr>
<tr>
<td>C/N=20</td>
</tr>
<tr>
<td>C/N=20</td>
</tr>
</tbody>
</table>

5.1.3 Analytical methods

TS and VS were tested following the standard methods for the examination of the water and wastewater (APHA, 2005). The pH was measured using a laboratory glass pH electrode (Mettler Toledo, DG 115-SC, USA) after the solid samples were diluted 11 times and mixed completely. Total carbon and total nitrogen were analyzed with a carbon, nitrogen and sulfur analyzer (VARIO MAS CNS, Elementar, German). The total VFA was measured by titration (Mettlor Toledo DL 22 Food and Beverage Analyzer, USA).

The volume of the biogas in the Tedlar gas bag was measured using wet-drum volumetric gas meter (Ritter, Bochum, Germany). Gas composition was determined using a GC (HP6890, Agilent Technologies, Wilmington, DE) equipped with a 30 m×0.53
mm×10 µm alumina/KCl deactivation column and a thermal conductivity detector (TCD). Helium was used as the carrier gas at a flow rate of 5.2 ml /min. The temperatures of the column, injector, and detector were 40, 150 and 200 °C, respectively.

The methane yield and the VS removal rate from the digestions were analyzed using analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons to determine which variables were significantly different. Significance will be assessed at alpha = 0.05.

5.2 Results and discussion

5.2.1 The effect of C/N ratio

The changes of specific methane yields and methane contents with respect to time are shown in Figure 15. Increasing C/N ratio yielded higher methane yield (Fig. 5.1A). The largest peak (8.09 mL/g VS<sub>added</sub>/day) was appeared on the 7<sup>th</sup> day for the digestion test with C/N ratio of 23. For digestion tests with C/N ratio of 20 and 18, the largest peaks were appeared on days 12 and 17, respectively. Decrease in peak value was observed with the decrease of C/N ratio. The peak value of methane yield at C/N ratio of 23 was 13.0% and 33.3% higher than that obtained at C/N ratio of 20 (7.16 mL/g VS<sub>added</sub>/day) and 18 (6.07 mL/g VS<sub>added</sub>/ day), respectively. In Fig. 15A, there were two obvious peaks observed from the digestion with C/N of 23. The first peak could be caused by the readily degradable substrates in the mixture. The microbes hydrolyzed the polysaccharides such as cellulose and hemi-cellulose in the corn stover, which could have caused the second peak. This indicated that the degradation of the polysaccharides was the rate-limiting step during the digestion of corn stover.
As shown in Fig 15B, the methane content profiles for the C/N ratios of 18 and 23 were similar. For the C/N ratio of 18, 20, and 23, lower methane content was obtained from test with higher C/N. The methane content increased with time at first and then stayed relatively stable at 50% and 44%, respectively, from the digestion of C/N ratios of 18 and 23. In the contrast, the methane content from the digestion with the C/N ratio of 20 decreased after reaching highest value (54.9%) in Fig. 15B. The methane content from each digestion reached the peak value on the same day as the peak specific methane yield.
Figure 15 Specific methane yields (A), methane content (B) and accumulated methane yields (C) of different C/N ratios from the solid-state co-digestions at the TS of 23%.
As is shown in Fig. 15C and Table 11, increase in C/N ratio caused increase in methane yield and VS removal rate and decrease in VFA concentration. At the C/N ratio of 23 and TS content of 23%, digestion of corn stover produced the highest biogas yield (387.6 mL/g VS added), methane yield (166.7 mL/g VS added) and VS removal rate (41.8%). Pang et al. (2008) reported the biogas yield of 376 mL/g VS added at the loading rate of 35g/L when the C/N ratio was 25 under mesophilic condition, which is comparable with the result in this study. In the co-digestion of algae waste with the corn stover at the TS of 23%, the digestion with the C/N ratio of 20 produced 23.4% higher methane yield and
4.6% more VS removal rate than the digestions with the C/N ratios of 18. The statistical analysis showed that the methane yields from the four digestions were significantly different with each other (p<0.05). The difference in the VS removal rates among the four digestions was also significant (p<0.05). A combination of low methane yield (8.33 mL/g VS\textsubscript{added}), methane content (up to 16%), and high VFA (27807 mg/L) indicated the digestion was upset when the C/N was 15 at the TS of 23% (Table 11). This failure and the high VFA could be caused by too much ammonia production during the degradation of nitrogenous matters. Samson and LeDuy (1985) had similar findings: VFA concentration as high as 23200 mg/L and methane yield as low as 0.037 mL/g VS\textsubscript{added} in the continuous digestion of algae slurry at a relatively high loading rate.

### Table 11 VS removal rates and pH, total VFA values before and after the solid-state digestions at the TS of 23%.

<table>
<thead>
<tr>
<th>C/N</th>
<th>pH Before</th>
<th>pH After</th>
<th>Total VFA (mg/L) Before</th>
<th>Total VFA (mg/L) After</th>
<th>VS removal rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>8.3(0.0)</td>
<td>7.3(0.6)</td>
<td>5548.3(513.8)</td>
<td>27807.3(360.1)</td>
<td>17.2(2.4)</td>
</tr>
<tr>
<td>18</td>
<td>8.3(0.0)</td>
<td>8.9(0.0)</td>
<td>4509.8(377.7)</td>
<td>6009.4(646.3)</td>
<td>24.9(0.8)</td>
</tr>
<tr>
<td>20</td>
<td>8.3(0.0)</td>
<td>8.7(0.1)</td>
<td>3953.8(172.4)</td>
<td>5057.7(140.9)</td>
<td>29.5(2.0)</td>
</tr>
<tr>
<td>23</td>
<td>8.5(0.0)</td>
<td>8.7(0.0)</td>
<td>4037.0(392.8)</td>
<td>4729.0(554.0)</td>
<td>41.8(1.0)</td>
</tr>
</tbody>
</table>
The value in this chapter was comparable in the literature. The continuous co-digestion of 50% waste paper and 50% algae wastes produced the methane yield of 292.5 mL/g VS$_{added}$ at the C/N ratios of 18 when the OLR was 4g/L/day (Yen and Brune, 2007).

5.2.2 The effect of TS

When the C/N ratio was maintained at 20, higher methane yield was obtained in digester with low TS content. For digestion test with the TS of 23 %, the peak value of 7.16 mL/g VS$_{added}$/day was appeared on the 11th day. For the digestion test with the TS of 26% nearly no biogas was produced in the first 15 days and a peak value of methane yield (3.61 mL/g VS$_{added}$/day) was observed on the 25th day. Nearly no biogas was produced for the digestion test with TS content of 31% (Fig. 16A). High VFA concentrations (19123.6mg/L and 26897.1 mg/L) were observed in the digesters with TS contents of 26% and 31% (Table 12). The high levels of VFA were produced due to the high organic content from the feedstock, thus inhibited the methane production. The TS of 26% did show the higher methane content (58.8%) at the end of the digestion than the TS of 23% (44%) and 31% (13%) (Fig. 16B). The higher carbon content in the TS of 26% may result in the higher methane content than the TS of 23%.
Figure 16 Specific methane yields (A), methane content (B) and accumulated methane yields (C) of different TS content from the solid-state co-digestions at the C/N of 20.
The cumulative methane yield and VS removal rates were significantly affected by the VS content (p<0.05). The highest cumulative methane yield of 119.5 mL/g VSadded was obtained from the digestion with the TS of 23%. The highest VS removal rate of 29.5% was also obtained from the digestion with the TS of 23%, which was about 2 and 4 times of the digestions with the TS of 26% and 31%, respectively (Table 12).
Table 12 VS removal rates and pH, total VFA values before and after solid-state digestions at the C/N of 20.

<table>
<thead>
<tr>
<th>TS%</th>
<th>pH Before</th>
<th>pH After</th>
<th>Total VFA (mg/L) Before</th>
<th>Total VFA (mg/L) After</th>
<th>VS removal rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>8.3(0.0)</td>
<td>8.7(0.1)</td>
<td>3953.8(172.4)</td>
<td>5057.7(140.9)</td>
<td>29.5(2.0)</td>
</tr>
<tr>
<td>26</td>
<td>8.7(0.0)</td>
<td>8.4(0.4)</td>
<td>5136.4(416.0)</td>
<td>19123.6(1388.1)</td>
<td>12.7(3.1)</td>
</tr>
<tr>
<td>31</td>
<td>8.8(0.1)</td>
<td>6.9(0.4)</td>
<td>5325.0(777.1)</td>
<td>26897.1(1365.8)</td>
<td>7.0(0.5)</td>
</tr>
</tbody>
</table>

5.3 Conclusions

In this chapter, the effects of TS and C/N ratio on the co-digestion of algae biomass residue and corn stover were studied. At TS of 23%, the methane yields decreased with decreasing C/N ratio. The highest methane yield (166.71 mL/g VS<sub>added</sub>) and VS removal rate (41.85%) was obtained from the digestion of corn stover only (C/N = 23) at TS content of 23%. Increase in TS content caused high level of VFA production. When the TS increased from 23% to 31%, the VFA increased from 5057.7 to 26897.1 mg/L. As a result, long lag time or even upset was observed when the TS was higher than 23% at the C/N of 20.

In conclusion, the addition of algae failed to improve the methane yield. The possible reason could be (1) the carbon content was relatively smaller when more algae waste was added and (2) the seed already provided sufficient nitrogen source.
Chapter 6: Conclusion

This research proves that the co-digestion of different wastes can enhance the biogas production, but it is also possible to inhibit the anaerobic digestion process if the nutrients are out of balance. This study provides important baseline data for the operation of commercial digester.

The introduction of food wastes enhanced the dairy manure digestion system by boosting both methane yield and methane content. The digestions containing food waste improved the methane yield by up to 5.2 times, compared to the control (dairy manure only). Digestion with beer produced methane faster than other feedstocks mostly because alcohol was converted into methane faster than other components such as lipid or protein. Beer and FOG had higher methane potential than FPW and creamer.

The addition of 0.34% hand sanitizer caused failure of the digestion process due to the accumulation of VFA and possible existence of anti-bacterial agents in the sanitizer. However, 0.17% sanitizer showed the highest methane yield at the HRT of 15 days. The co-digestion of more than 0.17% sanitizer was recommended. For the co-digestion of creamer and dairy manure, 69% creamer was suggested.
In the co-digestion of corn stover and algae wastes, the addition of algae failed to improve the methane yield. Long lag time and even failure occurred due to the high VFA concentration in the reactor.

Concerns in the projects lied in the feedstocks were not homogeneous and there were large difference between different batches of feedstocks coming in. Lacking of mixing in the batch tests and uncompleted mixing in the continuous tests may also affect the results when compared to literatures. Batch tests could be set up for longer time in the future to obtain the ultimate methane yield.
Reference


