THERMOPHILIC ANAEROBIC DIGESTION WITH ULTRAFILTRATION FOR DAIRY MANURE

By

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ABSTRACT

The purpose of this study was to determine volatile solids (VS) destruction and biogas production from pilot-scale thermophilic anaerobic digestion with ultrafiltration (TADU) utilizing dairy manure as the substrate. Previous studies have established that thermophilic digestion produces larger quantities of methane and inactivates pathogenic microorganisms more completely than mesophilic digestion. No previous reports of membrane ultrafiltration for dairy manure digestion have been published to the author’s knowledge. The study also provides information on anaerobic microorganism toxicity of chemicals commonly found on dairy farms and the effects of biomass storage temperature and storage time on biogas production.

The 90-gallon pilot-scale TADU was operated at a 23 day hydraulic retention time (HRT) and 30-day solids retention time (SRT). Solid/Liquid separation was accomplished through filtration and sedimentation to decouple the HRT and SRT. The influent VS to the digester were 4.35% after undigested cow feed was removed through screening. A full-scale mesophilic digester operated at Tinedale Farms (Wrightstown, WI) was examined for comparison. The Tinedale digester has a volume of 960,000 gallons, a 30-day HRT and SRT, and the non-screened influent contained 6.22% VS.

The TADU produced 86.5-ft³ methane/1000 gallons digester –day @ 35°C and the VS destruction was 0.053 lb VS/ ft³ digester -day. The mesophilic digester
produced 66.4 ft³ methane/1000 gallons digester –day @ 35°C and the VS destruction was 0.043 lb VS/ ft³ digester-day. Therefore, the TADU provided 23% higher VS destruction and 30% higher methane production than the mesophilic digester of comparison. The additional methane produced by the TADU system was estimated to be enough to justify the additional expense of heating based on a 20 day HRT. Both anaerobic digesters produced 12 ft³ CH₄/lb VS_{destroyed} @ 35°C.

Toxicity was determined by utilizing anaerobic toxicity assays (ATA). The 50% inhibition concentrations (IC₅₀) by volume of frequently used farm products ranged from 0.1% for Rumensin and quaternary ammonia chloride to 5.8% (mesophilic) and >14.3% (thermophilic) for Delaval, the only chemical to show significant IC₅₀ difference between mesophilic and thermophilic microorganisms. Several cleaners (Monarch Permorn Acid Cleaner, 1313-SD, and Tri Pfan), sanitizers (Mandate and Zinicin), and detergents (Della and Sheen Ezey) had IC₅₀ values of approximately 1.0% for both mesophilic and thermophilic microorganisms. Prime D pipeline cleaner had an IC₅₀ of approximately 2.4%. Copper sulfate, used as a footbath at 30 mg/L, and Artec teat conditioner both had an IC₅₀ of approximately 4.0%.

Optimum preservation of the methanogenic activity required storage temperatures at or below 20°C to recover the initial thermophilic biogas production rate after extended periods of storage. At storage temperatures of 35 and 55°C the
microorganisms remained active and may have exhibited signs of endogenous
decay after longer storage lengths. Storage at 70°C resulted in long lag times (8 to
16 days) to recover biogas production after 3 and 14 days of storage. After 28
days of storage at 70°C minimal biogas production was recovered when the
system temperature was subsequently returned to 55°C.
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Chapter 1: Introduction

Biological treatment of dairy manure has been used at full scale for many years to convert pollutants to biogas that may be combusted. There are several large-scale farms in Wisconsin currently operating or starting full-scale digesters and burning the biogas for energy. For example, Gordondale Farms (Nelsonville, WI), Tinedale Farms (Wrightstown, WI), Double S Dairy (Markesan, WI), Stencil Farm (Denmark, WI), and Wholesome Dairy (Hilbert, WI) operate mesophilic anaerobic digesters treating dairy manure (Kramer, 2002). The ultrafilter decouples the SRT and HRT, which may provide high rate digestion of the dairy manure. Anaerobic digestion is being used because of increasing concerns about the environmental hazards of manure, such as ozone layer damage, odor problems and pathogen content (Metcalf, 2001). Dependence on imported fossil fuels has also raised interest in alternate energy sources such as biogas from manure. There have been some significant improvements in the design, implementation and support of anaerobic digestion, including new materials, better monitoring devices and more reliable methane (CH₄) to electricity conversion from higher efficiency induction generators or absorption refrigeration units (Metcalf, 2001).

The key to successful economic implementation of manure digestion may involve a holistic view of its uses for pollution control, recovery of valuable resources in the digestion residue and utilization of energy in the biogas through combustion (Switzenbaum, 1995). Anaerobic digesters reduce odor problems and produce biogas, largely methane, which can be collected and burned to produce energy. In
addition, the methane produced is most valuable if it can be readily used, rather than converted into a liquid, stored on site, or purified and sent via pipeline to another site (Jewell et al., 1976). Burning the biogas in an engine-generator set reduces the deleterious effects of the greenhouse gases produced and provides two forms of energy: heat and electrical energy. The heat given off during combustion can warm the digester and influent as well as heat surrounding buildings. The electrical energy can be produced in surplus of farm requirements at large farms like Gordondale Farms in Nelsonville, WI (Kramer, 2002). The excess energy can be sold to the local energy company to recover equipment costs.

Anaerobically digested manure contains fewer pathogens, so the effluent can be dried in a press and used as bedding material (Kramer, 2002). Also, the digester effluent can supply nutrients when applied to cropland (Jewell et al., 1974).

The purpose of the study reported herein was to determine potential advantages of a thermophilic biological process in comparison to a mesophilic digester stabilizing dairy manure when the digester is coupled with an ultrafilter. The digester was operated at Frost Farms (Waterford, WI). Laboratory work was conducted at Marquette University’s Water Quality Center. The performance of a 0.2-micron titanium ultrafilter from Arbortech (McHenry, IL) was also studied for solids separation of digester contents. Transmembrane pressure, cleaning
frequency, cleaning solutions, velocity, flux, and run time were all parameters of interest.

Treating dairy waste with high solids concentration in a completely mixed thermophilic digester combined with an ultrafilter for increased solids retention and good volatile solids destruction appears to be unique to this study. Liao and Lo (1985) conducted a study of some similarity that investigated two-phase thermophilic anaerobic digestion of screened dairy waste with low solids concentrations and a 1 day HRT.

The study reported herein also provided information on the toxicity of commercial chemicals found on dairy farms to anaerobic microorganisms as well as the influence of biomass storage temperature and storage time on biomass activity. Laboratory experiments examining temperature and toxicant effects were conducted on the thermophilic microorganisms cultivated in the Frost Farms TADU as well as a mesophilic culture from Tinedale Farms (Wrightstown, WI) mesophilic digester treating similar dairy waste. Results can be used to predict outcome or recovery time of a digester after a chemical release or temperature change to an operating anaerobic digester.
Chapter 2: Thermophilic Anaerobic Digester with Ultrafiltration

2.A: Introduction

2.A.1 Anaerobic Digestion

During anaerobic digestion, complex organic materials are first hydrolyzed and fermented by facultative (i.e., those that live either in the presence or absence of oxygen) and anaerobic microorganisms into fatty acids (McCarty and Smith, 1986). McCarty and Smith (1986) describe how the fatty acids are oxidized by β-oxidation to produce H₂ and acetate, processes termed dehydrogenation and acetogenesis, respectively. Methanogenesis is the last step in the breakdown of the organics to methane and is accomplished by two different methanogenic organisms. One species of methanogens combines hydrogen and carbon dioxide to produce methane, whereas the other species converts acetic acid into carbon dioxide and methane (McCarty and Smith 1986).

Thermophilic digestion at 55°C is a biological process in which microorganisms convert waste while producing methane, carbon dioxide and traces of other gases. High methane production rate and good stability using completely mixed thermophilic digesters to digest screened dairy manure have been previously observed by others. For example, Liao and Lo (1985) studied single phase and two-phase mesophilic and thermophilic digesters and found no advantage in terms of biogas production in separating the acid and methane forming phases for digestion of dairy manure. Using screened dairy manure as the substrate, there
was no indication that a two-phase system would be superior to the one-phase system under thermophilic conditions (Liao and Lo, 1985). It should be noted that Liao and Lo (1985) screened the raw manure using a number 10 screen. The manure fed to the digester had a volatile solids content of only 3%.

Hydraulically flushed manure may present problems of high volume and low degradable solids concentrations. Liao and Lo (1987) operated a mesophilic fixed-film digester treating hydraulically flushed dairy manure. Three influent manures were prepared; the first was screened with No. 10-mesh (10 openings per inch), the second was the supernatant from settled manure, and the third was supernatant from settled and screened manure. All influents were fed to digesters with 1 day HRT values. The VS destruction was low, 22% for screened manure, 4.4% for settled manure, and 14.3% for screened-settled manure. The methane production was very good, 1.23 L CH₄/L_{digester-day} for screened manure, 1.17 L CH₄/L_{digester-day} for settled manure, 1.06 L CH₄/L_{digester-day} for screened-settled manure (Liao and Lo, 1987).

Another study found two-stage mesophilic anaerobic sequencing batch reactors (ASBR) removed 26-44% VS and destroyed 26-50% more VS than the mesophilic ASBR, both operating with a 6 day HRT and 13-18 day SRT. The two-stage ASBR produced 0.72 L CH₄/L_{digester-day} and the mesophilic ASBR produced 33.3% less methane (Dugba, et. al., 1997).
2.4.2 Membrane Bioreactors

Membrane or ultrafilter coupled biological digesters decouple the solids retention time (SRT) from the hydraulic retention time (HRT). SRT is calculated from the mass of solids in a digester divided by the mass of solids removed per day. HRT is calculated from the digester volume divided by the influent volume per day. Membrane separation uses selective permeability to allow selected components to pass. The processes include dialysis, electrodialysis, and reverse osmosis. Membranes have an effective range of separation from 0.0005 to 0.1 microns (Reynolds and Richards, 1996). Ultrafilters have pores that separate material by particle size, allowing particles smaller than the pore opening to pass. Ultrafiltration has an effective range of separation from 0.005 to 10 microns (Reynolds and Richards, 1996). In traditional systems, like plug flow anaerobic digesters, the SRT cannot be separated from the HRT, thus reducing the amount of control an operator can have over a system. With a membrane or ultrafilter, the effluent filtrate from the digester is theoretically all soluble and solids are retained in the digester. Therefore, the SRT can be controlled separately from the HRT. The extent of solids destruction depends on the rate at which the microorganisms are able to biodegrade the volatile solids and the length of time the solids remains in the system. Extending the SRT allows more complete solids destruction because the microorganisms have more time to perform their task.

Fouling trouble has been a problem for some membranes and filters in anaerobic systems. As more robust filters and membranes have been constructed, more
rigorous and frequent cleaning has eliminated some of the fouling trouble. Cleaning can be conducted using caustic or acidic solutions. When strong solutions are applied, the cleaning time is minimized while achieving thorough foulant removal (Lee et al., 2001).

Chemical cleaning must account for both the biological and inorganic fouling of the filter. Lee et al. (2001) reported in a study of anaerobic digestion of piggery waste that an alkali solution (NaOH) was effective for removal of biological foulants and an acidic solution (HCl) was effective for removal of inorganic foulants. After 50 days of operation the permeate flux dropped to 19% of that demonstrated by a new membrane. The flux recovered up to 24% when cleaned with 1 N NaOH alone. The flux recovered to 89% by cleaning the membrane with 1 N NaOH solution and 1 N HCl solution in series (Lee et al., 2001).

Membrane and ultrafiltration technologies have been applied to anaerobic digestion previously for brewery and alcohol distillery wastewater (Strohwald and Ross, 1992; Fakhru’l-Razi, 1994; Choo and Lee, 1998; Choo and Lee, 1996; Yoon et al., 1999; Kang et al., 2002; Ince et al., 1995; Ince et al., 1997), piggery wastewater (Lee et al., 2001), cellulose (Harada et al., 1994), activated sludge (Takashima et al., 1996; Pillay et al., 1994; Canales et al., 1994), municipal sewage (Kiriyama et al., 1992; Elmaleh and Abdilmoumni, 1998) and various industrial wastewaters (Strachan et al., 1996; van Dijk and Roncken, 1997). No
previous reports of membrane ultrafiltration for dairy manure digestion have been published to the author’s knowledge.

2.B: Methodology

A pilot-scale thermophilic anaerobic digester with ultrafilter (TADU) was constructed from a 31-inch-diameter polypropylene barrel (Figures 1 and 2). The barrel was reduced to a height of 33.6 inches creating a volume of 112 gallons (15 ft³). The sides were reinforced by sheet metal and timber. The lid was fashioned from marine-grade plywood and sealed with a rubber gasket and caulk. Liquid seals were used to separate the digester contents from the ambient air. The liquid seals were sections of polyvinyl chloride (PVC) pipe that were open at the top and submerged approximately 18 inches into the digester contents. The shafts of the mixer and heater were placed inside the PVC; therefore, the atmosphere only came into contact with the minimum amount of biomass inside the PVC pipe.
Mechanical components of the digester included a mixer, heater, pump, and ultrafilter. Triad Engineering (Milwaukee, WI) supplied the pump, heater, and Lightnin mixer (Wytheville, VA). The ultrafilter utilized for this study was a titanium coated stainless steel ultrafilter from Arbortech (McHenry, IL). The pore size of the ultrafilter was 0.2 microns.
Figure 2 Schematic of Frost Farm TADU
2.B.1 Acclimation of the microorganisms

Biomass was acquired from a mesophilic anaerobic digester treating waste from milk and cream processing at Kerry Ingredients, Inc. (Jackson, WI). Therefore, acclimation to both temperature and substrate was required. The biomass was allowed to gravity thicken for three days in 55-gallon drums. The total solids (TS) increased from 25,600 mg/L to 42,100 mg/L and the volatile solids (VS) from 10,900 mg/L to 19,800 mg/L. Gravity thickened biomass was added to the digester at ambient temperature on October 7, 2002. The 112-gallon digester was filled with 90 gallons of sludge, leaving 22 gallons of headspace. The temperature was increased over one day to 60°C on October 7th. On October 16th the temperature was reduced to 52°C. The temperature was lowered because heat generated while running the pump during effluent filtering operations raised digester content temperature by several degrees per hour, creating an environment that may have been too hot and may have inhibited the microorganisms. Initially 2.5 L of manure was fed to the digester. The feed volume was increased to 34.1 L over a 40-day period from October 11th to November 20th to slowly acclimate the digester to the new dairy waste substrate.

2.B.2 Dairy waste and preliminary treatment

To avoid clogging and excessive wear on the pump and filter surface, undigested cow feed and bedding sand were partially removed from the manure before it was
fed to the digester. The methods of removal are covered in the following paragraphs. Leaving undigested cow feed in the influent could cause clogging of pipes, since the digester-piping diameter to the filter was only 0.75 inches. Also, undigested cow feed and other cellulose-containing material typically degrades slowly under anaerobic conditions. The rate of digestion of raw cellulosics such as straw, corn stover, peat, and wood is limited in the hydrolyses step by the lignin sheath surrounding the cellulose (Speece, 1983). Removal of bedding sand reduced potential scouring of the pump and filter surface.

Influent manure was prepared in three steps. First the manure was diluted with an equal volume of well water and blended. Properties of the well water measured in the laboratory gave the following results: alkalinity 316 mg/L as CaCO₃, hardness 242 mg/L as CaCO₃, and pH 7.2. Next the influent was screened with a number 4 screen (4 openings per inch) to remove undigested cow feed. The mixture did not readily pass through the screen, so a water sprayer (Occasional Use Economy Sprayer, RL Flo-Master, Lowell, MI) was filled with well water and employed to force the feed through the screen. The last step was to macerate the feed with a hand held blender. The average influent to the digester contained 79,300 mg/L TCOD, 25,800 mg/L SCOD, and 42,600 mg/L volatile suspended solids (VSS). The average organic loading rate was 65 lb VSS / 10³ ft³-day. For comparison, Metcalf and Eddy suggest a range of 40-100 lb VSS / 10³ ft³-day for standard rate digestion and 100-200 lb VSS / 10³ ft³-day for high-rate digestion in municipal anaerobic sludge digesters (1991). It should be noted that screening dairy manure
can double the methane production rate based on volatile solids loading rate (Liao and Lo, 1985).

Several problems were encountered while using the first procedure for feed preparation. When the temperature dropped to near freezing, the sprayer was unable to separate the undigested cow feed from the rest of the manure on the screen. Also, the blender was not powerful enough to handle the consistency of the cold manure, so the motor burned out. To continue to facilitate blending, a metal mixer (Squirrel Mixer, Indco, New Albany, IN, Figure 3) and an electric drill were utilized. The mixer worked well to amalgamate the feed and had the added benefit of hay removal. When the mixer was spinning, the feed was pulled through the open ends of the cylindrical structure and the hay was caught on the louvered walls. Approximately 1 gallon of hay was removed from 5 gallons of water-manure mixture. Then the bucket was filled to 4.5 gallons with well water. The process was repeated on a second bucket of manure to acquire the 9-gallon volume of influent necessary for one feeding event.

Figure 3 Mixer Used for Blending
2. B. 3 Digester operation

After November 20th, the influent feed rate was maintained at 9 gal (34.1 L) per feeding, which was performed Monday, Wednesday, and Friday every week. Therefore, the average digester influent was 3.86 gal/day. Filtered or settled effluent was removed from the digester at 4.5 gallons per feeding and another 4.5 gallons of mixed biomass was removed directly from the digester. The aforementioned additions and removals gave the digester a theoretical 23-day HRT and a theoretical 46-day SRT if the filter was continuously utilized. During some periods of the investigation, either the pump or filter was non-operational, making it impossible to filter the effluent. Therefore, the effluent was periodically separated by gravity, rather than by filtering. Settling was accomplished by pumping digester contents into 5-gallon pails left undisturbed for approximately 2 hours. A clarified volume of 4.5 gallons was decanted and wasted, whereas the settled biomass was returned to the digester. Settling the effluent did not achieve solids separation as completely as filtration. During the time the digester was operated, the overall HRT remained at 23 days, but the SRT was 30 days since the effluent was not always filtered.

The volume of biogas produced by the pilot digester was initially measured on site with a gas meter (Wet Test Meter, Scientific Petroleum Instruments, San Antonio, TX). But leaks in the digester cover proved very difficult to permanently seal. An alternative method of estimating biogas production using serum bottles in the laboratory was relied upon. A 50-mL post-feeding aliquot of
the digester contents was placed into a 160-mL serum bottle, sparged with 70% N₂ and 30% CO₂, and incubated in a shaker table at 55°C. The biogas production was measured over time using a glass syringe. The rate of biogas production was determined by plotting cumulative volume of biogas produced versus time. The percent methane in the biogas was determined by gas chromatography as described below.

2.B.4 Monitored parameters

2.B.4.a Standard Methods

Chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS), volatile fatty acids (VFA), alkalinity, ammonia nitrogen (NH₃-N), total Kjeldahl nitrogen (TKN), total phosphorous (Total P), soluble phosphorous (Soluble P), fecal coliforms, percent methane (%CH₄) in biogas formed during BMP testing, temperature, and pH were all measured using standard methods (APHA et al., 1998). Table 1 contains the standard procedures utilized.

Gas chromatography was used to determine the percent methane in the biogas generated in the serum bottles. A 100-microliter sample of headspace gas was injected into a gas chromatograph (GC) with a thermal conductivity detector (Gow Mac, Bethlehem, Pennsylvania). Separation was accomplished with a CTR
Table 1 Standard Methods Utilized (APHA et al., 1998)

<table>
<thead>
<tr>
<th>Parameter Studied</th>
<th>Standard Methods Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>5220 D</td>
</tr>
<tr>
<td>TSS</td>
<td>2540 B</td>
</tr>
<tr>
<td>VSS</td>
<td>2540 E</td>
</tr>
<tr>
<td>VFA</td>
<td>5560 B</td>
</tr>
<tr>
<td>ALKALINITY</td>
<td>2320 B</td>
</tr>
<tr>
<td>NH$_3$-N</td>
<td>4500 NH$_3$ B &amp; C</td>
</tr>
<tr>
<td>TKN</td>
<td>4500 N$_{org}$ B</td>
</tr>
<tr>
<td>TOTAL P</td>
<td>4500 P B &amp; E</td>
</tr>
<tr>
<td>SOLUBLE P</td>
<td>4500 P E</td>
</tr>
<tr>
<td>FECAL COLIFORMS</td>
<td>9221 E 1</td>
</tr>
<tr>
<td>%CH$_4$</td>
<td>6200 C</td>
</tr>
<tr>
<td>Temperature</td>
<td>2550 B</td>
</tr>
<tr>
<td>pH</td>
<td>4500 B</td>
</tr>
</tbody>
</table>

1 column (Alltech, Deerfield, IL). Helium was used as the carrier gas, flowing at 30 ml/min, and the oven temperature was 38°C.

Temperature and pH were recorded each time before influent was introduced into the digester. A thermometer was lowered into the digester through the influent tube to measure the temperature. Extracted digester contents were transported to the Marquette University Water Quality Center for pH measurement. Initial attempts to measure pH in the field were inaccurate due to low ambient temperatures affecting the pH probe. Occasionally, the probe was frozen and inoperable.
2.B.4.b Other methods

Biochemical methane potential (BMP) was used to determine the methane production from the dairy manure. Since the digester was difficult to seal, it was impossible to monitor biogas production on site; therefore, the BMP aliquots were used to estimate digester biogas production.

For the BMP procedure, 50 mL of liquid was removed from the Frost Farm digester just after feeding and blending, and was placed in 160-mL serum bottles in the laboratory. The bottles were sparged for 20 seconds with 30% CO₂ and 70% N₂ to establish anaerobic conditions. The bottles were then placed into a shaker table that was heated to 55 °C. Using a wetted glass syringe, the biogas production was measured daily for the first week, then every two days for the next three weeks. Once during biogas measurement, the headspace gas was injected into a gas chromatograph to determine the percent methane.

Additional serum bottles were started, one per week, which included 4 mL of 16.7-g/L calcium acetate (to provide acetate substrate) to observe the maximum biogas production rate and determine aceticlastic methanogen activity (AMA). Procedures and additional information can be found in Owen et al. (1979).

The flow velocity was measured in the piping leading to the ultrafilter with a Doppler flow meter provided by Triad Engineering (Milwaukee, WI).
2.C: Results and Discussion

2.C.1: Solids Destruction and removal

The influent and effluent average values and standard deviations for quasi-steady state operation (February 28, 2003 to April 17, 2003) are presented in Table 2. Effluent values are reported for both samples that were filtered and that were settled because results varied based on the effluent removal method.

Table 2 Influent and Effluent Characteristics During Steady State

Average values are reported +/- one standard deviation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Settled</td>
<td>Filtered</td>
</tr>
<tr>
<td>TS</td>
<td>65,300 +/- 7,130</td>
<td>31,600 +/- 10,400</td>
</tr>
<tr>
<td>VS</td>
<td>43,500 +/- 5,860</td>
<td>20,600 +/- 7,200</td>
</tr>
<tr>
<td>COD</td>
<td>53,489 +/- 6,430</td>
<td>21,375 +/- 1,945</td>
</tr>
<tr>
<td>SCOD</td>
<td>21,000 +/- 8,655</td>
<td>17,563 +/- 1,808</td>
</tr>
<tr>
<td>NH3-N</td>
<td>1,054 +/- 213</td>
<td>1,695 +/- 126</td>
</tr>
<tr>
<td>N Organic</td>
<td>1,405 +/- 208</td>
<td>1,011 +/- 134</td>
</tr>
<tr>
<td>Total P</td>
<td>478 +/- 49</td>
<td>582 (1 sample)</td>
</tr>
<tr>
<td>Soluble P</td>
<td>41 +/- 18</td>
<td>112 +/- 16</td>
</tr>
<tr>
<td>Fecal Coliforms</td>
<td>42 x 10^6 +/- 30.6 x 10^6</td>
<td>110 (1 sample)</td>
</tr>
</tbody>
</table>
2.C.1.a: Solids destruction over the six-month operating period

Volatile solids (VS) destruction was calculated to be 49% over the 26-week operation of the digester using an overall, non steady-state equation as follows:

\[ \text{VS}_{\text{destroyed}} = \text{VS}_i - \text{VS}_e - \text{VS}_r \]

Where:
- \( \text{VS}_i \) = total sum of lbs VS in influent over 26 weeks
- \( \text{VS}_e \) = total sum of lbs VS in effluent (filtered and settled) and wasted digester contents over 26 weeks
- \( \text{VS}_r \) = lbs VS gained in digester over 26 weeks (\( \text{VS}_{26} - \text{VS}_0 \))
- \( \text{VS}_{26} \) = lbs VS in digester during week 26
- \( \text{VS}_0 \) = lbs VS in digester during week 0
- \( \text{VS}_{\text{destroyed}} \) = lbs VS destroyed during 26 weeks

Total VS introduced into the digester equaled 206 lbs. Removals from the digester included 1 lb VS in the filtered effluent, 43 lbs VS in the settled effluent, and 59 lbs VS in the wasted digester contents. The VS concentration in the digester increased from 19,775 mg/L to 23,642 mg/L, which was a 3-lb VS gain in the system. Subtracting the effluent and system increase in VS from the influent leaves 100 lbs VS destroyed. Dividing VS destroyed (100 lbs) by influent VS (206 lbs) gives 49% VS destruction.
2.C.1.b: Solids destruction over quasi-steady-state period

Consistent operation of a digester for 3 SRTs is commonly considered the length of time required to reach a steady state. To reach steady state, this digester was fed consistently for 105 days, which was 15 days beyond 3 SRTs. While the volume of feed did not deviate during the 3 SRT period, the feed manure concentration did vary somewhat due to natural variation in manure constituents over time at the dairy facility.

Volatile solids destruction was calculated over the last 7 weeks of consistent digester feeding and wasting. The coefficient of variation for the VS destruction was only 6% during this period. Therefore, a quasi-steady state was achieved. The average volatile solids destruction was 0.64 lbs/day or 49%. Also, an average of 17.5 +/- 1.1 lbs VS was maintained in the digester during this period, the coefficient of variation was 6%. As an indication of the stability of the process, the relatively consistent, overall (quasi-steady state and non-steady state) average of 18.3 lbs VS was maintained in the digester.

The SRT of the overall operation was 30 +/- 5.3 days. The SRT for the last 7-week quasi steady state period was also 30 +/- 1.2 days. Therefore, the solids retention time did not change significantly over the course of operation.
2.C.1.c: Solids destruction estimate from biogas production

Theoretically the VS destruction can also be estimated based on biogas production. The total average biogas production in the laboratory serum bottles was 50 mL/day per 50 mL of biomass (1 ft³ biogas/ ft³ digester volume-day) at 55°C. Metcalf and Eddy (1991) have reported typical biogas production values from 12 – 18 ft³ biogas/ lb of volatile solids destroyed at 35°C for municipal sludge digestion. Correcting for temperature to 55°C, the typical values range from 14 – 21 ft³ biogas/ lb of volatile solids destroyed. Using an estimated value of 18- ft³ biogas/ lb of VS destroyed for the dairy waste, VS destruction can be estimated from biogas production as follows:

\[
\frac{1 \text{ ft}^3 \text{ biogas}}{\text{ft}^3 \text{ digester-d}} \times \frac{\text{lb VS_{destroyed}}}{18 \text{ ft}^3 \text{ biogas}} \times \frac{90 \text{ gal}_{\text{digester}}}{7.48 \text{ gal}} \times \frac{\text{ft}^3}{\text{day}} = 0.67 \text{ lb VS}_{\text{destroyed}}
\]

The total VS added per day was 1.37 lbs based on the average concentration of 42,600 mg/L VS in the 3.86 gallons of influent added per day. Therefore, the theoretical VS destruction was also estimated to be 49% based on gas production.

\[
\frac{(0.67 \text{ lbs VS}_{\text{destroyed/day}})}{(1.37 \text{ lbs VS}_{\text{applied/day}})} \times 100\% = 49\%
\]

COD removal in the digester was about 50% of the influent. The calculation was based on the laboratory value of 50 mL biogas / day from 50 mL of biomass and the stoichiometric value of 350 mL CH₄ / g COD\text{removed} @ 0°C (420 mL CH₄ / g COD\text{removed} @ 55°C) (Speece, 1996).
From serum bottle data the digester was estimated to have produced 62 gallons (235 L) of methane per day. Based upon this a calculation can be made to show a theoretical 557 g COD$_r$ / day removal rate. The actual COD removal rate calculated from COD data was 484 g COD$_r$ / day. This value was calculated as the difference between COD added to the digester (383 lbs) and the total COD taken out of the digester as waste (117 lbs) and as effluent (75 lbs).

Soluble COD (SCOD) values were measured for the digester as well. The average influent SCOD was 21,000 +/- 8,650 mg/L and the filtered effluent had an average SCOD of 4,400 +/- 2,180 mg/L and a settled average SCOD of 17,600 +/- 1,800 mg/L.

Influent nitrogen concentrations were 1050 +/- 210 mg/L for ammonia nitrogen (NH$_3$–N) and 1400 +/- 210 mg/L for organic nitrogen (org-N). The effluent contained 1380 +/- 140 mg/L NH$_3$–N in the filtered samples and 1700 +/- 130 mg/L NH$_3$–N in the settled samples. Organic nitrogen samples for the effluent are 110 +/- 10 and 1010 +/- 130 mg/L for filtered and settled samples, respectively.

Phosphorous (P) concentrations were measured as total and soluble phosphorous. The influent values were 480 +/- 50 and 40 +/- 20 mg/L, respectively (8.6% soluble). The effluent measurements were also for total and soluble P, but were categorized as either filtered or settled. The filtered samples must pass through the Arbortech ultrafilter, which had a pore size of 0.2 microns. The standard
methods procedure followed to measure soluble P dictates that the sample be filtered using a 0.45-micron filter. Since the sample has already been filtered at a smaller pore size, the percent soluble data for filtered and settled effluent P are not comparable. The filtered values are 17 +/- 5 mg/L total P and 12 +/- 4 mg/L soluble P, or 71% soluble. The settled values are 582 mg/L total P (1 sample) and 112 +/- 16 mg/L soluble P, or 19% soluble.

Fecal coliform inactivation during quasi-steady state reached 5 log removal for filtered and settled effluent. Influent levels of fecal coliforms averaged 42 x 10^6 +/- 30.6 x 10^6 colony-forming units (CFUs) per 100 mL. During the quasi-steady state operation, the average filtered effluent contained 62 +/- 119 CFUs per 100 mL and the average settled effluent contained 110 CFUs (1 sample) per 100 mL. A minimum filtered count of zero CFUs was recorded on March 26, 2003 during quasi-steady state as the first run of the newly installed ultrafilter. The maximum effluent count of 16 x 10^3 CFUs per 100 mL was recorded from a settled effluent on December 11, 2002 before quasi-steady state.

Volatile fatty acid (VFA) concentrations varied in both influent and effluent samples (Figure 4). Variations in feed, filtered verses settled effluent, and holes in the filter could have all contributed to the variations.
From the end of January, 2003 (day 98), the effluent VFA concentrations reach a somewhat steady concentration that averaged 217 +/- 79 mg/L for the duration of the study, whereas the influent concentrations continued to fluctuate with an average of 1248 +/- 809 mg/L. For municipal wastewater anaerobic treatment, when digestion is proceeding satisfactorily, the VFA concentration will typically be less than 250 mg/L (Metcalf and Eddy, 1991).

2.C.2: Biogas production and percent methane

Biogas production in the Frost Farm digester was estimated in the laboratory using serum bottles. An aliquot of the digester was obtained approximately 30
minutes after the influent was introduced into the digester. The 30-minute delay was to achieve sufficient mixing to homogenize the digester contents. The sample was transported to Marquette University’s Water Quality Center, where 50 mL of the sludge was placed into each of two serum bottles. The first serum bottle was a methanogen activity assay (MAA), which included 50 mL of sludge and 10,000 mg/L of acetate. The second serum bottle did not receive acetate so as to estimate gas production in the digester. The acetate supplied sufficient substrate to observe the maximum aceticlastic methanogen biogas production rate.

In the serum bottles with 50 mL of sludge and no acetate, the rate of biogas production averaged approximately 50 mL/day (see Table 3). The plots of Figure 5 show an initial period when the rate was increasing as the microorganisms probably recovered from oxygen toxicity during pouring and handling. Over the operating time of the digester, some higher and lower rates were observed. Notably, biogas production was at its highest in February (starting on day 110, Figure 5), which corresponds to an influent VS concentration increase. The thicker-than-normal feed created an abundance of substrate, which in turn encouraged growth of the microorganism population. Increased microbial population was probably the reason for the increased rate of biogas production in the serum bottles, which was 54 mL/day with no acetate and 153 mL/day with acetate. Unfortunately the digester contents and feed solids concentration had to be decreased to allow the recycle pump to function properly.
Table 3 Thermophilic Anaerobic Biogas Production at Frost Farm

Gas Production of 160-mL Serum Bottles Not Containing Acetate Over the Operation of the Digester

<table>
<thead>
<tr>
<th>Date</th>
<th>Days</th>
<th>Biogas Production Rate (mL/day)</th>
<th>Date</th>
<th>Days</th>
<th>Biogas Production Rate (mL/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-Oct</td>
<td>3</td>
<td>6.1</td>
<td>3-Feb</td>
<td>110</td>
<td>47</td>
</tr>
<tr>
<td>23-Oct</td>
<td>7</td>
<td>9.4</td>
<td>5-Feb</td>
<td>112</td>
<td>53</td>
</tr>
<tr>
<td>30-Oct</td>
<td>14</td>
<td>12</td>
<td>7-Feb</td>
<td>114</td>
<td>64</td>
</tr>
<tr>
<td>6-Nov</td>
<td>21</td>
<td>10.6</td>
<td>9-Feb</td>
<td>116</td>
<td>59</td>
</tr>
<tr>
<td>22-Nov</td>
<td>37</td>
<td>45</td>
<td>17-Feb</td>
<td>124</td>
<td>51</td>
</tr>
<tr>
<td>25-Nov</td>
<td>40</td>
<td>43</td>
<td>19-Feb</td>
<td>126</td>
<td>54</td>
</tr>
<tr>
<td>27-Nov</td>
<td>42</td>
<td>46</td>
<td>21-Feb</td>
<td>128</td>
<td>65</td>
</tr>
<tr>
<td>4-Dec</td>
<td>49</td>
<td>44</td>
<td>24-Feb</td>
<td>131</td>
<td>62</td>
</tr>
<tr>
<td>11-Dec</td>
<td>56</td>
<td>79</td>
<td>26-Feb</td>
<td>133</td>
<td>53</td>
</tr>
<tr>
<td>16-Dec</td>
<td>61</td>
<td>42</td>
<td>28-Feb</td>
<td>135</td>
<td>73</td>
</tr>
<tr>
<td>18-Dec</td>
<td>63</td>
<td>49</td>
<td>5-Mar</td>
<td>140</td>
<td>48</td>
</tr>
<tr>
<td>2-Jan</td>
<td>78</td>
<td>41</td>
<td>7-Mar</td>
<td>142</td>
<td>42</td>
</tr>
<tr>
<td>9-Jan</td>
<td>85</td>
<td>38</td>
<td>12-Mar</td>
<td>147</td>
<td>49</td>
</tr>
<tr>
<td>13-Jan</td>
<td>89</td>
<td>43</td>
<td>17-Mar</td>
<td>152</td>
<td>45</td>
</tr>
<tr>
<td>15-Jan</td>
<td>91</td>
<td>49</td>
<td>26-Mar</td>
<td>161</td>
<td>40</td>
</tr>
<tr>
<td>17-Jan</td>
<td>93</td>
<td>39</td>
<td>7-Apr</td>
<td>173</td>
<td>37</td>
</tr>
<tr>
<td>22-Jan</td>
<td>98</td>
<td>39</td>
<td>9-Apr</td>
<td>175</td>
<td>44</td>
</tr>
<tr>
<td>29-Jan</td>
<td>105</td>
<td>41</td>
<td>14-Apr</td>
<td>180</td>
<td>51</td>
</tr>
</tbody>
</table>

*Average = 49.2

* Average excludes the first 4 values in table which were measured while the digester feed was being increased.
Proportionally, the Frost Farm TADU containing 90 gallons (341 L) of sludge should produce 90 gallons (341 L) of biogas per day based upon laboratory serum bottle data. With acetate, the maximum rate in the 160-mL serum bottles exceeded 150-mL biogas/day (Figure 6), again from 50 mL of sludge.
Figure 6 TADU Biogas Production from Serum Bottles with Acetate

ATA Results

ml gas

0 50 100 150 200 250 300 350 400 450

days

0 50 100 150 200

y = 59x

y = 116x

y = 123.5x

y = 130x

y = 102.4x

y = 134x

y = 153x

y = 151x

y = 138x

1113

1023RC2

1106RC2

1127RC2

1204RC2

1211RC2

0102RC2

0109RC2

0115RC2

0122RC2

0129RC2

0207RC2

224

226

312

317

326

409

414
Gas chromatography (GC) was used to determine the percent methane in the biogas generated in the serum bottles. The original amounts of gases used to generate anaerobic conditions in the headspace are accounted for by taking the difference between ending and starting amounts of nitrogen and carbon dioxide. The average percent methane in the biogas was 69% without acetate and 71% with acetate based on GC results. The percent methane standard deviations were 6.5% and 9.2% for 36 and 15 serum bottles without and with acetate, respectively.

The lower biogas production rates usually correspond to lower influent VS concentrations (Table 3 and Figure 7). The second half of January and the period from the end of March to the beginning of April demonstrated low biogas production coinciding with some of the lowest influent VS concentrations. The highest influent VS concentration corresponded to the highest biogas production rate on December 11, 2002. The biogas production rate without acetate for the serum bottle reach a maximum of 79 mL/day. That same day, the influent VS concentration was at an all time high of 7.4%, which is significantly higher than the average of 4.2% volatile solids.

Biogas production during start-up was much lower than average, shown in the first 21 days of Figure 7. After the full influent volume of 3.86 gal/day was reached on day 37, the biogas production rate increased to 45 mL/day in the serum bottle containing a 50-mL digester contents sample.
2.C.3: Ultrafilter flux and cleaning

Arbortech (McHenry, IL) supplied the titanium ultrafilter, which was a five-foot pervious pipe with I.D. (inside diameter) of 0.75 inches and O.D. (outside diameter) of 0.875 inches. The surface area was 0.8 ft$^2$ with a pore size of 0.2 microns. Digester contents were recycled through the inside of the ultrafilter, forcing liquid out of the pores. A 2.5-inch-diameter encasement pipe collected the filtrate. The flow velocity in the piping leading to the ultrafilter was measured with a Doppler flow meter provided by Triad Engineering. The flow was 15 gpm (10.9 ft/sec) through the 0.75 inch diameter pipe.
Flux data show an unexpected increase in flux rate without cleaning over the first 70 liters of filtrate produced (Figure 8). After reaching a maximum of 182 L/m²-hr (228 mL/min), the flux rate quickly fell below 80 L/m²-hr.

Figure 8 Ultrafilter Performance

The cleaning solution, 10% sulfuric acid in distilled water, was introduced into the system by pumping it into the filter effluent tube. The cleaning solution filled the chamber on the permeate side of the filter. Therefore, little cleaning was
accomplished on the inside of the filter where the sludge adhered to the surface. When cleaning failed to substantially increase the flux rate after 126 L were filtered (day 63), the filter was removed for inspection. Clogs in the 90° elbows leading to the filter were discovered. The filter was removed from the encasement, which revealed two holes worn completely through the side of the filter (Figure 9). Scouring action of bedding sand in the digester and extended contact with the acidic cleaning solution may have caused the damage.

**Figure 9 Damaged Ultrafilter**
The damaged filter may explain the unexpected increase in flux during early filter operation. The cleaning solution and sand gradually reduced filter wall width and possibly increased the pore size. Higher flux rates may have resulted because the damaged filter provided less resistance for the effluent to navigate.

Eventually the digester contents high solids concentration clogged the recycle piping and minimized the flux. Cleaning the filter had minimal impact because the obstructed recycle flow was not reaching the filter (Figure 8, 110 – 130 L of filtrate). The lack of flux recovery after cleaning and the low flux rate prompted the removal of the filter, which in turn exposed the piping clogs and the filter damage. Efforts to correct these problems included digester content dilution, filter replacement, alternative cleaning solutions, and modifying the method of cleaning solution application.

Membrane fouling could be attributed to the adsorption of organic species, the precipitation of less soluble inorganic species, and the adhesion of microbial cells at the membrane surface (Choo and Lee, 1996). Shearing of microbial floc and reducing the size of digester contents due to mechanical pumping may contribute to fouling as smaller and smaller particles are deposited on the filter.

Improved flux recovery from cleaning was achieved by reconfiguring the connections around the filter to allow the cleaning solution to come in direct contact with the inside surface of the filter. Cleaning solutions were investigated
to find a less corrosive alternative. Since the high strength of the 10% sulfuric acid cleaning solution may have been partially responsible for the filter damage, weaker cleaning solutions were used. First a caustic solution of 3.5% sodium hydroxide (NaOH) was used to remove biological foulants. Then the filter was rinsed with distilled water. Next an acidic solution of 3.0% phosphoric acid was used to remove inorganic foulants. This cleaning process gave a maximum 78% recovery of the original flux rate and in less time than the original cleaning process.

2.D: Conclusions and Recommendations

The Tinedale mesophilic digester and the Frost TADU both produced 12-cft methane/ lb VS\textsubscript{destroyed}. The advantage to thermophilic digestion observed herein was a higher VS destruction and methane production from equivalent digester volumes. The mesophilic digester had 0.043 lb VS\textsubscript{destroyed} / ft\textsuperscript{3} digester – day and produced 66.4 ft\textsuperscript{3} CH\textsubscript{4} / 1000 gallons of digester volume - day. The TADU had 0.053 lb VS\textsubscript{destroyed} / ft\textsuperscript{3} digester – day and produced 86.5 ft\textsuperscript{3} CH\textsubscript{4} / 1000 gallons of digester volume - day. Therefore, the TADU provided 23% more VS destruction and produced 30% more methane than mesophilic digestion from equivalent digester volumes.

Thermophilic digestion will potentially be cost effective since the extra methane supplies ample British thermal units (BTUs) to increase the temperature from 35°C to 55°C. One cubic foot (ft\textsuperscript{3}) of thermophilic digester produces an extra 0.15
ft³ of methane/day, which has 994 BTUs / ft³. Therefore, the thermophilic
digester supplied an additional 149 BTUs / ft³ - day. Assuming a HRT of 20
days means that the influent is 1/20th the digester volume. Therefore, every 20 ft³
of digester may be applied to heat every 1 ft³ of influent. The influent requires
2257 BTUs / ft³ and the thermophilic digester supplies an extra 2990 BTUs /
20 ft³. Therefore, the additional methane produced from TADU is sufficient
to increase the influent temperature by 47.7 °F. This may justify the cost of
thermophilic operation. An anaerobic digestion system that includes heat
recovery will decrease the influent heating requirement, making thermophilic
digestion more appealing. This estimate does not include the $270 cost of the
ultrafilter and, therefore, does not apply to a TADU system, but applies to a
conventional thermophilic digester.

Regarding the ultrafilter, once the proper cleaning configuration was determined,
it appears that an expected minimum flux for the titanium ultrafilter would be
about 80 ml/min or 60 L/m²-hr based on the 0.8 ft² filter surface area. For the
short time that the second ultrafilter was operated, the average flux was about 88
L/m²-hr provided regular cleaning was conducted. From literature, common flux
values are 10-40 L/m²-hr (Choo, K. and Lee, C., 1998 and Norddahl, B. and
Rohold, L., 1998), 40-70 L/m²-hr (Elmaleh, S. and Abdimoumni, L., 1998), and
40-50 L/m²-hr (Pillay, V. L., 1994). Given the time required to produce 4.5
gallons of filtered effluent, this 90-gallon digester would have benefited from two
0.8 ft² Arbortech titanium ultrafilters. Two filters run in parallel would have
reduced the run time of the pump, thereby reducing the shearing of floc and minimizing the temperature increase observed during pump operation. Also, providing two filters would allow cleaning or repair/replacement of one to occur while the other was utilized.

Preparation of feed and equipment selection is very important to the longevity of a digester and the mechanical components. Bedding sand caused problems in the pilot digester by creating excessive wear on the pump, pump seals, and filter. A peristaltic pump could be employed so that the sludge avoids contact with the mechanical parts of the pump. This would have solved the problem with the pump. Sand removal would still be necessary to avoid scouring the filter. In the future, sand could be replaced with dried digester biosolids, eliminating the need for sand removal from a full-scale digester. Drying digested solids and using it for animal bedding was a planned benefit of operating an anaerobic digester, but replacing the sand as bedding before the digester is started would help enormously.

Motor selection also needs special attention. The motor used on the recycle pump may have been undersized to handle the thickness of the sludge. Overworking the motor added extra heat to the digester and, as seen in the temperature effects, overheating thermophilic microorganisms greatly reduces biogas production.
Maintaining influent total solids (TS) concentrations at a manageable level is also important to sustain proper digester operation. Today’s complete-mix digesters can handle manures with TS concentrations of 3%-10%, and generally can handle substantial manure volumes (Biogas Works, 2003). A plug flow digester could be used if higher TS are generated. In the study reported herein, the influent TS concentration was over 10% and the digester contents TS was 9.7% in early January 2003 (day 78). When the filter was removed, clogs in the piping upstream of the filter were discovered. The influent was diluted more than usual to reduce the TS concentration in the digester. However, it is significant that the piping used was only 0.75 inches in diameter and, at full scale, larger pipe would probably be used and not as easily clogged. Digester TS ranged from 4 – 6% much of the time with no clogging problems.

During disassembly of the digester, an accumulation of 6 to 8 inches (20 gallons) of sand was discovered at the bottom of the tank. As the sand would have deposited over time the impact on data acquired early in the study may have been negligible. Once the sand accounted for a significant percentage of the digester volume, the values of gas production, solids destruction, and COD removal may have decreased. The difference the sand made on calculations may have been minor compared to the effect on the system. Most likely the sand was the major cause of pump seal failure as well as being responsible for the filter damage.
Table 4 summarizes and compares the Frost Farm TADU operated for this thesis at steady state and non-steady state to the conventional mesophilic digester operated at Tinedale Farms (Katers and Schultz, 2003). The mesophilic digester at Tinedale also treats dairy manure. However, the raw manure was not screened before it was pumped to the digester.

Table 4 TADU and Mesophilic Parameter Comparison

Frost Farms Steady State and Non-Steady State Parameters Compared to Tinedale Farms Mesophilic Non-Steady State Digestion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>TADU Steady State</th>
<th>TADU Non-Steady State</th>
<th>Mesophilic Digestion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT</td>
<td>days</td>
<td>23</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>SRT</td>
<td>days</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Methane</td>
<td>%</td>
<td>66</td>
<td>69</td>
<td>64</td>
</tr>
<tr>
<td>Inf TS</td>
<td>%</td>
<td>7.0</td>
<td>6.9</td>
<td>7.6</td>
</tr>
<tr>
<td>Inf VS</td>
<td>%</td>
<td>4.35</td>
<td>4.26</td>
<td>6.22</td>
</tr>
<tr>
<td>VS Destroyed</td>
<td>%</td>
<td>49</td>
<td>49</td>
<td>20-45</td>
</tr>
<tr>
<td>Biogas Prod.</td>
<td>mL @ 35°C/mL digester-day</td>
<td>0.90</td>
<td>0.94</td>
<td>0.78</td>
</tr>
</tbody>
</table>


In general, the TADU described herein achieved from 9 to 145% greater VS destruction and approximately 19 - 30% more methane production than the conventional mesophilic digester at Tinedale Farms. TADU and other anaerobic systems handling dairy manure require constant monitoring and regular maintenance. Therefore, they are most applicable to large-scale farms where an operator can monitor and service the digester on a regular routine.
Chapter 3: Toxicant and Temperature Influences on Anaerobic Cultures

3.A: Introduction

The anaerobic toxicity assay (ATA) is a method used to establish the potential inhibitory effects of a chemical or substance to methane production (Owen et al., 1979). Blum and Speece (1991) used the ATA procedure to test a range of chemicals for toxicity, focusing on suites of substances such as aromatics, chlorinated benzene, and halogenated phenols. The concentration of a chemical that inhibited methane production by 50% (IC$_{50}$) of over 50 chemicals was determined for aerobic heterotrophs, methanogens, *Nitrosomonas*, and microtox (Blum and Speece, 1991; Speece, 1996).

Others have used ATA procedure to test the toxicity of a variety of chemicals. For example, the ATA procedure was used to determine the ammonia nitrogen IC$_{50}$ between 7000 and 8000 mg/L for an anaerobic digester with a 25 or 40 day SRT, and ammonia nitrogen IC$_{50}$ between 8000 and 9000 mg/L for an anaerobic digester with a 15-day SRT (Bhattacharya and Parkin, 1989). Completely mixed anaerobic digesters fed acetate were inhibited by dissolved sulfide concentrations of 150-200 mg/L (Maillacheruvu *et al.*, 1993). Ethanol was determined to have an IC$_{50}$ of 43,000 mg/L (Speece, 1996). Inhibition of *Methanosarcina* cultures in anaerobic batch reactors prior to acclimation for acrolein, acrylic acid, and allay alcohol were determined to be 20, 60, and 3000 mg/L, respectively (Demirer and Speece, 1998).
Regarding industrial mixtures, Nagel et al., (1999) studied mesophilic methanogenic toxicity of commercial chemicals coming from brewery clean-in-place (CIP) systems. Cleaning materials tested included a detergent and a disinfectant that were both acidic. The detergent IC$_{50}$ was 0.45% and the disinfectant IC$_{50}$ was 0.25% (Nagel et al., 1999).

Taking the time and expense to determine IC$_{50}$ values for large numbers of chemicals is not common. Regarding the mixture of chemicals, it is not economically feasible to determine the specific toxicity of each of the thousands of potentially toxic substances in complex effluents. Therefore, whole-effluent toxicity testing using aquatic organisms is often employed to determine effluent toxicity (Metcalf and Eddy, 1991).

The complex effluent from the stanchion barn contains manure, feed, feed additives, sanitizers, detergents, cleaners, and other chemicals. This slurry of substances becomes the influent to the digester at a dairy farm. Experiments were conducted as part of this study to determine the thermophilic and mesophilic 50% inhibition concentration (IC$_{50}$) of thirteen cleaning agents and one feed additive that are utilized on dairy farms, as well as ammonia.

The cleaning agents have a high probability of contacting microorganisms in an anaerobic digester stabilizing dairy waste because of proximity and usage. Minimal amounts of the chemicals tested can end up in the digester every day.
Cleaners, sanitizers, and feed additives can collect on the barn floor and may then be mixed with the manure.

The digester operator should be concerned with potential anaerobic toxicity. Toxicant concentration spikes can occur from over-use, leaking containers, spills, improper disposal of unused chemicals, or other mechanisms. Knowing IC$_{50}$ values can help agricultural professionals operate a digester to its fullest potential. In addition, if a chemical spill occurs, knowing inhibition concentrations would be valuable when determining a course of action to minimize any adverse impact on an anaerobic digester.

In addition to chemical toxicity, temperature change in a digester can result from the incapacitation of a heating device or thermostat, or the introduction of excess heat from a malfunctioning pump or other mechanical component prone to heat generation. Therefore, the affects of storage temperature and temperature change on methane production were investigated. The duration of storage at various temperatures and its impact on biogas production were examined, as well as the time required by a biomass sample to recover maximum biogas production after storage.
3.B: Methodology

3.B.1: ATA analysis of potential toxicant

For the Anaerobic Toxicity Assay (ATA) procedure, 50 mL of TADU biomass, 4 mL of 16.7 g/L calcium acetate (to provide acetate as substrate), and a 0.2 gram maximum dose of NaHCO₃ (to maintain pH of approximately 7.0) were added to 160-mL serum bottles. A control group of bottles was prepared as described above and run in triplicate with no potential toxicant addition. The remaining bottles received various doses of potential toxicant to produce a series of bottles with increasing doses. All potential toxicants tested were applied at a % volume/volume concentration except for Rumensin, which was applied at a % weight/weight concentration. Two dosed systems (Della and Delaval) were also run in triplicate for statistical analysis. Biogas production rate data could then be used to define the dose-response relationship and the dose of toxicant required to inhibit methane production by 50% as compared to the maximum (uninhibited) value (i.e., the 50% inhibitory concentration, IC₅₀).

The bottles were sparged for 20 seconds with 30% CO₂ and 70% N₂ to help establish anaerobic conditions. The bottles were then placed in a temperature-controlled shaker table at 55°C (thermophilic). Using a wetted glass syringe, the gas production was measured daily for the first week, and then every two days for the next three weeks. A headspace biogas sample was injected into a gas chromatograph to determine the percent methane in the biogas. The process was
repeated for the 15 potential toxicants studied using mesophilic biomass incubated
in a shaker table at 35°C. The mesophilic biomass was from a full-scale
mesophilic anaerobic digester stabilizing dairy waste (Tinedale Farms,
Wrightstown, WI) (Katers and Schultz, 2003).

3.B.2: Temperature influences on anaerobic biomass

The methanogen activity assay (MAA) test was employed to study the affects of
temperature and unmixed storage duration on thermophilic biomass removed
from the Frost Farm TADU. Mesophilic biomass was not investigated. Storage
temperatures examined were 4, 10, 20, 35, 55, and 70°C. Storage durations
included 3, 14, and 28 days. The 160-mL serum bottles were seeded with 50 mL
of TADU biomass and 4 mL of calcium acetate solution (to supply 10,000 mg/L
of acetate). The bottles were then sparged with 70% nitrogen and 30% carbon
dioxide to help create anaerobic conditions. Then the bottles were sealed and
stored unshaken at their respective storage temperature. During storage, the
biogas production was measured using a wetted glass syringe. After 3 days, one
bottle from each storage temperature was given an additional 2 mL of calcium
acetate (to provide 5,000 mg/L acetate) and placed into the shaker table at 55°C.
The additional calcium acetate was added to supply substrate to serum bottles that
remained biologically active after storage. According to Lawrence and McCarty,
the half-saturation acetate utilization rate constant (K_s) is 154 mg/L (Speece,
1996). Therefore, a dose of 5,000 mg/L was assumed to be non-limiting.
In anaerobic biotechnology, the rate-controlling step in the overall process is related to temperature as well as substrate nature, process configuration, and loading rate (Speece, 1983). Typically, a Monod-type equation is assumed to model substrate utilization over a broad range of substrate concentrations. However, a zero order equation will represent the consumption of acetate and production rate \((R)\) of biogas at high substrate concentrations because the concentration \((C)\) is much higher than the half-saturation rate constant \((K_s)\):

\[
R = \frac{k \times C}{K_s + C}
\]

\[
R = k, \text{ when } C \gg K_s
\]

where \(R\) = biogas production rate

and \(k\) = maximum utilization rate

The original 10,000 mg/L and the 5,000 mg/L added upon transfer of the serum bottles are much greater than the reported \(K_s\) value of 154 mg/L. Therefore, \(R\) is theoretically a function of the maximum utilization rate \((k)\) and is independent of the concentration \((C)\). It is therefore assumed that the methane production rate of various cultures fed excess acetate at a defined temperature is a measure of the aceticlastic methanogen activity.
3.C: Results and Discussion

3.C.1: Inhibition concentrations of potential toxicants (IC₅₀)

The Anaerobic Toxicity Assay results were used to determine the IC₅₀ of the potential toxicants tested. The IC₅₀ values and potential toxicants tested are presented in Table 5. Some of the systems dosed with potential toxicants developed a maximum biogas production rate after a lag period of little or no biogas production. The ability of the microorganisms to produce gas after lag periods indicates the potential for acclimation.

The biogas data collected during the ATA incubation were used to prepare plots of thermophilic methane production versus time. A typical plot can be found in Figure 10 for Della Super Liquid Milking Detergent. Remaining plots are presented in the Appendix. The values in the legend of Figure 10 represent the control group, run in triplicate (B-1, B-2, & B-3) and the numerical values in the figure legend are the percent volume (percent weight for Rumensin) concentration of the tested potential toxicants.
### Table 5 Potential Toxicant $IC_{50}$ Values

<table>
<thead>
<tr>
<th>Potential Toxicants</th>
<th>Description</th>
<th>$IC_{50}$ % Mesophilic</th>
<th>$IC_{50}$ % Thermophilic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumensin</td>
<td>Specific methanogen blocker 80 ppm Monensin (Elanco, Indianapolis, IN)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Geron IV</td>
<td>Quaternary Ammonia Chloride and Ethanol (1000 ppm) (Anderson Chemical. Co., Litchfield, MN)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Barn Wash</td>
<td>Monarch Permon Acid Cleaner</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Teat Dip</td>
<td>Pre Dip Sporicidin, 9.59% Phenol mixed at 1:4 v/v</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Della</td>
<td>Super Liquid Milking Detergent</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Sheen Ezy</td>
<td>Heavy Duty Acid Based Detergent</td>
<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Zinicin</td>
<td>Chlorinated Sanitizer, Sodium Hypochlorite (Westfalia-Surge, Naperville, IL)</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Mandate</td>
<td>CIP Acid Sanitizer, Phosphoric, Octanoic, Citric and Decanoic Acids (Ecolab, St. Paul, MN)</td>
<td>2.1</td>
<td>1.1</td>
</tr>
<tr>
<td>1313-SD</td>
<td>Mechanical &amp; CIP Cleaner, Sodium Hydroxide &amp; Hypochlorite</td>
<td>1.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Tri Pfan</td>
<td>Caustic Chlorinated Cleaner, Sodium Hypochlorite (Westfalia-Surge, Naperville, IL)</td>
<td>0.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Prime D</td>
<td>Pipeline Cleaner, Phosphoric, Sulfuric, and Nitric Acids</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Copper Sulfate (CuSO₄)</td>
<td>Foot bath-hoof sanitizer used as 30 mg/L CuSO₄</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Artec</td>
<td>Post Dip Ultra Conditioner, 1.5% Heptanoic Acid (Ecolab, St. Paul, MN)</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Delaval</td>
<td>Iodine 1% concentrate 2% Glycerin mixed with water</td>
<td>5.8</td>
<td>&gt;14.3</td>
</tr>
<tr>
<td>Ammonia Nitrogen (NH₃-N)</td>
<td>Fed as NH₄Cl</td>
<td>7500 mg/L</td>
<td>7500 mg/L</td>
</tr>
<tr>
<td>Calcium Chloride (CaCl₂)</td>
<td>Checking to be sure chloride was not the inhibitory chemical in ammonium chloride (NH₄Cl)</td>
<td>&gt;8000 mg/L</td>
<td>&gt;8000 mg/L</td>
</tr>
</tbody>
</table>
The concentrations of 0.4% Della and 3.5% Delaval were run in triplicate to gain statistical insight. The maximum methane production rates for the control group (zero dose), 0.4% Della, and 3.5% Delaval were 88 +/- 2.1, 113 +/- 1.2, and 127 +/- 0.9 mL methane/50 mL biomass-day, respectively. The systems demonstrated coefficients of variation of 2.4, 1.1, and 0.7% for the maximum methane production, respectively. The low coefficients of variation support the assumption that the results are repeatable.

Material safety data sheets were reviewed to supply additional information about the potential toxicants tested. Artec, a conditioning teat dip, contains heptanoic acid and is highly soluble in water. Mandate, a liquid acid sanitizer, contains 23%
phosphoric acid, reacts with metals, is categorized corrosive (D002) by the Resource Conservation and Recovery Act (RCRA), reacts vigorously with alkaline chemicals, and causes hazardous vapors when mixed with chlorinated detergents or sanitizers. Both Artec and Mandate are products of Ecolab, Food and Beverage Division (St. Paul, MN).

Rumensin, a dietary supplement, is a product of Elanco Animal Health (Indianapolis, IN) and is also called Romensin, Rumensin Premix, Monensin Formulation, and Monensin Premix. Rumensin contains 2-22% monensin sodium, 65-83% diluents, and 1-3% anti-dusting oil. Monensin sodium is toxic and may cause burns or permanent tissue damage to the eyes. Other effects may include changes in heart and muscle tissue or change in heart rate/rhythm. Diluent may include rice hulls, limestone, corn meal, soybean millrun, wheat bran, or semolina. The anti-dusting oil reduces potential inhalation exposure under normal conditions of use.

Copper sulfate, used as hoof cleaner, is delivered as a granular solid and is dissolved with tap water at 30 mg/L on site at many farms. Copper sulfate is considered an immediate health hazard under the Superfund Amendments and Reauthorization Act (SARA) and the lethal dose (LD₅₀) is 1000mg/kg in rats.

Geron IV, a product of Anderson Chemical Company (Litchfield, MN), contains quaternary ammonia chlorides (QAC) and ethanol, which is flammable. QAC is
corrosive and contains 1-2% N-Dimethylammonia Chloride (LD$_{50}$ 366mg/kg in rats).

Zinicin, a corrosive product, contains 12-15% sodium hypochlorite and 0.2-2.0% sodium hydroxide. Zinicin is a pesticide and is harmful or fatal if ingested. Tri-Pfan, a liquid caustic chlorinated cleaner, is corrosive and contains 10-30% sodium hypochlorite and 10-30% sodium hydroxide. Tri-Pfan is harmful or fatal if ingested and will cause severe burns to eyes and skin. Both Zinicin and Tri-Pfan are products of Westfalia-Surge (Naperville, IL).

No discernable difference was observed in the response of mesophilic and thermophilic biomass. The exception to the negligible differences was that of Delaval, which produced no inhibition of methane production in the thermophilic biomass at the concentrations tested, resulting in an IC$_{50}$ greater than 14.3%. To exceed 14.3% contamination of a full scale anaerobic digester would require the discharge of tens of thousands of gallons of Delaval in a large dairy waste anaerobic digester. Therefore, the specific IC$_{50}$ value was not determined because the probability of such a high-dose event is unlikely.

A linear regression was used to determine the maximum rate of methane production for each concentration dose from Figure 10. An example of the linear regression for Della Super Liquid Milking Detergent is presented in Figure 11.
The methane production rate is theoretically proportional to the aceticlastic methanogen activity in the culture and is indicated by the slope of the line, which is 88 mL CH₄/day for the thermophilic culture that did not receive a toxicant dose (Figure 11, slope = 88) and 19.6 mL CH₄/day for the mesophilic culture that did not receive a toxicant dose (data not shown). The different rates were used to plot the rate of methane production versus the potential toxicant concentration. An example dose-response plot for Della Super Milking Detergent is presented in Figure 12. The dose-response plots for the other potential toxicants are presented in the Appendix.
In the case of Della Super Milking Detergent, methane production was inhibited at concentrations greater than 0.4%. A concentration of 0.4% (0.2 ml of Della in 50 ml of anaerobic biomass and 4 mL of acetate solution) was the highest percent dose that showed no adverse affects to the thermophilic biomass. The IC$_{50}$ concentration for the thermophilic biomass was 0.8% (0.45 ml of Della in 50 ml of anaerobic biomass and 4 mL of acetate solution). The IC$_{50}$ concentration for the mesophilic biomass was 0.7% as shown in Figure 13.
3.C.1.a Inhibition from Rumensin

Rumensin is presently a dietary supplement for calves only and is pending approval by the US Food and Drug Administration for use in lactating dairy cows in the US. Rumensin is approved for use in Canada and New Zealand for lactating dairy cows. Monensin is the active ingredient in Rumensin, which is produced by Elanco (Indianapolis, IN). Monensin is an ionophore antibiotic that blocks methanogens in the digestive tract of cows from consuming substrate,
allowing the cow to uptake the maximum amount of nutrition from the feed, rather than have it shunted to methane production by digestive tract methanogens.

The intestinal bacteria do not degrade monensin, allowing the majority of the chemical to be excreted (Hobson et al., 1993). Other dietary supplement antibiotics, such as aureomycin are commercially available but are less effective at inhibiting methane production. Varel and Hashimoto (1981) found that anaerobic digestion of waste from cattle fed aureomycin was only slightly (20%) inhibited when cattle feeding was performed at the manufacturers’ suggested rate. When the digester influent was changed from normal cattle waste to waste from cattle given monensin, the methane production was completely inhibited. However, in batch digesters run over a period of three months, the biomass in the anaerobic digester acclimated to a waste containing monensin and the total methane production was the same as that from the digestion of waste without monensin (Varel and Hashimoto, 1981). Speece et al. (1979) reported similar results, such that monensin at 1 mg/L completely inhibited methane production from acetate in unacclimated cultures. However, gradual acclimation has been demonstrated with no inhibition at 100 mg/L (Speece et al., 1979). Figure 14 shows that some biogas was produced even though Rumensin was present. The system identified in Figure 14 as B-1, B-2, and B-3 represent biogas production from control bottles that received no potential toxicant dose. The percentages in the legend refer to the concentration by weight of Rumensin in the serum bottle.
When biogas samples from the headspace of the controls and Rumensin-containing ATA bottles were injected into a gas chromatograph, it was discovered that the majority of the biogas in bottles containing Rumensin was carbon dioxide. The biogas contained 35-40% methane in all the bottles with Rumensin. For the control group (no toxicant dose), a typical value of 69% methane was calculated (Table 6). Looking at just the methane production instead of total biogas production reveals low methanogen activity (Figure 15).
Table 6 Percent Methane in Biogas Produced in Control & Rumensin Systems

<table>
<thead>
<tr>
<th>Sample %w/w Rumensin</th>
<th>Methane (% produced by biomass)</th>
<th>Carbon Dioxide (% produced by biomass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td>0.18</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>0.9</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>1.8</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>5.3</td>
<td>35</td>
<td>65</td>
</tr>
</tbody>
</table>

Figure 15 Methane Production of Serum Bottles Containing Rumensin

Rumensin demonstrated thermophilic and mesophilic IC₅₀ values of 0.1% by weight (Figure 16 & 17).
The managers at the dairy farm where the thermophilic biomass was cultured (Frost Farms) do not use Rumensin. Therefore, the feed to the TADU did not contain monensin. The supplier of the mesophilic sludge at Tinedale Farms does use Rumensin at 10 lbs per day for the entire dairy. A temperature-phased
digester at Tinedale Farms was in start-up, but over the course of nine months did not successfully operate in the thermophilic section. A possible reason for the lack of biogas production from the thermophilic phase was the inhibitory effect of monensin. The digester was operated longer than the acclimation periods reported earlier by Speece et al., (1979) and Varel and Hashimoto (1981), but it was not operated at steady state. Operational fluctuations may have prevented the microorganisms from acclimating.

3.C.1.b Inhibition from detergents and disinfectants

Nagel et al., (1999) studied mesophilic methanogenic toxicity of commercial chemicals coming from brewery clean-in-place (CIP) systems. Cleaning materials tested included a detergent and a disinfectant that were both acidic. The detergent IC\textsubscript{50} was 0.45\% and the disinfectant IC\textsubscript{50} was 0.25\% (Nagel et al., 1999). The percent concentration was based on volume per volume and the values were determined graphically. Detergents tested for this thesis were similar to the detergent tested by Nagel et al., (1999) and resulted in comparable mesophilic IC\textsubscript{50} values. The mesophilic IC\textsubscript{50} of Della Super Liquid Milking Detergent and Sheen Ezey heavy-duty acid detergent were 0.7\% and 1.1\%, respectively. Comparable disinfectants include Mandate, a CIP acid sanitizer, Monarch Permorn Acid Cleaner Barn Wash, and 1313-SD a mechanical and CIP Cleaner, which had mesophilic IC\textsubscript{50} values 2.1\%, 0.8\%, 1.8\%, respectively.
3.C.1.c Inhibition from ammonia-nitrogen

Laboratory results herein demonstrate an ammonia-nitrogen IC$_{50}$ of 7500 mg/L (approximately 0.75 %) for thermophilic biomass. In comparison, others have reported that under optimal experimental conditions, 8500 mg/L of total NH$_4^+$-N (at neutral pH this would result in 84 mg/L NH$_3$-N) could be tolerated by mesophilic biomass with no decrease in process performance (Speece, 1996). With slug additions, up to 8 g/L of total ammonia-N (TAN) was tolerated at a 15-day solids retention time (SRT) in mesophilic systems (Bhattacharya, S.K. and Parkin, G.F., 1989). It should be noted that, during the ATA test of ammonia toxicity in the laboratory, ammonia was added as ammonium chloride. To confirm that the concentration of chloride was not causing the inhibition, an ATA was conducted with calcium chloride. The results of the calcium chloride test confirmed that the toxicity of ammonium chloride was not a result of the chloride concentration.

3.C.1.d Thermophilic potential of mesophilic biomass

Mesophilic sludge was obtained from Tinedale Farms and incubated in serum bottles at the thermophilic temperature of 55°C in the laboratory. The bottles were run in triplicate both with and without acetate. For the bottles not containing acetate, a maximum rate of 19 +/- 0.6 mL of methane/day was observed and the total biogas was 61 +/- 1.1% methane. The lag time between placing the serum bottles not containing acetate in the shaker table and maximum biogas production
rate was 14 days. For the bottles containing acetate, a maximum rate of 60 +/- 5.3 mL of methane/day was observed, and the total biogas was 79 +/- 4.3% methane. The lag time between placing the serum bottles containing acetate in the shaker table and maximum biogas production rate was 7 days. In comparison, thermophilic culture from the TADU demonstrated methane production rates of 35 +/- 0.6 and 107 +/- 1.0 mL of methane/day with methane percents of 69 +/- 1.5 and 71 +/- 0.4% for bottles without acetate and with acetate, respectively. Therefore, in comparison, the mesophilic biomass from the digester at Tinedale Farms demonstrated thermophilic methanogen activity after a brief period of acclimation.

3.C.2: Biogas Production, a Function of Storage Length and Temperature

Results of the temperature and storage study show that storage at the three coldest temperatures led to approximately a one-day lag before the maximum gas production rate was achieved (Table 7).

The shortest lag of a single bottle was 0 days for storage temperature of 55°C that spent 3 days in storage. The shortest average lag for all three storage lengths at a single storage temperature was observed at 35°C, which averaged roughly 0.3 days. At 55 and 70°C the recovery lag time increased as the storage length increased, although more dramatically at 70°C. The lag time to recover maximum biogas production was fairly consistent between storage lengths for the temperatures of 4, 10, 20, and 35°C.
### Table 7 Variable Storage Temperature and Duration Data

<table>
<thead>
<tr>
<th>Bottle Number</th>
<th>Storage Temp (°C)</th>
<th>Max Rate During Storage (mL/day)</th>
<th>Storage Length (day)</th>
<th>55°C Max Rate a (mL/day)</th>
<th>Lag After Removing From Storage b (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>418</td>
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<td>2</td>
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<td>3</td>
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<td>1</td>
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<tr>
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<td>1</td>
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<td>1.3</td>
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<td>204</td>
<td>1.3</td>
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<td>70</td>
<td>7</td>
<td>28</td>
<td>26</td>
<td>16</td>
</tr>
</tbody>
</table>

a The maximum rate of biogas production after the serum bottle was transferred from the storage temperature to the 55°C shaker table.
b The lag time from the time the serum bottle was placed in a shaker table until the 55°C maximum rate was achieved.
c The rate for this bottle was uncharacteristically low and suggests a loss of biogas.

The maximum rates of biogas production after transferring the serum bottles to the thermophilic shaker table decrease as the storage temperature increased above 55°C (Table 7). In addition, at 35 and 55°C a substantial amount of the calcium acetate was metabolized while the samples were in storage because these temperatures were conducive to microorganism biological activity. The reduction in activity of the microorganisms stored at 35 and 55°C can be divided into two
possible types: low substrate concentration due to utilization and endogenous decay. The maximum biogas production rates decreased with increased storage length. Some of this decrease is potentially the result of endogenous decay. Endogenous decay became more significant during longer storage lengths, thereby reducing the active microbial population and slowing the overall methane production rate.

The systems for 4°C storage temperature and 28 day storage length, and 20°C storage temperature and 14 day storage length were run in triplicate to gain statistical insight. The 4°C storage temperature and 28 day storage length, and 20°C storage temperature and 14 day storage length had biogas production averages of 381 +/- 9.8 and 199 +/- 4.6 mL/day, respectively.

Biogas production rate values after transferring the bottles to the 55°C shaker table show no discernable correlation with storage length for the three coldest temperatures. Therefore, biogas production activity can be considered independent of storage lengths shorter than 28 days at temperatures below 20°C (Figure 18). The previous statement disregards the 14-day, 4°C bottle because it is probable that the data for this bottle are erroneous. Reduced biogas production is apparent at 35 and 55°C in Figure 18. This may be due to endogenous decay during storage. An acetate-rich environment was provided to each serum bottle when the cultures were transferred to the thermophilic shaker table. But the biogas production diminished significantly as the storage length increased for
those bottles maintained at temperatures greater than $20^\circ C$, which remained biologically active during storage.

The decrease in biogas production with storage time corresponding to storage length at $70^\circ C$ appears graphically similar to the reduction present at $35$ and $55^\circ C$ (Figure 18). The theoretical maximum temperature conducive to thermophilic digestion has been reported to be $65^\circ C$ (Metcalf and Eddy, 1991). Exceeding the thermophilic range for more than 14 days appears to have caused significant inhibition of the microorganisms stored at $70^\circ C$.

**Figure 18 Biogas Productions for Various Temperatures and Storage Lengths**

The $4^\circ C$, 14-day serum bottle has an uncharacteristically low biogas production rate and suggests a leak or loss in biogas.
Figure 19 shows the rate loss per degree C (slope of line) for the three storage lengths. Both three and fourteen-day storage lengths show a loss of approximately 2 mL biogas/day-°C. Rate loss for 28-day storage is 5.3 mL biogas/day-°C.

**Figure 19 Biogas Production Rate Loss Variations with Temperature**

![Graph showing rate vs. storage temp](image)

Plotting the rate loss per °C versus storage time showed the trend of increased rate loss for increased storage temperature as the storage time increased (Figure 20).

The temperature data before the serum bottles were transferred to the thermophilic shaker table were observed to determine a rate equation dependent on temperature. An approximate Arrhenius relationship temperature correction
theta (Θ) value was calculated for the temperature range of 20 to 55°C. Temperatures outside this range produced little or no biogas and were not considered. The Θ value was 1.1147, which was calculated from the slope (ln Θ) of the plot of ln (rate) versus T-55°C (Figure 21).

The approximated Arrhenius equation for temperature correction was as follows:

\[ r_T = r_{55} \times \Theta^{(T - 55)} \]

Where \( r_T \) is the rate at temperature T (mL of biogas produced/day) and \( r_{55} \) is the rate at 55°C.
3.D: Conclusions and Recommendations

Optimum preservation of the methanogenic activity requires a storage temperature at or below 20 °C to recovery the initial biogas production rate after extended periods of storage. For storage temperatures between 35 and 55°C the biomass can acclimate to the temperature and remain actively consuming substrate. The reduction in microbial population due to extended storage and endogenous decay at temperatures greater than 20°C caused decreased biogas production when the substrate was re-introduced at 55°C. For temperatures greater than 20°C, shorter periods of storage have a lesser impact on biogas production rate and recovery time than longer storage periods, whereas storage lengths up to 28 days did not affect recovery after storage at temperatures less than approximately 20°C. Therefore, careful monitoring of the digester is
necessary to provide quick response to malfunctions that cause temperature increases.

Typical practice and use of sanitizers, feed additives, and detergents require scrutiny to operate a properly functioning digester. Rumensin and quaternary ammonia chloride have the ability to severely inhibit methane production in both thermophilic and mesophilic digesters at concentrations less than approximately 0.1%. Other cleaners, sanitizers and detergents including Della, Prime D, Artec, Teat Dip, Mandate, Delaval, Copper Sulfate, Monarch, 1313-SD, Sheen Ezey, Zinicin, and Tri Pfan can be significantly toxic at concentrations from 0.4 – 6%.
Chapter 4: Overall Conclusions and Recommendations

1) Thermophilic digestion of screened dairy waste at HRT of 23 days and SRT of 30 days resulted in 49% volatile solids destruction, biogas production of 1 L/L-d @ 55°C, and 5 log removal of fecal coliform.

2) The ultrafilter provided solid/liquid separation and uncoupled the SRT from the HRT. The ultrafilter average flux was 88 L/m²-hour and cleaning was performed when flux declined to 60 L/m²-hour. More work is needed to define sustainable flux rates.

3) Rumensin and quaternary ammonia chloride severely inhibit methane production at concentrations less than approximately 0.1%. Other cleaners, sanitizers and detergents including Della, Prime D, Artec, Teat Dip, Mandate, Delaval, Copper Sulfate, Monarch, 1313-SD, Sheen Ezey, Zinicin, and Tri Pfan can be significantly toxic at concentrations from 0.4 – 6%.

4) Storage temperature at or below 20°C was observed to be necessary to recovery the initial biogas production rate after extended periods of storage. During longer periods of storage at 35 and 55°C, decreased biogas production rates were observed when influent feeding resumed.

5) Technology and understanding of thermophilic anaerobic digestion has advanced to a point that it can be considered reliable and beneficial. But implementation and operation of a system requires meticulous planning for the full potential to be realized. For example, materials processing,
sand and grit, and excess heat from pumps and other equipment can be problematic.

6) The TADU provided good volatile solids destruction and methane production and should be considered for future applications to stabilize dairy waste at large-scale farms. Further research could be conducted to establish the potential benefit of ultrafiltration at a dairy utilizing an alternative to sand for bedding. Insufficient data were collected during this study to fully characterize ultrafilter performance because of sand damage to the ultrafilter.
REFERENCES:


APPENDIX
Thermophilic ATA Methane Production of Potential Toxicants

### ATA of Thermophilic Biomass for Quarternary Ammonium Chloride

- **BQ-1**
- **BQ-2**
- **0.002%**
- **0.02%**
- **0.18%**
- **1.8%**
- **3.6%**
- **6.9%

### ATA of Thermophilic Biomass for Della Super Liquid Milking Detergent

- **B-1**
- **B-2**
- **B-3**
- **0.4%**
- **0.4%-a**
- **0.4%-b**
- **0.4%-c**
- **0.6%**
- **0.8%**
ATA of Thermophilic for Copper Sulfate

ATA of Thermophilic Biomass for Delaval

ATA of Thermophilic Biomass for Mandate-CIP Acid Sanitizer
ATA of Thermophilic Biomass for Sheen Ezey Detergent

ATA of Thermophilic Biomass for Barn Wash Monarch Cleaner

ATA of Thermophilic Biomass for Rumensin, 80 ppm Monensin
Mesophilic ATA Methane Production of Potential Toxicants

ATA of Mesophilic Biomass for Della Super Liquid Milking Detergent

ATA of Mesophilic Biomass for Delaval

ATA of Mesophilic Biomass for Mandate-CIP Acid Sanitizer
Thermophilic Dose-Response Curves for Potential Toxicants

**Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Della Super Liquid Milking Detergent**

- Max rate = 88
- $\frac{1}{2}$ Max rate = 44
- IC$_{50}$ = 0.8%

**Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Rumensin, 80 ppm Monensin**

- Max rate = 88
- $\frac{1}{2}$ Max rate = 44
- IC$_{50}$ = 0.1%

**Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Quaternary Ammonium Chloride**

- Max rate = 88
- $\frac{1}{2}$ Max rate = 44
- IC$_{50}$ = 0.1%
Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Copper Sulfate

Max rate = 88
½ Max rate = 44
IC$_{50}$ = 3.0%

Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Delaval

Max rate = 88
½ Max rate = 44
IC$_{50}$ > 14.3%

Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Mandate

Max rate = 88
½ Max rate = 44
IC$_{50}$ = 1.1%
Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Teat Dip

Max rate = 88
½ Max rate = 44
IC\textsubscript{50} = 0.5%

Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Artec

Max rate = 88
½ Max rate = 44
IC\textsubscript{50} = 4.0%

Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Prime D-Pipeline Cleaner

Max rate = 88
½ Max rate = 44
IC\textsubscript{50} = 2.4%
Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for 1313-SD Mechanical & CIP Cleaner

Max rate = 107
½ Max rate = 53.5
IC₅₀ = 1.2%

Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Tri Pfan

Max rate = 107
½ Max rate = 53.5
IC₅₀ = 1.4%

Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Zinicin Chlorinated Sanitizer

Max rate = 107
½ Max rate = 53.5
IC₅₀ = 1.0%
Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Sheen Ezey Detergent

Max rate = 107
½ Max rate = 53.5
IC₅₀ = 0.9%

Toxicant Concentration (%) vs Rate of Thermophilic Methane Production for Barn Wash Monarch Cleaner

Max rate = 107
½ Max rate = 53.5
IC₅₀ = 0.4%
Mesophilic Dose-Response Curves for Potential Toxicants

Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Della Super Liquid Milking Detergent

- Max rate = 19.6
- £ Max rate = 9.8
- IC$_{50}$ = 0.7%

Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Prime D-Pipeline Cleaner

- Max rate = 19.6
- £ Max rate = 9.8
- IC$_{50}$ = 2.5%

Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Delaval

- Max rate = 19.6
- £ Max rate = 9.8
- IC$_{50}$ = 5.8%
Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Mandate-CIP Acid Sanitizer

Max rate = 19.6
½ Max rate = 9.8
IC₅₀ = 2.1%

Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Teat Dip-Pre Dip Sporicidin

Max rate = 19.6
½ Max rate = 9.8
IC₅₀ = 1.0%

Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Artec

Max rate = 36
½ Max rate = 18
IC₅₀ = 4.0%
Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Rumensin

- Max rate = 36
- ½ Max rate = 18
- IC₅₀ = 0.1%

Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Quaternary Ammonium Chloride

- Max rate = 36
- ½ Max rate = 18
- IC₅₀ = 0.1%

Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Copper Sulfate

- Max rate = 36
- ½ Max rate = 18
- IC₅₀ = 4.0%
Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Tri Pfan

Max rate = 36  
½ Max rate = 18  
IC$_{50}$ = 0.8%

Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Zinicin

Max rate = 36  
½ Max rate = 18  
IC$_{50}$ = 1.1%

Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Sheen Ezey

Max rate = 36  
½ Max rate = 18  
IC$_{50}$ = 1.1%
Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for 1313-SD

Max rate = 36
½ Max rate = 18
IC\textsubscript{50} = 1.8 %

Toxicant Concentration (%) vs Rate of Mesophilic Methane Production for Barn Wash

Max rate = 36
½ Max rate = 18
IC\textsubscript{50} = 0.8 %
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This is to certify that we have examined this copy of the master’s thesis by

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and have found that it is complete and satisfactory in all respects.

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Approved on __________________________