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DESIGN IMPLICATIONS FOR ANAEROBIC MEMBRANE BIOREACTORS AND THE METABOLIC INFLUENCE OF CYCLE TIME FOR THE TREATMENT OF LIQUID DAIRY MANURE

presented by

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has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Biosystems and Agricultural Engineering

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May 4, 2009
Date

DESIGN IMPLICATIONS FOR ANAEROBIC MEMBRANE BIOREACTORS AND THE METABOLIC INFLUENCE OF CYCLE TIME FOR THE TREATMENT OF LIQUID DAIRY MANURE

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Biosystems and Agricultural Engineering

2009

ABSTRACT

DESIGN IMPLICATIONS FOR ANAEROBIC MEMBRANE BIOREACTORS AND THE METABOLIC INFLUENCE OF CYCLE TIME FOR THE TREATMENT OF LIQUID DAIRY MANURE

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James M. Wallace

This research developed a design approach for an anaerobic membrane bioreactor (AnMBR) treating liquid dairy manure with consideration of the cycle time impact on microbial activity. The research builds from initial comparison experiments with an AnMBR and a complete mix digester (CMD) and concludes with testing of various cycle time conditions necessary to develop a qualitative understanding of the associated microbiology. The results from this research and those of previous researchers were integrated into specific design considerations for an AnMBR treating liquid dairy manure.

A pilot-scale AnMBR and an identically sized CMD were designed and constructed to treat a sand-separated dairy manure. The CMD produced 54% more methane than the AnMBR operating at a cycle time of 84. Despite the apparent negative impact on microbial activity, the AnMBR produced an effluent permeate devoid of suspended solids with a COD reduction of 89%. There was also a strong correlation between membrane flux rate and the total solids (TS) concentration of the digester system that indicated declining flux rate with increasing digester TS concentration.

Based on the initial results, a combined CMD/AnMBR digester configuration was studied where the CMD effluent was used as the AnMBR influent. Metabolic evaluation of the biomass from the CMD and the AnMBR using a respirometer setup indicated a

reduction in the interaction between fatty acid oxidizing bacteria and hydrogen consuming methanogens (syntrophic relationship); however, some activity remained.

A final set of experiments evaluated the impact of cycle time, digester volatile solid concentration and cross-flow velocity on the rate of methane production for two AnMBR systems and a control CMD. All digesters received the same sand and solid-liquid separated manure feedstock.

Cycle times as high as 27/day and cross flow velocities up to 4.5 m/s did not produce a negative effect on methane production compared to a CMD control while total VFA concentration for the AnMBR digesters was lower than that of the CMD. Metabolic evaluation illustrated a reduction in syntrophic activity compared to the CMD; however, even at a cycle time of 27/day, the AnMBR biomass retained approximately 25% of the syntrophic activity of the CMD biomass.

Operation at the higher VS concentration of the AnMBR did not confer a methane production advantage compared to the CMD for the operating conditions tested. Considering low VFA concentrations in all of the systems, it was theorized that once steady-state operation was attained, hydrolysis mass transfer limitations controlled available substrate for anaerobic degradation.

Based on the findings of this research, the AnMBR process, when operated at cycle times of 27/day or less, provided equal gas production to a CMD while reducing the COD, phosphorus and pathogen/virus loading by approximately 90%, 95% and 99.96% respectively.

Dedicated to my wife Amy and daughters Abigail, Margaret and Lucille

ACKNOWLEDGEMENTS

I would like to express my gratitude to my major professor and research advisor, Dr. Steve Safferman, for his guidance and support during my Ph.D. study. Special thanks are also extended to my other research advisor, Dr. Bill Bickert, for his support throughout the entire process.

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KEY TO ABBREVIATIONS

AnMBR	Anaerobic membrane bioreactor
CFV	Cross-flow velocity
CMD	Complete mix digester
COD	Chemical oxygen demand
HRT	Hydraulic retention time
IMMS	Integrate manure management system
MLTSS	Mixed liquor total suspended solids
MLVSS	Mixed liquor volatile suspended solids
OLR	Organic loading rate
PFD	Plug flow digester
SRT	Solids retention time
TS	Total solids
TSS	Total suspended solids
VS	Volatile solids
VSS	Volatile suspended solids
VFA	Volatile fatty acids

Chapter 1

INTRODUCTION

During the last four decades, there has been a steep decline in the number of dairy farms in the United States. Thirty-five years ago, there were 124,000 dairy farms. By the mid-1980s, the number decreased to 42,000 and from 1986 to 2006, to 15,500. Projections suggest that only 8,000 dairies will remain by the year 2018 (Hoard's Dairyman, June 2008). This decline is due to increased milk production per cow coupled with increasing herd sizes resulting from industry consolidation.

Land application is the traditional method of animal manure management; however, increasing animal density, growing regulatory oversight and negative public perceptions are acting to shift the manure management paradigm. Three specific characteristics of animal waste management present significant technical challenges. Animal manure has a high organic strength and can exert a chemical oxygen demand in excess of 75,000 mg/L (Pain, West et al. 1984; Demirer and Chen 2005) . Animal manure has a high nutrient concentration that presents serious water quality concerns because it promotes excessive algal growth in receiving waters. Excessive algal growth leads to a depression of dissolved oxygen which can have a deleterious impact on aquatic biota (Vesilind and Peirce 1983). Dairy manure derived wastewater phosphorus concentrations are typically in the range of 300–600 mg/L as P (Vogel 2003; Demirer and Chen 2005) and total nitrogen concentrations are often in excess 2,000 mg/L (Lo, Bulley et al. 1983; Lo and Liao 1985; Ghaly and Echiegu 1992) . Finally, animal manure contains pathogens and viruses (Wong, Xagoraraki et al. 2009).

Manure management is a critical component of every animal agriculture operation and influences the farm's management structure. A farmer's ability to remain economically competitive is often predicated on effective manure management. Manure is spread consistent with required crop nutrient uptake. However, in some cases, fields are nutrient saturated and the farmer must increase the distance that manure is transported, adding cost and complexity. As a result, livestock farmers are seeking new methods to effectively and efficiently manage the manure generated by their operations (Bickert 2006).

Many farmers are turning to anaerobic digestion to enhance their manure management performance (Knight 2003). Anaerobic digestion is a renewable energy technology that has gained significant popular appeal related to the benefits of energy production, manure treatment cost savings, nutrient conversion, odor and pathogen control and co-product recovery (Moser, Mattocks et al. 1998).

The long-term viability of animal agriculture in the United States is largely dependent on integrated manure management systems that incorporate new technologies to provide effective treatment of livestock manure (Bickert 2006). One such technology is the adaptation of the conventional anaerobic digester with a membrane system to form a process commonly known as an anaerobic membrane bioreactor or AnMBR.

Many studies have evaluated the use of AnMBRs for the treatment of a variety of waste streams with the vast majority focused on the operational and water quality outcomes. A few have investigated the impact of the membrane system on microbial activity and the potential for nutrient management or pathogen and virus removal. None has presented a framework for the design of a farm-based AnMBR system. The concept

of coupling an anaerobic process with a cross-flow ultrafiltration membrane process holds promise for enhanced organic treatment, nutrient management and pathogen and virus removal. The objective of this work was to develop a design approach for an anaerobic membrane bioreactor for the treatment of dairy manure with consideration for the metabolic impacts associated with the pump/membrane system.

Chapter 2

BACKGROUND

The basic anaerobic processes to convert organic substrate to its most reduced state, carbon dioxide and methane is first presented. A review of digestion technology, with a specific emphasis on the difference between the complete mix digester and high rate digester systems follows. High rate digester systems are distinguished by equipment configurations that enable the separation of hydraulic retention time (HRT) from solids retention time (SRT). The last portion of this chapter presents specific detail of a high rate digester system that couples a complete mix digester with an ultrafiltration (UF) membrane to produce what is commonly referred to as an anaerobic membrane bioreactor (AnMBR). Chapters 4-6 provide further literature specific to the content.

2.1 Anaerobic digestion process

Anaerobic digestion is a multi-faceted and complex process. No one organism is capable of completely reducing carbonaceous matter to methane. A four step process is required to complete this transformation (Bryant 1979; Speece 1996). Complex organic matter such as proteins, carbohydrates and lipids are first hydrolyzed into less complex compounds such as sugars, amino acids and peptides and these are further fermented to fatty acids by acidogenesis. Long-chained fatty acids (> C2) are converted to acetate, H_2 and CO_2 by acetogenesis. Lastly, acetate and H_2 are converted to methane and carbon dioxide by methanogenesis (McInerney 1979; McCarty and Smith 1986; Samsoon, Loewenthal et al. 1987; Oremland 1988; Speece 1996). Figure 2.1 presents a graphical representation of the process flow and the following subsections discuss hydrolysis, acidogenesis, acetogenesis and methanogenesis in more detail.

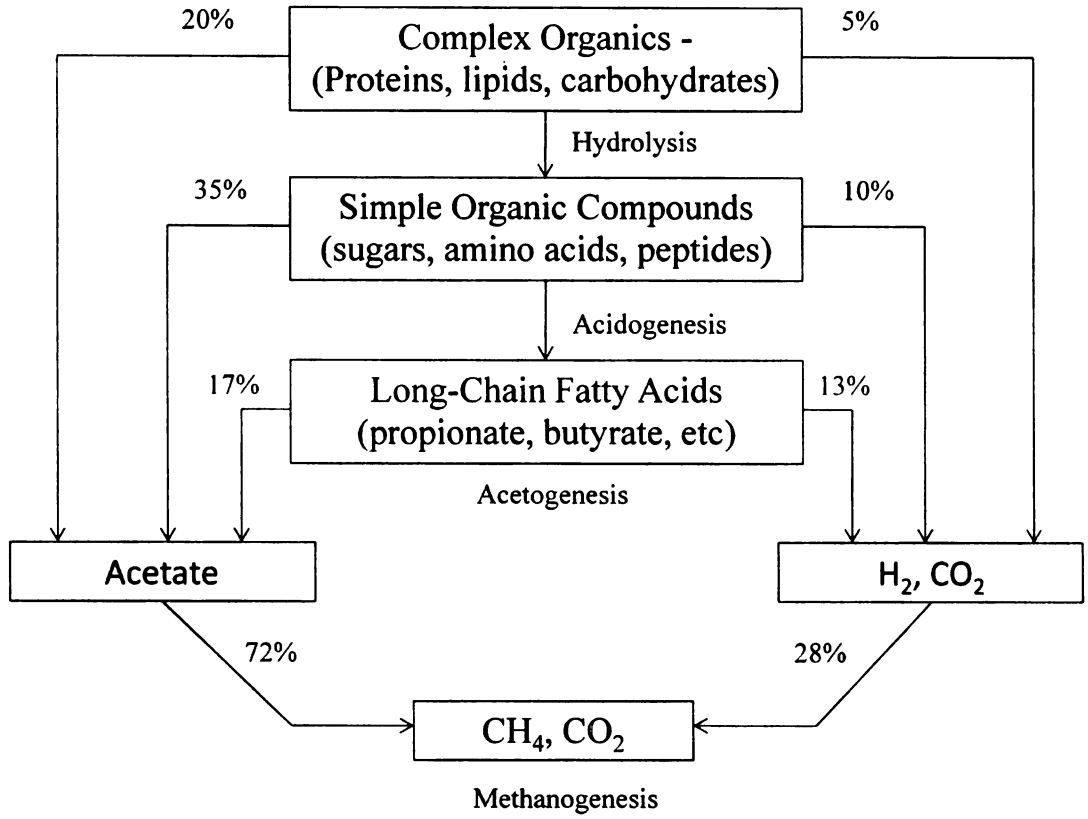


Figure 2.1 Stages of methanogenesis
McCarty and Smith reprinted with permission from Environ. Sci. Technol. Copyright 1986, American Chemical Society

2.2 Hydrolysis

Cellulose and hemicellulose compose a significant portion of the digestable fraction of dairy manure (Amon 2007). Hydrolysis is catalyzed by a variety of different bacteria secreted enzymes such as proteases, lipases and cellulases. In anaerobic environments, the initial enzymatic attack of cellulose is dependent on the activity of a relatively select group of microorganisms (Chayovan, Gerrish et al. 1988). Noike et al. (1985) found that the percentage removal of cellulose fed to a reactor apparatus increased as the solids retention time (SRT) increased. Only 2% of the cellulose fed was removed at an SRT of

1.94 days, while 54% was removed at an SRT of 13.7 days. Based on these results, it was concluded that cellulose is slowly broken down in the hydrolysis phase of anaerobic digestion.

The hydrolysis step is typically rate controlling when the substrate contains a high concentration of particulate matter (Eastman and Ferguson 1981; Vavilin, Rytov et al. 1996; Miron, Zeeman et al. 2000; Rittmann and McCarty 2001; Mahmoud, Zeeman et al. 2004; Zhang, He et al. 2007). Veeken et al. (2000) illustrated that hydrolysis proceeds at pH values between 5.0-7.0; however, they illustrated that lowering the pH below neutral did not provide a hydrolysis rate advantage.

2.3 Acidogenesis

Acidogens ferment the less complex compounds to acetate, formate or to other volatile fatty acids (VFA) and H_2 (Kaspar and Wuhrmann 1978; Boone and Bryant 1980; McCarty and Smith 1986). The optimum pH for acidogenic bacteria is 5.2 – 6.5 and they exhibit doubling times of approximately 2 days (Demirer and Chen 2004).

2.4 Acetogenesis

Acetogenic bacteria represent a complex of species involved in ß-oxidation of fatty acids of even numbered carbons to acetate and H_2, conversion of fatty acids of odd-numbered carbons to acetate, propionate and H_2 and decarboxylation of propionate to acetate, CO_2 and H_2 (Boone and Bryant 1980). As an example, according to Boone and Bryan (1980), propionate is fermented per Equation 2.1.

$$\text{Propionate} + 3H_2O \rightarrow \text{Acetate} + HCO_3^- + H+ + 3H_2, \quad \Delta G^0 = +76.1 \text{ kJ /reaction} \quad (2.1)$$

Propionate conversion is endergonic under standard conditions and only proceeds under low concentrations of H_2 below 10^{-4} atmospheres, while H_2 conversion to methane is only thermodynamically possible at concentrations above 10^{-6} atmospheres (Speece 1996). The H_2 concentration is typically kept low by hydrogentrophic methanogens working in partnership with acetogenic propionate degrading fermenters (Kaspar and Wuhrmann 1978; Boone and Bryant 1980; McCarty and Smith 1986). This relationship is referred to as a syntrophic interaction. The term syntrophic was coined to describe the close cooperation of fatty acid-oxidizing fermenting bacteria with hydrogentrophic methanogens (McInerney 1979; Boone and Bryant 1980). This process is also known as "interspecies hydrogen Transfer (Ianotti 1973) and, in the absence of this syntrophic relationship, fatty acids accumulate. Kasper and Wuhrman (1978) reported that propionate-degrading systems were saturated to only 10-15% of their capacity. This suggests that in a well operating digester system, there should not be a build-up of propionate.

Ideal conditions for acetogenic bacteria are quite different than those favored by hydrolysis and acidogenesis and more closely mirror the conditions under which methanogens thrive. The optimum pH for acetogenic bacteria is 6.6 – 7.6 and they exhibit a minimum doubling time of 3.6 days (Speece 1996).

2.5 Methanogenesis

Methanogens form a unique group of Archae capable of metabolizing a limited number of simple organic compounds, primarily acetate, H_2 and CO_2 to methane. Acetate and H_2 are the two immediate precursors of CH_4 (Yao and Conrad 2001). There are two primary methane forming paths that are relevant to a manure-based anaerobic digestion process, methanogenic respiration and acetate fermentation (McCarty and Smith 1986), and each is discussed below.

For methanogenic respiration, hydrogen acts as the electron donor and CO_2 acts as the electron acceptor as illustrated in Equation 2.2.

$$2H_2\,(g) + \tfrac{1}{2}\,CO_2\,(g) + H^+\,(aq) \rightarrow \tfrac{1}{2}\,CH_4\,(g) + H_2O\,(l) \qquad \Delta G^\circ = -65.37 \qquad (2.2)$$

Kaspar and Wuhrmann (1978) reported that hydrogen removal by hydrogen consuming methanogens (or hydrogentrophic methanogens) was less than 1% of the maximum possible rate, suggesting a large unused capacity able to buffer the partial pressure of dissolved hydrogen in the system. Approximately 30% of methane produced in the anaerobic digestion process results from methanogenic respiration of H_2 and CO_2 (Smith and Mah 1966).

Methanogenesis by acetate fermentation forms CH_4 and CO_2 per Equation 2.3.

$$CH_3COO^-(aq) + H^+\,(aq) \rightarrow CH_4\,(g) + CO_2\,(g) \qquad \Delta G^\circ = -35.83 \qquad (2.3)$$

There are only two known genera of methanogens capable of degrading acetate including the species *Methanosarcina,* which is also capable of utilizing H_2/CO_2 and *Methanosaeta,* which is only able to convert acetate to methane (Harper 1985). Despite a limited number of known organisms capable of degrading it, acetate fermentation accounts for 70% of the total methane produced (Smith and Mah 1966).

Speece (1996) indicated that the generally accepted pH range for methanogenic bacteria is 6.5-8.2. Rittmann and McCarty (2001) suggest a similar range of 6.6 to 7.6.

Doubling times for hydrogen consuming methanogens have been reported between 6-24 hours (Archer and Powell 1985; Rittmann and McCarty 2001), a rate that is considerably greater than that for the acetate consuming methanogens which exhibit reported doubling times ranging between 2 and 9 days, as summarized by Harper and Pohland (1985). Due to the very slow kinetics associated with the methanogenic process, adequate digester retention of the methanogenic consortia is critical to successful anaerobic treatment.

2.6 High Rate Anaerobic Digestion

A significant advantage of the complete mix digester is the simplicity of design and operation. There are minimal internal components required. Submersible or external mounted mixers provide satisfactory agitation with minimal power consumption. Typical organic loading rates for complete mix systems range from 1-4 g COD/L/day (Rittmann and McCarty 2001). Further, complete mix systems are capable of handling total solids loadings consistent with those found in most animal agricultural operations with an influent total solid concentration of 4-10% (Hills and Roberts 1981; Lo, Liao et al. 1984;

Pain, West et al. 1984; Oliver, Pain et al. 1986; Chapman, Phillips et al. 1990; Moller, Sommer et al. 2004).

Hydraulic retention times in standard complete mix digesters are commonly in the range of 10 to 20 days. This is considerably greater than the minimum detention time of 4 days required for acetate using methanogens (Rittmann and McCarty 2001). Dague et al. (1970) reported that the critical solids retention time for anaerobic waste treatment systems was 10 days and that virtually no waste stabilization occurred at solids retention times of 3 days or less. Due to the slow growing nature of methanogens, long retention times are necessary, without which, the anaerobic digestion process will come to a halt.

A negative attribute of the complete mix system is the biomass retention time (or SRT) is equal to the HRT. As a result, the active biomass concentration available to convert substrate entering the digester is limited by its growth rate within the operating HRT. Theoretically, if the SRT is de-coupled from the HRT, a higher concentration of active biomass is available for treatment and a greater degree of substrate conversion achievable.

The development of the anaerobic contact process (Schroepfer, Fullen et al. 1955) resulted from an effort to enhance digester performance by segregating SRT from HRT. By adding a settling tank and recycling the biomass back to the digester tank, separation of HRT from SRT resulted. This process is analogous to the aerobic activated sludge process. Typical organic loading rates associated with the contact process range between 2 — 8 g COD/L/day (Schroepfer, Fullen et al. 1955; Hamdi and Garcia 1991; Hickey 2007). However, entrained biogas in the anaerobic effluent leads to poor settling

characteristics and washout of biomass, degrading effluent quality (Hawkes, Donnelly et al. 1995).

In the late 1960s, the anaerobic filter was developed by Young and McCarty (1969). This process originally used a rock medium for attaching the biosolids, which was eventually replaced with plastic media. Design loadings are often in the 6 to 16 g COD/L/day range (Hawkes, Donnelly et al. 1995; Powers, Wilkie et al. 1997; Rittmann and McCarty 2001). Anaerobic filter systems, also known as fixed-film systems, are particularly well suited for the treatment of soluble organic waste streams. Powers et al. (1997) employed a fixed film process for treating dilute dairy manure resulting from a flush manure collection system that operated at HRTs of 1.5 and 2.3 days and had approximate VS and TS reductions equivalent to a CSTR operated at a HRT of 10 days.

Lettinga et al. (1980) introduced a novel mechanism for segregating HRT from SRT through the use of a process known as the upflow anaerobic sludge blanket reactor (UASB). In a UASB "granules" naturally form after several weeks of digester operation. These compact spherical particles are about 0.5 mm in diameter and consist primarily of a dense mixed population of microorganisms necessary to carry out anaerobic digestion (Rittmann and McCarty 2001). The UASB process is capable of managing organic loading rates as high as 16 g COD/L/day (Lettinga, Vanvelsen et al. 1980). Like the anaerobic filter process, the UASB is particularly well suited for waste streams with high concentrations of soluble COD, but have little tolerance for suspended solids (Hickey 2007). However, Castrillon et al. (2002) used a lab-scale UASB to treat cattle manure and operated this system continuously for approximately one year. During this period, the UASB operated at organic loading rates were between 1.67 and 5.06 g/L/day and

influent TS concentrations between 22.38 and 39.94%. The total sludge accumulation in the UASB was controlled through wasting.

The anaerobic sequential batch reactor (ASBR) was developed at Iowa State University in the late 1990s. Operation is similar to the contact process with the exception that the solids are separated directly in the reactor rather than in an external clarifier. The operation of an ASBR involves four distinct stages: feed, reaction, settling and decanting. The purpose of the settling and decanting stage is to allow the biomass to settle and remain in the tank while removing the digested effluent such that HRT is decoupled from SRT. Biomass granulation has been reported to occur with this process producing a highly active granular mass with good settling properties (Zhang, Yin et al. 1997). This design has been demonstrated for treating swine waste at organic loading rates of 1.6 to 4.5 g VS/L/day with VS reduction ranging from 55 to 61% and BOD reduction of 81 to 86% (Zhang, Yin et al. 1997). With dairy manure, VS reductions of 26.1 to 44.2% have been reported at organic loading rates of 2 to 6 g VS/L/day (Dugba and Zhang 1999). Considering the typical characteristics of swine and dairy manure, this suggests the ASBR is capable of treating waste streams containing relatively high concentrations of suspended solids. Table 2.1 presents a summary of the common high rate digester systems that use various mechanisms to segregate SRT from HRT. Irrespective of configuration, digesters are typically operated in the mesophilic range with an optimum temperature around 35°C or the thermophilic range with an optimum temperature of 55-60°C.

2.7 Anaerobic Reactors Coupled with External UF Membranes (AnMBR)

All of the high rate digester systems described above rely on settling of biomass or adhesion of biomass to media in the digester tank. As a result, each of these systems, to varying degrees, allow biomass to exit the system with the treated effluent. Coupling an anaerobic reactor with an external UF membrane to create an AnMBR is another adaption of traditional digestion technology that seeks to decouple SRT from HRT to improve reactor substrate conversion efficiency. Figure 2.2 is a schematic of the AnMBR and illustrates the placement of the membrane external to the digester tank.

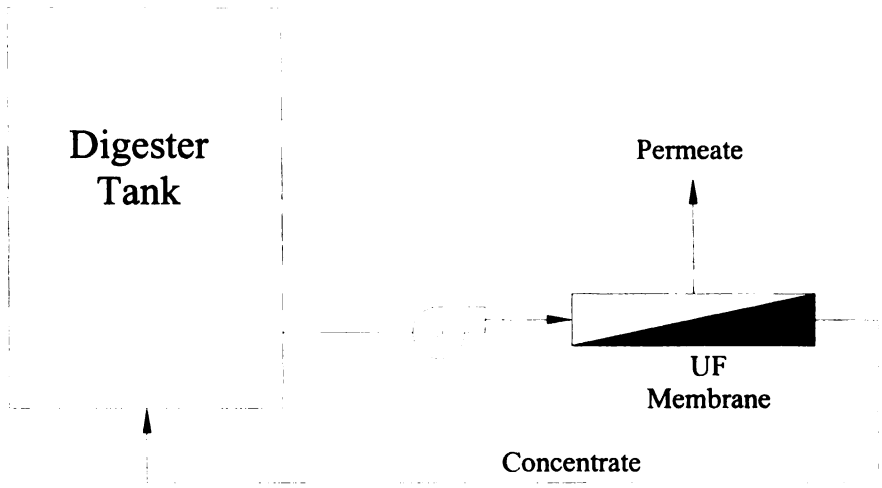


Figure 2.2 AnMBR schematic

A full-scale system will be comprised of multiple membranes, placed in series, parallel or a combination with the placement of the membranes referenced as the membrane configuration. Biomass from the anaerobic reactor is pumped through the UF membrane which provides a physical barrier to prevent wash-out of biomass. Clarified effluent, or

permeate, which is devoid of solids, is removed from the system and concentrated biomass is returned to the digester tank. The biomass concentration in the digester is described by the mixed liquor volatile suspended solids (MLVSS) concentration and is defined based on design conditions. Other parameters commonly used to describe the biomass concentration in the digester include mixed liquor total solids (MLTSS), total solids (TS) and volatile solids (VS). The rate that biomass is pumped through the membrane is known as the cross-flow velocity (CFV) and is determined based on system design.

Much work has been conducted related to the advantages of AnMBRs. The first known research took place in the United States in the mid to late 1970s (Grethlein 1978; Sutton, Berube et al. 2004) and employed a membrane filter coupled to a domestic septic tank system. In the early 1980s, Epstein and Korchin et al. (1981) and Choate, Houldsworth et al. (1983) conducted research with a combined anaerobic reactor and UF membrane in an industrial wastewater treatment capacity. This was followed by the development of the anaerobic digestion ultrafiltration (ADUF) process (Ross, Barnard et al. 1992; Strohwald and Ross 1992; Ross 1994) which utilized an unsupported tubular UF membrane and organic loading rates of 10 g COD/L/day and greater were reported, up to four times that of conventional processes at reduced volume and capital requirements. The ADUF work was followed by the development of the cross-flow ultrafiltration membrane anaerobic reactor (CUMAR) system (Anderson, Kasapgil et al. 1994; Ince, Anderson et al. 1995; Anderson, Kasapgil et al. 1996; Ince 1998; Ince, Ince et al. 2000; Ince, Ince et al. 2001). In a series of publications, a CUMAR system was evaluated for treating brewery wastewater with COD removal efficiencies no lower than 97% while

operating at organic loading rates as high as 28.5 kg COD/m^3/day (Anderson, Kasapgil et al. 1996; Ince, Ince et al. 2000; Ince, Ince et al. 2001). Fakhru'l-Razi (1994) reported operating an AnMBR to treat high strength industrial wastewater at an organic loading rate as high as 19.7 g COD/L/day and achieving COD removal of greater than 96%. Cadi, Huyard et al.(1994) achieved an organic loading rate of 24 g/L/day with a COD removal yield of 87% using starch as the sole carbon source for the study. Fuchs, Binder et al. (2003) achieved COD removal rates of 90% for an artificial wastewater (loading rate of 20 g COD/L/d), sauerkraut brine (8 g COD/L/d) and an animal slaughterhouse wastewater (6-8 g COD/L/d).

Much research supports the AnMBR as an effective process capable of producing excellent effluent quality while providing a very high level of organic conversion. However, other research suggests microbial inhibition due to the shearing impacts associated with turbulent transport of biomass through the membrane system or other high shear applications (Brockmann 1995; Brockmann and Seyfried 1996; Choo and Lee 1996; Brockmann and Seyfried 1997; Ghyoot and Verstraete 1997; He, Xu et al. 2005; Padmasiri, Zhang et al. 2007). Brockman and Seyfried conducted methane potential testing on the biomass from an AnMBR and demonstrated a 50% reduction of microbial activity when the entire contents of the reactor were pumped through the membrane 20 times per day. They theorized that this reduction was due to an interruption in syntrophic activity resulting in an accumulation of VFA. Ghyoot and Verstraete (1997) subjected biomass to displacement through the membrane system (treated biomass) of an AnMBR and compared its activity to that of a control sample (untreated). The treated biomass exhibited a lower biogas production potential and it was concluded that the mechanical

stress of the AnMBR damaged the interaction between the different species in the anaerobic consortia. Padmasiri et al. (2007) also reported a reduction in microbial activity with an anaerobic membrane bioreactor used for the treatment of swine waste. The deterioration in reactor performance was manifested by increased VFA, in excess of the metabolic capacity of the methanogens, and thought to be a direct result of an increase in the hydrolysis rate due to the high shear environment of the AnMBR. Choo and Lee (1996) reported a dramatic reduction in the reactor biomass concentration while operating an AnMBR (3,000 mg/L to 300 mg/L as MLVSS) . A significant amount of biomass was observed attached to the membrane surface during the experimental run and it was theorized that the microbial cells moved from the reactor to the membrane surface to avoid the shear stress of the pump.

Evaluating a similar phenomenon, Stroot, McMahon et al. (2001) evaluated the impact of various mixing conditions on the digestion of municipal solid waste and found that vigorous and continuous mixing had a detrimental impact on microbial activity and caused a disruption in the syntrophic interaction or an increase in hydrolysis leading to an excess of fermentation intermediates (in excess of the methanogens capacity to process). However, in a similar manner, Hoffman, Garcia et al. (2008) evaluated the effect of mixing shear on performance and microbial ecology of continuously stirred anaerobic digesters treating dairy manure and concluded that at four different mixing intensities (50,250, 500 and 1,500 RPM), with the exception of at startup, there was no effect on the biogas production rates and yields at steady-state conditions.

Table 2.2 provides a detailed summary of the AnMBRs described above as well as other AnMBR work of interest to this research. The heading "cycle time", a concept

introduced by Seyfried and Brockmann (1995; 1996; 1997), is used as a metric to compare various levels of AnMBR pump circulation rates. Specifically, cycle time is defined as the period of time required for a discreet particle to travel from the digester tank, through the pump/membrane and return to its initial starting location in the tank. Cycle time is typically presented as number of cycles completed in a 24 hour period. For example, a cycle time of 10 indicates the biomass has, on average, been completely circulated through the pump, membrane and digester tank 10 times in a 24 hour period. In a number of cases, cycle time (or necessary data to calculate cycle time) was not provided and often, the research indicated that excess permeate was returned to the digester tank. This is a typical situation for a laboratory setup because the membranes used are often industrial size units; therefore, the biomass pumping rate is high in relation to the digester tank size and results in excess permeate production. Where permeate is returned to the digester tank, it is likely that the system is operating at a high cycle time.

2.8 Advantages of AnMBR for Nutrient and Pathogen Management

Recent surveys suggest that for many Midwestern dairy farms, phosphorus inputs are greater than phosphorus outputs. This leads to a buildup of phosphorus in the soil and the potential for phosphorus runoff to surface water exists (Beede 2003). Understanding both the fate and chemical composition of nutrients existing in an anaerobic digester is of great interest and importance, particularly to dairy farmers in the Midwest. Converse and Karthikeyan (2002) conducted a series of settling tests to evaluate both flushed dairy manure and effluent from a screw press. After long-term settling (49 days), approximately 75-80% of the total phosphorus was concentrated in the bottom 25% of

the test vessel. Inglis et al. (Inglis 2007) evaluated the phosphorus content of a plug-flow digester during a cleanout operation. The phosphorus concentration of the supernatant was 465 mg/kg, the crust phosphorus level was 686 mg/kg and the bottom phosphorus concentration was 874 mg/kg. Qureshi Lo et al. (2006) evaluated the nutrient recovery balance for a sequencing batch reactor (SBR) treating dairy manure. The phosphorus remaining in the settled fraction of the SBR ranged between 45% - 59%. Masse and Droste (2000) evaluated the phosphorus fate for a psychrophilic anaerobic sequencing batch reactor and, after two cycles, the bioreactors retained on average, 25.5% of the total phosphorus. These findings suggest that phosphorus tends to partition with the solid fraction in an animal manure digester.

The literature contains a little detail regarding the impact of the AnMBR system on the removal of nutrients. Ghyoot and Verstraete (1997) reported 82% removal of total phosphorus, 56% ortho-phosphorus, 66% organic nitrogen and 32% ammonia nitrogen in the AnMBR permeate. Vogel (2003), using a thermophilic AnMBR to treat dairy manure, reported an influent total phosphorus concentration of 478 mg/L and a permeate concentration of 17 mg/L. Wong et al. (Wong, Xagoraraki et al. 2009), using an AnMBR for the treatment of dairy manure found a phosphorus reduction of 96%, a TKN reduction of 31% and no ammonia reduction. Ammonia is soluble and therefore would be expected to pass through the membrane, whereas, organic nitrogen could partition with the solid fraction and would be excluded. The membrane in the AnMBR acts as a very efficient filter precluding solid particles larger than 0.03 μm and therefore, high removal efficiency should be anticipated for constituents that tend to partition with the solid fraction such as phosphorus and organic nitrogen.

Following the same line of reasoning, pathogens and viruses should be excluded based on the membrane pore size. Cicek, Franco et al. (1998) reported operation of an aerobic MBR treating simulated municipal wastewater that completely excluded viruses from the MBR permeate. Grethlein (1978) coupled a membrane with a septic tank system and reported treated effluent that contained no E. *coli*. Total coliforms were removed with an efficiency greater than 99% from liquid pig manure (Fugere, Mameri et al. 2005). Vogel et al. (2003) operated a thermophilic AnMBR for the treatment of liquid dairy manure and reported a 5 log removal of fecal coliform for both filtered and settled (no membrane) effluent. Work conducted by Wong et al. (2008) evaluated the removal of pathogen and virus indicators in the effluent of the AnMBR used for the present research and results are presented in Chapter 3, Section 3.4.

2.9 Objective

Much of the published research related to coupling anaerobic digesters with membranes has focused on water quality outcomes. Little is presented related to specific criteria needed to design an AnMBR. Further, there is also uncertainty in the literature regarding the impact of the AnMBR system on methane productivity.

The purpose of this research work is to develop a design approach for an AnMBR treating liquid dairy manure. Cycle time is thought to exert significant influence over the methane productivity of an AnMBR system and, understanding this impact, is central in the effort to define its role in the AnMBR design. Much effort is dedicated in this research to evaluating the effect of cycle time on biogas production and exploring the

potential mechanism(s) influencing the microbial biota under various digester configurations.

2.10 Research Outline

The research builds from initial comparison experiments with an AnMBR and CMD to testing of specific cycle time conditions that incorporate a qualitative understanding of the associated microbiology, followed by an integration of these findings into design considerations for an AnMBR treating liquid dairy manure. A brief summary of the content of each chapter is presented below.

Chapter 3 - Acquire a general applied knowledge of AnMBR performance and operating characteristics necessary to provide basis for future work.

Chapter 4 – Evaluate methane production at cycle times consistent with anticipated full-scale design and incorporate important design parameters fundamental to the definition of cycle time including digester VS concentration, CFV and membrane configuration.

Chapter 5 – Use activity measurements to characterize and evaluate the microbial pathways associated with the digester configurations presented in Chapters 3 and 4 and explain the affect of cycle time at a metabolic level.

Chapter 6 – Formulate AnMBR design considerations based on the findings of Chapters 3, 4 and 5.

Chapter 7 - Conclusions and Future work

Table 2.1 High rate anaerobic digester configurations

Design	Method of Biomass Retention	Typical Organic Loading Rate, COD g COD/L/d	References
Contact	External settling	2 - 8	(Hamdi and Garcia 1991; Ince, Ince et al. 2001; Hickey 2007)
Anaerobic filters	Packing media	10 -16	(Rittmann and McCarty 2001)
Upflow anaerobic sludge blanket	Methanogenic sludge granules	7.5 - 33	(Lettinga, Vanvelsen et al. 1980; Gavala, Kopsinis et al. 1999; Gao, She et al. 2007)
Sequencing batch reactor	Fill/Settle/Decant	2 – 4 g VS/L/d	(Zhang, Tao et al. 2000)

Table 2.2 Summary of anaerobic membrane bioreactor systems

Parameter	(Ghyoot and Verstraete 1997)	(Fuchs, Binder et al. 2003)	(Cadi, Huyard et al. 1994)	(Fakhru'l-Razi 1994)	(Strohwald and Ross 1992)
Cycle Time, cycles/day	122-162	Excess to digester	Excess to digester	NA	288
Cross flow velocity, m/s	0.6-2.5	NA	2.5	NA	1.5-2.6
Substrate	Anaerobically digested primary sludge	Artificial Slaughterhouse Sauerkraut Brine	Starch	Brewery waste	Brewery waste
Digester size, L	120	7	NA	120	50
Membrane pore size, µm	0.1 (ceramic)	0.2	0.2	NA	NA
Molecular weight cutoff, Dalton	60,000 (polymeric)	NA	NA	10,000	40,000
MLVSS, mg/L	NA	NA	NA	37,150	NA
MLSS, mg/L	8,600	60,000	NA	NA	50,000
VS, mg/L	NA	NA	74,000	NA	NA
TS, mg/L	NA	NA	NA	NA	NA
Feed COD, mg/L	40.2	29,100 20,150 64,600	10,500	84,010	NA
COD destruction, %	29-54	90	87	96	96-99
OLR g COD/L/day	1.2	20 8 6-8	24	19.7	15
Flux rate, $L/m^2/hr$	200-250 (ceramic) 25 (polymeric)	NA	4	NA	10-18
HRT, days	20	NA	0.25	3.98	0.5-0.8
SRT	NA	NA	NA	58.8	NA
Operating pressure, kPa	300-460	NA	160	100-200	140-340
Pump type	Centrifugal	NA	Positive Displacement	Positive Displacement	Positive Displacement
Type of digester operation	Mesophilic	Mesophilic	Mesophilic	Mesophilic	Mesophilic

[1] Calculated from presented data

[2] Average cycle time over feeding cycle of 3 times per week

Table 2.2Continued

Parameter	(Ross, Barnard et al. 1992)	(Ross, Barnard et al. 1990)	(Ince, Ince et al. 2001)	(Brockmann 1995; Brockmann and Seyfried 1996; Brockmann and Seyfried 1997)	(Padmasiri, Zhang et al. 2007)
Cycle Time, cycles/day	1.3	Excess to digester	NA	0 – 150	1,7902[1]
Cross flow velocity, m/s	1.6	2.0	2.4-3.2	1.5-2.0	1.1
Substrate	Maize-processing effluent	Wine distillery waste	Brewery wastewater	Bleaching water	Swine manure
Digester size, L	2,600,000	2,300	120	4,000	6
Membrane pore size, µm	0.1[1]	NA	NA	0.1	NA
Molecular weight cutoff, dalton	NA	NA	200,000	NA	20,000
MLVSS, mg/L	NA	NA	8,000 – 50,000	NA	NA
MLSS, mg/L	21,000	50,000	NA	NA	NA
VS, mg/L	NA	NA	NA	NA	NA
TS, mg/L	NA	NA	NA	NA	NA
Feed COD, mg/L	15,000	37,000	80,000 - 90,000	NA	NA
COD destruction, %	97	93	97	NA	96
OLR g COD/L/day	2.9	11	28.5	>6.0	1.0
Flux rate, L/m^2/hr	8.1-37	37.5	NA	NA	NA
HRT, days	5.2	3.3	4.2	NA	6
SRT	NA	NA	NA	NA	NA
Operating pressure, kPa	450	400	NA	100	NA
Pump type	NA	NA	Positive displacement	NA	Positive displacement
Type of digester operation	Mesophilic	Mesophilic	Mesophilic	Mesophilic	Mesophilic

[1]Calculated from presented data

[2]Average cycle time over feeding cycle of 3 times per week

Table 2.2 Continued

Parameter	(du Preez, Norddahl et al. 2005)	(Zitomer, Bachman et al. 2005)	(Pierkiel and Lanting 2005)	(Ross, Barnard et al. 1992)	(Nagano, Arikawa et al. 1992)
Cycle Time, cycles/day	2.9 – 6.8	42[2]	NA	NA	NA
Cross flow velocity, m/s	1.5 – 3.5	3.3	NA	1.6	NA
Substrate	Pig manure	Dairy manure	Municipal solids	Maize-processing effluent	Liquor wastewater
Digester size, L	5,000	340	NA	3,000	NA
Membrane pore size, µm	NA	0.2	0.1	NA	NA
Molecular Weight Cutoff, dalton	40,000	NA	NA	NA	2,000,000
MLVSS, mg/L	NA	NA	NA	NA	NA
MLSS, mg/L	NA	NA	NA	NA	NA
VS, mg/L	NA	NA	NA	NA	NA
TS, mg/L	40,000 – 80,000	NA	10,000	NA	NA
Feed COD, mg/L	NA	53,000	NA	80,000	NA
COD destruction, %	NA	NA	NA	90	98
VS destruction, %	NA	49	59	NA	NA
OLR g COD/L/day	NA	2.3	NA	5.0	7.0
Flux rate, L/m^2/hr	20 - 38	40 - 80	145	8 - 32	NA
HRT, days	NA	23	1.7 – 11.8	1.6	NA
SRT	NA	30	4.2 – 70.5	NA	NA
Operating pressure, kPa	NA	NA	NA	560	147
Pump type	NA	NA	NA	NA	NA
Type of digester operation	Thermophilic	Thermophilic	Mesophilic	Mesophilic	Mesophilic

[1] Calculated from presented data

[2] Average cycle time over feeding cycle of 3 times per week

CHAPTER 3

UTILITY OF THE ANAEROBIC MEMBRANE BIOREACTOR

3.1 Introduction

An initial plan was developed to assess the AnMBR operating characteristics and conduct a side-by-side comparison with a CMD (CMD and AnMBR comparison study), including gas production. Based on the results of the CMD and AnMBR comparison study, a second experiment was conducted in which the AnMBR was coupled with the CMD such that the effluent of the CMD was the influent to the AnMBR (CMD/AnMBR study). This CMD/AnMBR study emphasized understanding the impact of cycle time on biogas production, enhancing the flux rate of the AnMBR system (including long-term fouling/cleaning impacts) and evaluating the fate of nitrogen and phosphorus for the CMD/AnMBR system.

3.2 Materials and Methods

3.2.1 Analytical Methods

Total solids (TS) and volatile solids (VS) were measured according to AWWA Standard Methods 2540 B and 2540 E respectively. Chemical oxygen demand (COD) was evaluated using Hach (Loveland, Colorado) high range COD test kits. Total Kjeldahl nitrogen (TKN), ammonium nitrogen and total phosphate (TP) were conducted according to "Recommended Methods of Manure Analysis", Bulletin A3769, University of Wisconsin Extension (2003). Methane was measured by gas chromatography using a SRI 310C equipped with a high temperature TCD and an AllTech Porapak Q 80/100

column (6' x 1/8" x 0.85 stainless steel) column (SRI, Torrance, CA). Volatile acid, total alkalinity and bicarbonate alkalinity were measured using a titration method adopted from O'Brien and Donlan (1977), procedure is detailed in Appendix B.

3.2.2 Substrate

The substrate used for the studies was collected on a weekly basis from an operating 3200 cow dairy that uses sand to bed their cows. At this dairy, the manure is scraped from the alleys into reception pits and then processed through sand-manure separators (McLanahan Corporation, Hollidaysburg, PA) for primary sand and grit removal. Samples were collected at the discharge of the sand-manure separator. The manure was collected one time per week and stored in 5 gallon carboys at room temperature. During warm weather, the 5 gallon carboys were stored in a freezer and defrosted as needed. Typical manure characteristics at collection for the CMD and AnMBR comparison study are presented in Table 3.1. Table 3.2 presents the typical substrate characteristics for the CMD/AnMBR study.

Table 3.1 Substrate characteristics for initial CMD and AnMBR comparison

Parameter	Value	Standard Deviation
COD, mg/L	53,700	11,900
TS, %	4.7	1.4
VS, %	3.4	0.6
pH	7.30	0.07

Table 3.2 Substrate characteristics for Combined CMD/AnMBR

Parameter	Value	Standard Deviation
COD, mg/L	35,700	10,500
Total solids, %	3.7	0.8
Volatile solids, %	2.4	0.6
pH	7.04	0.22

3.2.3 CMD System

Figure 3.1 provides a schematic of the CMD system. The CMD system consisted of a 175 cm tall x 30.5 cm diameter section of schedule 40 PVC with flanged ends, a working volume of 105 liters and approximately 30 cm of headspace for gas collection. Mixing was achieved with a 1"x1.5" centrifugal pump (AMT, Inc. Mansfield, OH), operated 6 times per day for 5 minutes. The circulation rate was approximately 45 LPM. A 0.64 cm diameter tube directed the biogas in the digester headspace to a wet tip meter (Wet Tip Meter Company, Nashville, TN). The digester was heated using an external heat blanket and thermostat (BriskHeat, Columbus, OH). Gas samples for GC analysis were collected between the digester and the wet tip meter via a luer-style, 3-way valve. Digested manure was removed from the system once per day based on mass and the same amount of fresh manure was added to the system once per day from a 100-L mix tank. The mix

tank was agitated with a submersible pump for approximately 5 minutes prior to feeding the digester systems.

3.2.4 AnMBR System

A schematic of the AnMBR is presented in Figure 3.2. The digester portion of the AnMBR was constructed identical to the CMD. The membrane was a 0.03 micron, 14.4 mm diameter, 0.079 m^2 PVDF tubular product (X-Flow, Inc., Netherlands) and was operated in a cross-flow configuration using a centrifugal pump (AMT, 1.5" self-priming centrifugal) to generate a circulation rate of approximately 33 L/min (cross flow velocity = 3.4 m/s).

3.2.5 Combined CMD/AnMBR

A schematic of the combined system is presented in Figure 3.3 and consisted of the AnMBR and CMD described in Sections 3.2.3 and 3.2.4 respectively. The AnMBR circulation pump was energized by a timer to achieve a specific cycle time and permeate was removed from the digester during these periods of pump operation. The difference between the mass of substrate fed to the digester (based on design HRT) and the mass of permeate removed from the digester was wasted directly from the digester (See Figure 3.3).

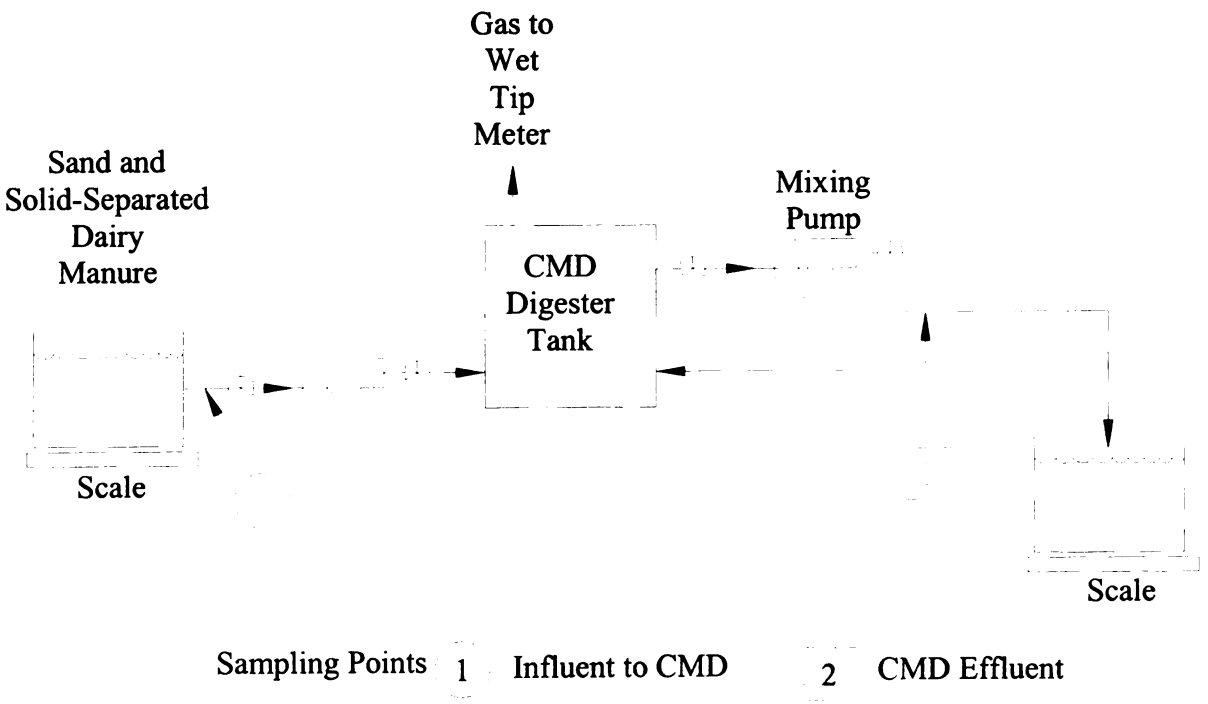


Figure 3.1 CMD schematic

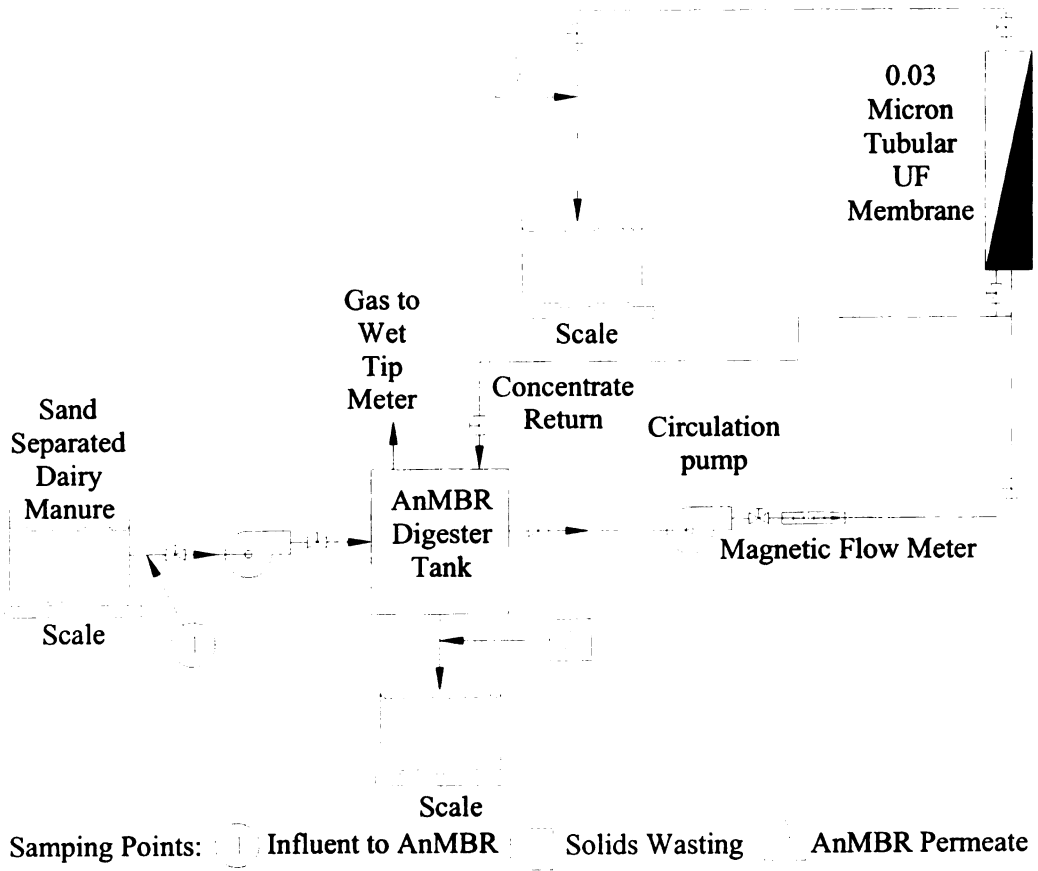


Figure 3.2 AnMBR schematic

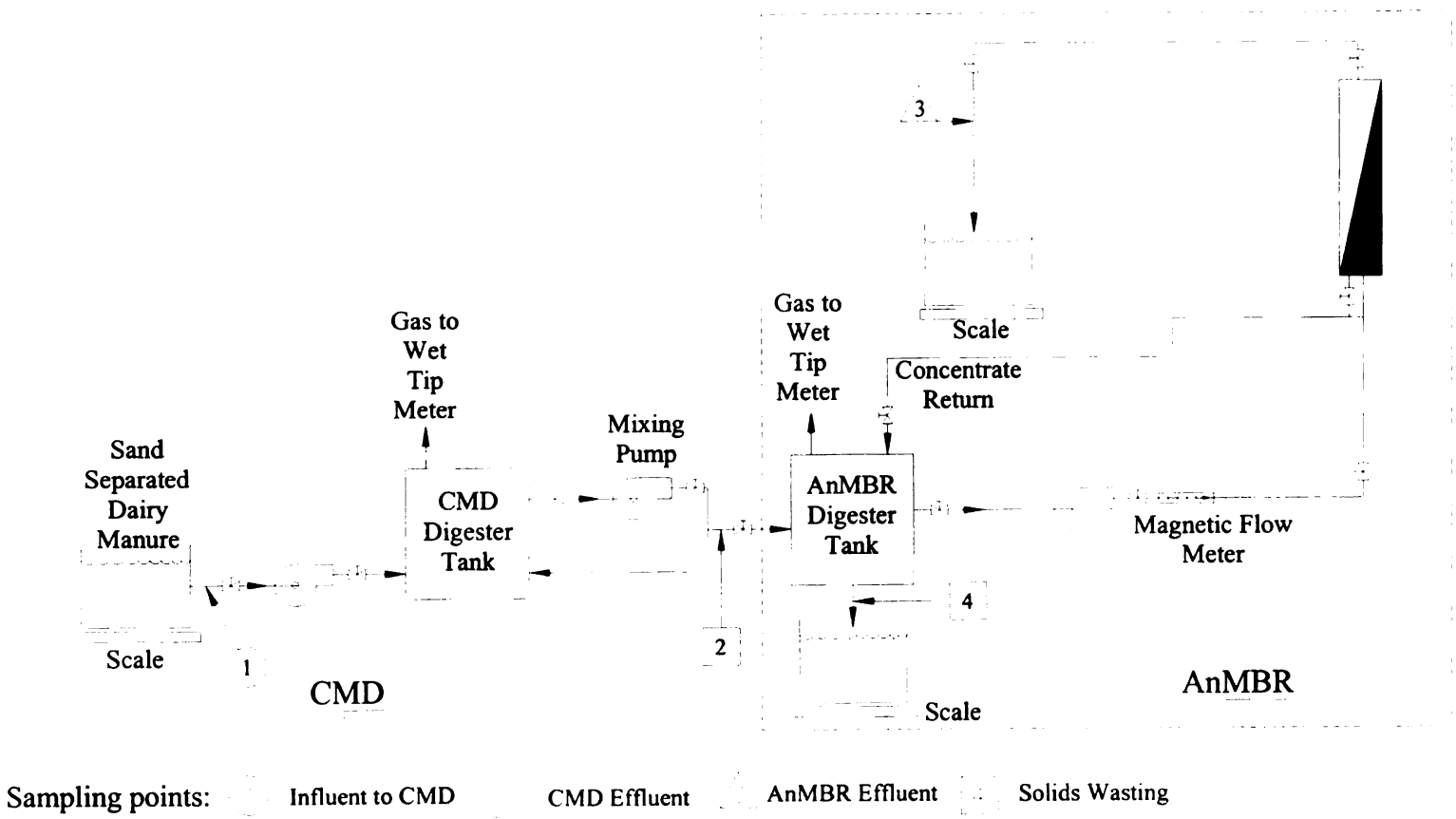


Figure 3.3 CMD/AnMBR schematic

3.3 Results and Discussion

3.3.1 Comparison of an AnMBR and a CMD

The AnMBR was operated for 108 days in parallel with a CMD. During this period, both systems had a HRT of 19 days. The AnMBR cross-flow pump was operated on a timer energized approximately ½ of the time until day 45. This was necessary because a digester heating system had not yet been installed and the pump was used to both circulate biomass through the membrane and provide heat to the system (thermal energy imparted from the pump to the circulating biomass). When the circulation pump was energized, the permeate was continuously returned to the digester tank with the exception of one time per day when it was discharged from the AnMBR for approximately 1.5 hours (to match the quantity of fresh manure added). Beginning on day 41, an external heating blanket was added to the digester to maintain system temperature. At this time, the pump circulation rate was reduced to 15 minutes every two hours to provide periodic mixing plus an additional 1.5 hours per day for permeate removal.

A summary of the operating data is shown in Table 3.3. Table 3.4 presents the water quality data. Figures 3.4 and 3.5 present the organic loading rate for COD and VS and influent/effluent COD and VS concentrations for the digesters.

Table 3.3 Summary of operating data for AnMBR and CMD

Parameter	AnMBR Days 1-40	AnMBR Days 41-108	CMD Days 1-40	CMD Days 41- 108
UF Rate, /min	36	31	NA	NA
Perm. Rate, mL/min	78	48	NA	NA
Flux, $L/M^{2}/hr^{1}$	59	36	NA	NA
Avg. TS in reactor, %	8.0	10.2	4.0	3.6
L CH_4/kg VS fed/day*	118	177	245	272
VS Destruction, %	NA	NA	15	29
Cycle Time	256	84	NA	NA
HRT, days	19	19	19	19

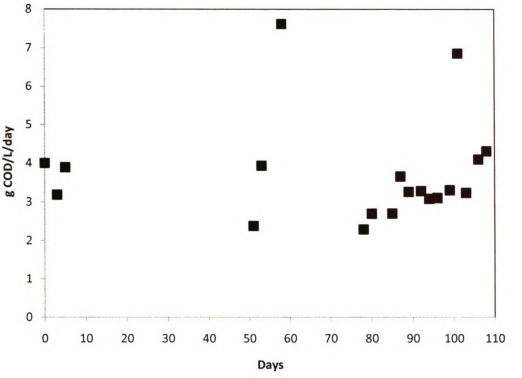
*Actual conditions

Table 3.4 Summary of water quality data for AnMBR and CMD

Parameter	Influent	AnMBR Digester	AnMBR Permeate	CMD
COD, mg/L	53,700 ± 18,200	94,900 ± 12,400	6,600 ± 2,400	32,600 ± 8,100
TS, %	4.7 ± 1.4	9.3 ± 1.6	1.1 ± 0.135	3.6 ± 0.8
VS, %	3.2 ± 0.9	6.7 ± 1.1	0.5 ± 0.09	2.4 ± 0.6
pH	7.32 ± 0.27	7.67 ± 0.11	7.81 ± 0.09	7.75 ± 0.10

Figure 3.6 illustrates the impact of AnMBR total solids concentration on the flux rate. This is consistent with the findings of other researchers (Anderson 1986; Beaubien, Baty et al. 1996; Brockmann and Seyfried 1996) who also reported a degradation in flux with corresponding to increasing digester TS. There was a steady increase in AnMBR digester total solids concentration from day 1 to approximately day 46 and a steady decay in the

permeate flux to approximately day 25 at which time flux held stable to day 51. The TS content of the digester increased to above 10% at day 53, and the flux rate experienced a stepwise reduction from approximately 60 $L/m^2/hr$ to 40-50 $L/m^2/hr$ with a steady decline beginning day 99 to the end of the experiment at day 108. The membrane was not cleaned during this study and likely lead to the slow decay in flux rate beginning after day 51. The step reduction in the flux rate at day 51 appears to be related to attainment of a critical TS concentration in the digester.



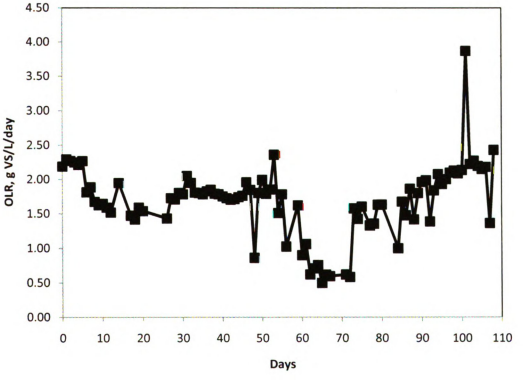
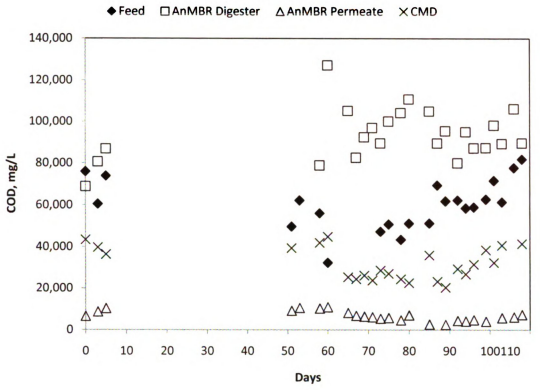


Figure 3.4 CMD and AnMBR, organic loading rate for COD and VS



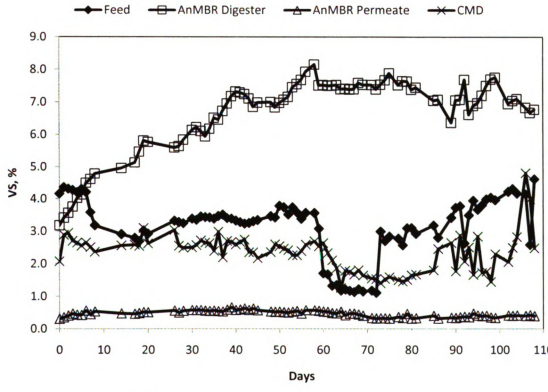


Figure 3.5 CMD and AnMBR, COD and VS

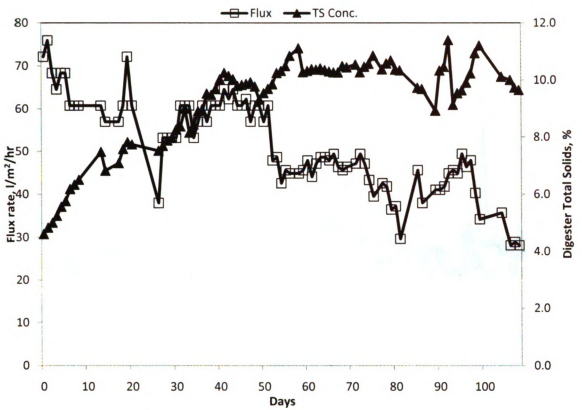


Figure 3.6 AnMBR (from CMD and AnMBR comparison study), permeate rate and digester TS concentration

Figures 3.7 and 3.8 present a comparison of pH and methane production. During the period of days 60 through 72, there was an unknown condition at the dairy that resulted in collected manure with a VS content considerably lower than the previous average. Once the VS content recovered, the AnMBR gas production returned to a value consistent with previous readings. During this period of low VS organic loading (Figure 3.4), the AnMBR produced more methane (per mass VS fed) than the CMD. As it was operated at a VS concentration that was approximately 3 times higher than the CMD, the probable explanation is that the residual VS of the AnMBR was converted to methane during this period, accounting for the apparent increase in methane production.

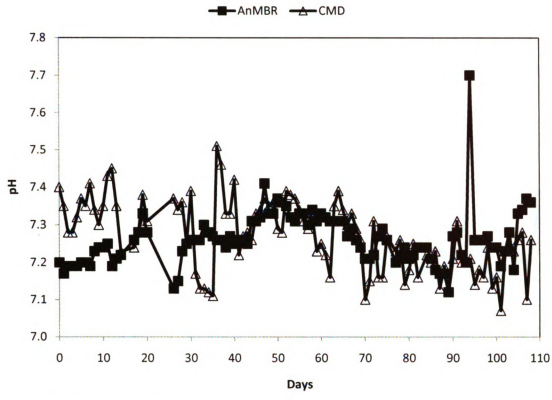


Figure 3.7 CMD and AnMBR, pH

Despite the greater biomass concentration of the AnMBR, the average methane production for the CMD over the period of the experiment was 262 L CH_4/kg VS fed/day compared to 155 L CH_4/kg VS fed/day for the AnMBR. These results suggest a negative impact on methane productivity related to the cross-flow membrane system. However, the impact is not as extreme as the findings of Brockmann and Seyfried (1995; 1996; 1997) who reported a 50% reduction in microbial activity at a cycle time of 20 with only 10 to 15% of the activity remaining after 120 to150 cycles/day.

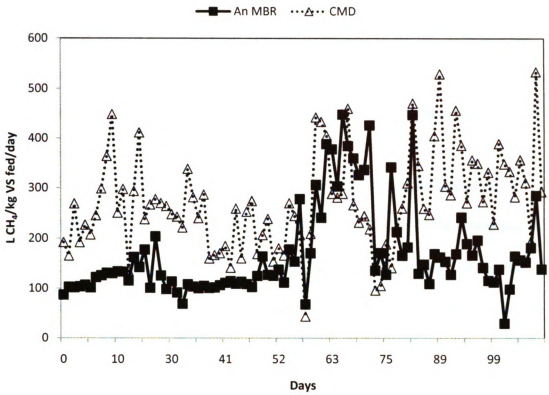


Figure 3.8 CMD and AnMBR, methane production

Ghyoot and Verstraete (1997) compared the methane generating potential for biosolids subjected to the shearing impact of an AnMBR ("treated") operated at a cycle time of 245/day, with biosolids that had not been impacted by an AnMBR system ("untreated") and found a 18% increase in biogas production for the untreated biosolids. The results for the present research fall between the findings of Brockmann and Seyfried and those of Ghyoot and Verstraete.

VS destruction during the period of study for the CMD was 24%. The calculated VS destruction for the AnMBR was skewed upwards by apparent solids settling in the digester tank, likely caused by operating for an extended period of time without wasting. As a result, the data are not valid. Vogel (2003), who conducted the only known

AnMBR work on dairy manure, reported a VS reduction of 49% and a COD reduction of 50% for the operation of a thermophilic AnMBR treating dairy manure at an average cycle time of 42 (system was fed three times per week). Gas production estimates were simulated in Vogel's work using serum bottles and the basis for predicting gas production for the pilot anaerobic digester was not clear.

The average methane production for the AnMBR during the first phase of the experiment (cycle time of 256/day, days 1-40) was 118 L CH_4/kg VS/day compared to the CMD during the same period which produced 245 L CH_4/kg VS/day. When the AnMBR cycle time was reduced to 84/day (days 41-108), the biogas production for the AnMBR improved (Figure 3.8). As previously discussed, the manure fed to the digesters for days 60-72 was very low in VS (Figure 3.5) and was inconsistent with previous and future data and the methane production per kg VS fed to the AnMBR during this period was unusually high, likely due to endogenous decay of existing VS retained in the digester tank. Comparing days 41-108 skews the AnMBR methane production upwards; therefore, days 75-108 were used for the methane production comparison between a cycle time of 256/day and 84/day. The CMD produced 319 L CH_4/kg VS fed/day during days 75-108, a 30% increase from the days 1-40. The AnMBR produced 171 L CH_4/kg VS fed/day during days 75-108, a 45% increase. Therefore, the AnMBR experienced a significant increase in methane production when the cycle time was reduced from 245/day to 84/day indicating an apparent positive impact with cycle time reduction.

The AnMBR COD removal efficiency, as measured by comparing the feed to the permeate, during the course of the 108 day experimental period equaled 88% (average feed COD = 53,700 mg/L and average permeate COD = 6,570 mg/L and when evaluated

independently for the two cycle times, also resulted in the same removal efficiency). The CMD removal efficiency during this same period equaled 39%. The AnMBR findings are consistent with the findings of previous researchers (Cadi, Huyard et al. 1994; Fakhrulrazi 1994; Anderson, Kasapgil et al. 1996; Ince, Ince et al. 2000; Ince, Ince et al. 2001; Fuchs, Binder et al. 2003). However, it is important to note that this does not speak to the overall COD removal for the process as biomass was not wasted during this period of study and, as a result, COD accumulated in the digester tank (per Figure 4.9).

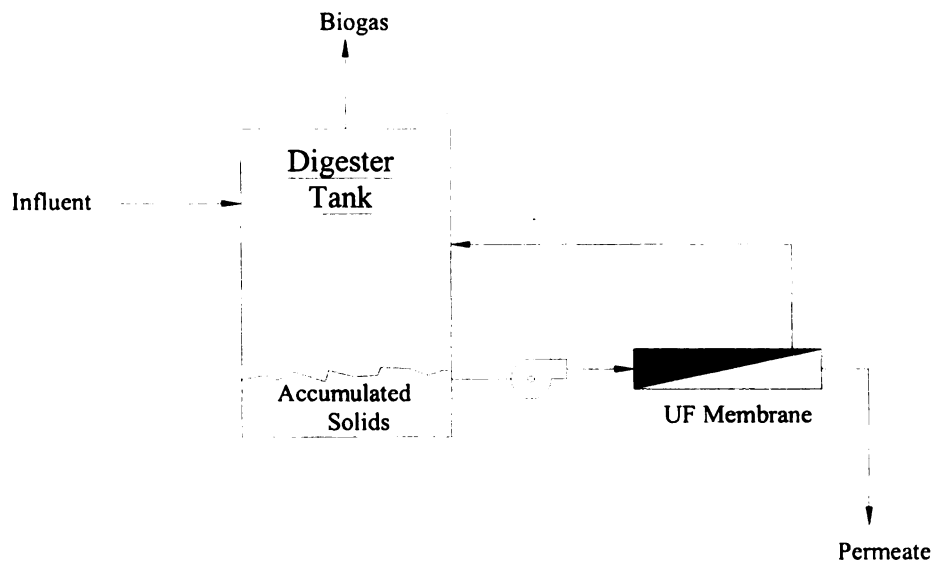


Figure 3.9 AnMBR without solids wasting

3.3.2 Combined CMD/AnMBR

Following the comparison experiment, the CMD and AnMBR were placed in series so that the effluent from the CMD was acting as the influent to the AnMBR. This was conducted to determine if an operational advantage could be leveraged by reducing the total solids loading to the AnMBR with the expectation of increasing the flux rate while diminishing the cost of operation. The flux rate is directly related to the solids loading applied to the membrane (Beaubien, Baty et al. 1996; Madaeni 1997), and, as a result,

reducing the solids loading will improve the flux rate and reduce the energy required per unit of permeate produced. A second potential advantage is that the readily degradable substrate will be available for conversion by the CMD and the AnMBR, due to its longer SRT, will be more effective at converting the more recalcitrant organic matter.

The CMD/AnMBR was operated at a cycle time of 34 for days 1 through 69 and a cycle time of 56 for days 70 through 282. Table 3.5 outlines the general operating conditions and Tables 3.6 and 3.7 provide a summary of the water quality data.

Figure 3.10 provides a summary of the organic loading rate in terms of COD and VS applied to the system. The VS concentration in the feed to the CMD began to decline around day 215, most likely caused by a problem with operation of the sand-manure separators at the dairy farm where the manure was collected, the manure feed tank provided some equalization and, as a result, the decline in the feed VS concentration (and corresponding organic loading rates) slowed until day 247 before beginning to slowly increase to a value consistent with the balance of the data.

Table 3.5 Summary of operating data for combined CMD/AnMBR

Parameter	AnMBR Days 1-69	AnMBR Days 70-282	CMD Days 1-69	CMD Days 70- 282
UF Rate, LPM	36.3	34.8	NA	NA
Perm. Rate, mL/min	73	41	NA	NA
Flux, $L/M^2/hr$[1]	55	31	NA	NA
Avg. TS in reactor, %	5.5	5.7	3.4	2.8
VS destruction, %	20	27	22	26
COD destruction, %	NA	NA	29	39
L CH_4/Kg VS fed/day*	82	83	133	190
Cycle Time	34	56	NA	NA
HRT, days	10.7	9.7	9.5	9.4
SRT, days	23	25	9.5	9.4

*Actual conditions

Table 3.6 Summary of water quality data for combined CMD/AnMBR Days 1 - 69

Parameter	CMD Influent	CMD Effluent/AnMBR Influent	AnMBR Contents	Permeate
COD, mg/L	42,900 ± 9,200	29,500 ± 5,800	53,000 ± 9,800	3,054 ± 730
TS, %	4.3 ± 0.5	3.4 ± 0.5	5.5 ± 1.2	0.9 ± 0.1
VS, %	2.9 ± 0.3	2.2 ± 0.4	3.6 ± 0.8	0.3 ± 0.1
pH	7.02 ± 0.15	7.73 ± 0.08	7.66 ± 0.10	7.77 ± 0.12

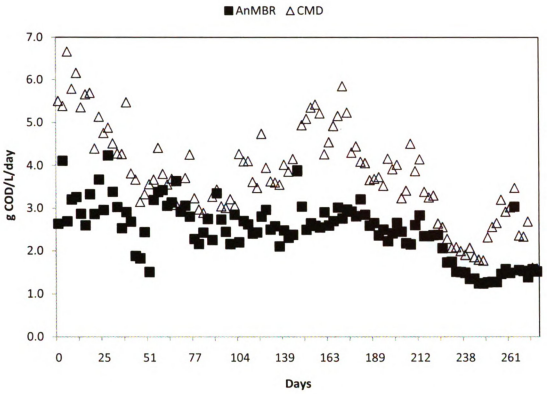
Table 3.7 Summary of water quality data for combined CMD/AnMBR Days 70-282

Parameter	CMD Influent	CMD Effluent/AnMBR Influent	AnMBR Contents	Permeate
COD, mg/L	33,100 ± 9,400	22,600 ± 5,700	53,800 ± 11,500	2,450 ± 800
TS, %	3.5 ± 0.8	2.8 ± 0.7	5.7 ± 1.2	0.8 ± 0.10
VS, %	2.3 ± 0.6	1.7± 0.4	3.6 ± 0.8	0.3 ± 0.3
pH	6.98 ± 0.27	7.62 ± 0.1	7.59 ± 0.1	7.68 ± 0.10

Figure 3.11 presents influent and effluent COD and VS data for the digester systems and Figure 3.12 shows pH and methane production and, though the pH declined during the period of reduced organic loading to the digester systems, the gas production remained reasonably consistent over the course of the experiment.

Figure 3.13 shows the titrated volatile acid concentration for samples removed from the AnMBR. The volatile acid concentration was between 200-400 mg/L as HAc during the experiment. Volatile acid analysis for samples from the CMD, with few exceptions, were non-detect (data is not shown). A common metric used to assess the relative health of a digester system is the ratio of volatile acids to total alkalinity (Speece 1996) . Manure contains a high concentration of ammonia and organic nitrogen from protein. During the anaerobic digestion process, the organic nitrogen is degraded and forms ammonium bicarbonate alkalinity (Speece 1996). Consequently, the volatile acids/total alkalinity ratio never exceeded 0.06 at any time for the present systems. Vogel (Vogel 2003) operated a thermophilic AnMBR with dairy manure as the substrate at similar HRTs and SRTs to the present work but at approximately double the organic loading rate and reported effluent VFA concentrations between 1,000 and 2,000 mg/L during the first 100 days of operation with a steady reduction to values less than 250 mg/L. Padmasiri, Zhang et al. (2007) reported volatile acid excursions for swine manure greater than 3,000 mg/L as HAc at an organic loading rate of 2.0 g COD/L/day when cross-flow velocity was increased from 0.9-1.9 m/s but reported VFA concentrations of less than 500 mg/L as HAc during periods of stable operation. Brockmann and Seyfried (1995; 1996; 1997), when treating potato waste with an AnMBR, experienced a volatile acid concentration of

4,000 mg/L when the organic loading rate was increased from the range of 1.5-3.0 g COD/L/day to 4.0 g COD/L/day and with a reduction in the organic loading rate, stabilized at approximately 3,500 mg/L as HAc. Though the AnMBR gas production lagged the CMD, the system was stable, as measured by VFA production, and consistent with other reported AnMBR research.



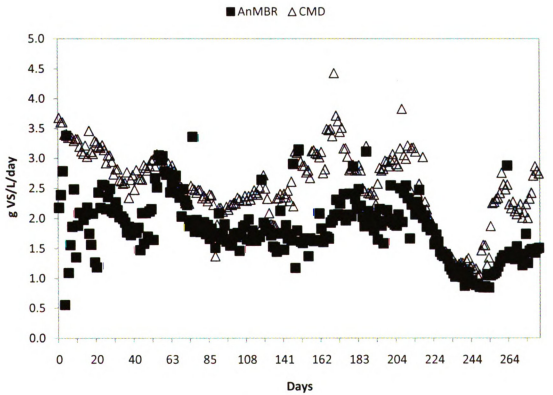


Figure 3.10 Combined CMD/AnMBR, organic loading rates for COD and VS

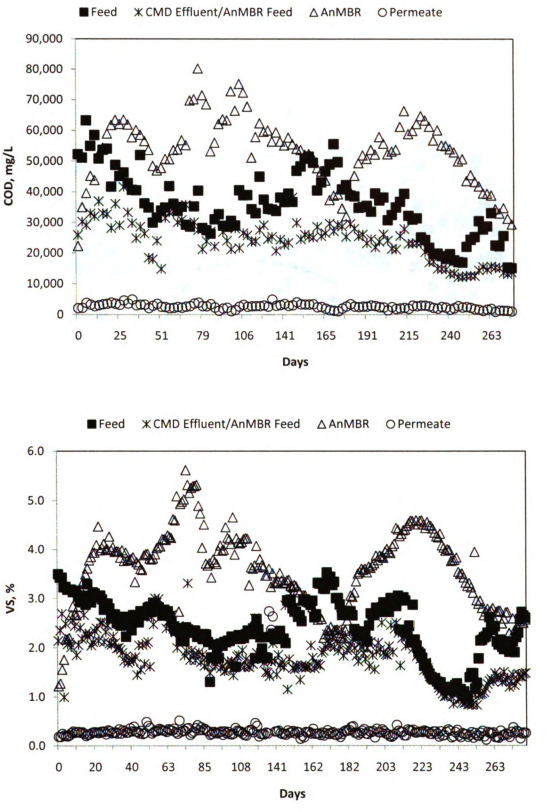


Figure 3.11Combined CMD/AnMBR, COD and VS

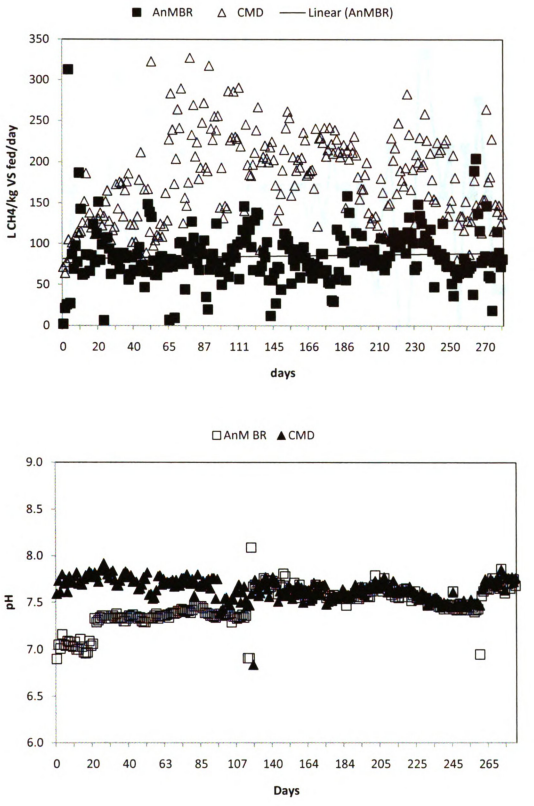


Figure 3.12 Combined CMD/AnMBR, gas production and pH

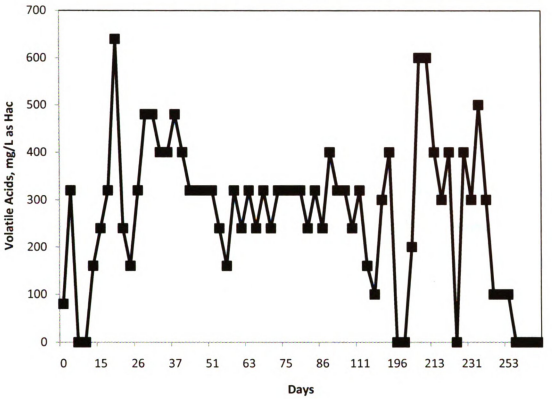


Figure 3.13 Combined CMD/AnMBR, volatile acid concentration

The methane production rate of the AnMBR, during days 1-69 and operating at a cycle time of 34/day, equaled 82 L CH_4/kg VS/day. Following the increase in cycle time to 56, the methane production was nearly identical at 83 L CH_4/kg VS/day for days 70-282. During the period between days 1-69, the CMD methane production rate equaled 133 L CH_4/kg VS/day but increased to 190 L CH_4/kg/day between days 70-282.

AnMBR VS destruction for days 1-69 was 20% and the CMD VS destruction for this same period was 22%. VS destruction for days 70-282 for the AnMBR was 27% while the CMD VS destruction for this same period was 26%. The VS destruction results are at odds with the methane production rate. One potential explanation relates to the type of

VS being converted to methane. Methane is produced consistent with the chemical makeup of the substrate (Bryant 1979). It is possible that the VS degraded by the CMD contained greater methane potential per mass than the residual VS fed to the AnMBR. Therefore, because the AnMBR was fed with the CMD effluent, VS destruction may not provide a good metric for comparing the systems. Also, because the AnMBR is operated at a higher biomass concentration, there is a greater propensity for solids to settle in the digester tank. The increase in AnMBR VS destruction from days 1- 69 compared to days 70- 282 could be partially the result of increased solids settling and an overstatement of the VS destruction.

There was also a difference in the VS content of the manure for days 70-282 which averaged 2.3% compared to 2.9% for days 1-69. The resulting gas production per mass VS fed to the CMD was significantly greater for days 70-282. Though there was less total mass of VS during the second period, it is possible that a higher percentage of this VS was readily degradable and this may account for the increased gas production of the CMD. As a result, it cannot be concluded that there was a difference in gas production between a cycle time of 34 and 56.

The COD reduction based on the difference between the COD concentration of the CMD feed and the AnMBR permeate equals 93% (average permeate COD equals 2,450 mg/L and average CMD feed equals 33,127 mg/L). It is important to note that this does not represent the total system COD reduction as it does not account for COD wasted from the AnMBR. COD reduction data is presented in this fashion for consistency with previous AnMBR research.

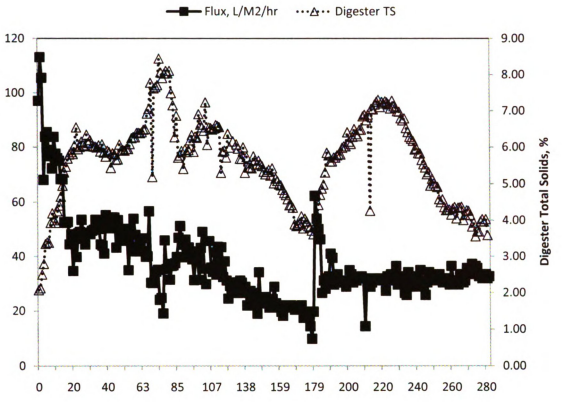


Figure 3.14 Combined CMD/AnMBR, flux rate and digester TS concentration

AnMBR TS concentration (in the AnMBR digester tank, reference Figure 3.3) versus flux rate is shown in Figure 3.14. The digester TS concentration started at 2% and increased to approximately 6% by day 24 with a corresponding decrease in the flux rate from approximately 100 L/m^2/day to 50 L/m^2/day. The digester TS and the flux rate were stable between days 24 to 60. After day 60, there was a spike in digester TS concentration and a corresponding decrease in flux rate followed by a decline in digester TS and an increase in flux rate between days 77 and 87. Following day 87 until day 179, there was a steady degradation in flux, despite a decline in the TS content of the digester,

mostly likely caused by membrane fouling. At day 179, the membrane was cleaned. There was an immediate increase in flux from the range of 10-20 L/m^2/day to a little more than 60 L/m^2/day. For the next 100 days, the digester TS increased to approximately 7% and then steadily decreased from day 218 to 282 to approximately 4% while the flux rate was stable. It is possible that the decreasing TS content was balanced by a corresponding increase in membrane fouling resulting in a stable flux during this period.

3.3.3 Nutrients

Table 4.8 provides data for various nutrients of interest. The AnMBR permeate phosphorus concentration equaled 16 mg/L (approximately 95% removal efficiency). The permeate TKN concentration equaled 1,454 mg/L (approximately 30% TKN removal efficiency). The AnMBR membrane did not significantly impact the ammonium N or potassium concentration because both were present in the soluble state and thus passed through the membrane.

Table 3.8 Water quality at each sampling point

Parameter	n	CMD Influent	CMD Effluent	AnMBR Permeate	AnMBR Digester
Total P, mg/L	7	339±27	322±68	16±5	791±156
TKN, mg/L	7	2,070±205	2,130±131	1,450±88	3,090±550
Organic N, mg/L	7	871±325	848±69	121±23	1,810±339
Ammonium N, mg/L	7	1,200±416	1,280±91	1,330±84	1,570±76
Potassium, mg/L	7	1,840±189	1,790±205	1,780±148	1,900±113

During the period of study, the average input feed to the combined CMD/AnMBR was 11.0 kg/day of raw manure, the average permeate discharge rate was 6.8 kg/day and the

average wasting rate was 4.2 kg/day. A consistent HRT was maintained throughout the experiment. Based on this mass balance accounting, nearly 97% of the total phosphorus can be accounted for in the system. This is consistent with other researchers who reported near perfect mass balances for total phosphorus for plug flow digestion systems (Wright 2004; Martin 2007).

3.3.4 Removal of Virus and Pathogen Indicators

The membrane provides a barrier that excludes suspended solids that are larger than the pore size of the membrane. Limited work has been conducted with respect to quantifying the ability of AnMBR systems to exclude viruses and pathogens. A study was conducted by Wong et al. (2009) to evaluate the removal of bacterial and virus indicators in the effluent of the AnMBR used for the present research. E. *coli*, Enterococci and C. *Perfringens* were the bacterial indicators and somatic coliphage was the viral indicator monitored in this study. The paper published from this work is attached, with the permission of the Journal of Environmental Quality, as Appendix A. Referencing Table 2 from Appendix A, the influent tested positive in all 8 sampling events for E. *coli*, Enterococci, C. *perfringens* and coliphage. The CMD effluent tested positive for E. *coli* (8/8), Enterococci (7/8), C. *perfringens* (8/8) and coliphage (8/8). The AnMBR effluent tested positive for E. *coli* (2 of 8 samples), Enterococci (3 of 8 samples), *C. perfringens* was not detected in any of the AnMBR samples and Coliphage (5 of 8 samples). The average values for E. coli, Enterococci, C. *perfringens* and Coliphage in the combined CMD/AnMBR effluent were 0.31, 0.51, ND and 2.47 $\log_{10}$cfu/L respectively with log10 removals of 6.7, 7.3, 6.5 and 4.2, respectively. The

coliphage exhibited the highest occurrence frequency and concentration in the AnMBR effluent which is to be expected because viruses are generally smaller than bacteria, with diameters as small as 0.01 μm. The average pore size for the AnMBR membrane was 0.03 μm. The log10 removals of the indicators are illustrated in Figure 2 of Appendix A and the results illustrate that most of the removal was due to the AnMBR.

The high rate of removal attributed to the AnMBR was a direct result of the membrane. However, it is not clear if the membrane pore size is solely responsible for the rejection of the virus and pathogen indicators or if the removal efficiency is due to the filtering impact of the gel layer due to concentration polarization. He et al. (2005) reported bacteria removal ranging from 5.65 $\log_{10}$ removal to 5.14 $\log_{10}$ removal (>99.9%) with membrane pore sizes ranging between 20,000 and 70,000 Da. 20,000 Da is approximately equal to 0.01 μm and 70,000 Da is approximately equal to 0.06 μm. Concentration polarization is described as the formation of a gel layer at the membrane surface due to retained solutes. This gel layer forms a secondary barrier to flow through the membrane (Baker 2000). It seems likely that the gel layer provides additional filtration capability and may explain the relatively small difference between the bacterial removal of 20,000 Da membrane versus the 70,000 Da membrane. Nevertheless, the bacteria removal rate is consistent with that reported by Wong et al. (2009).

CHAPTER 4

CYCLE TIME COMPARISON

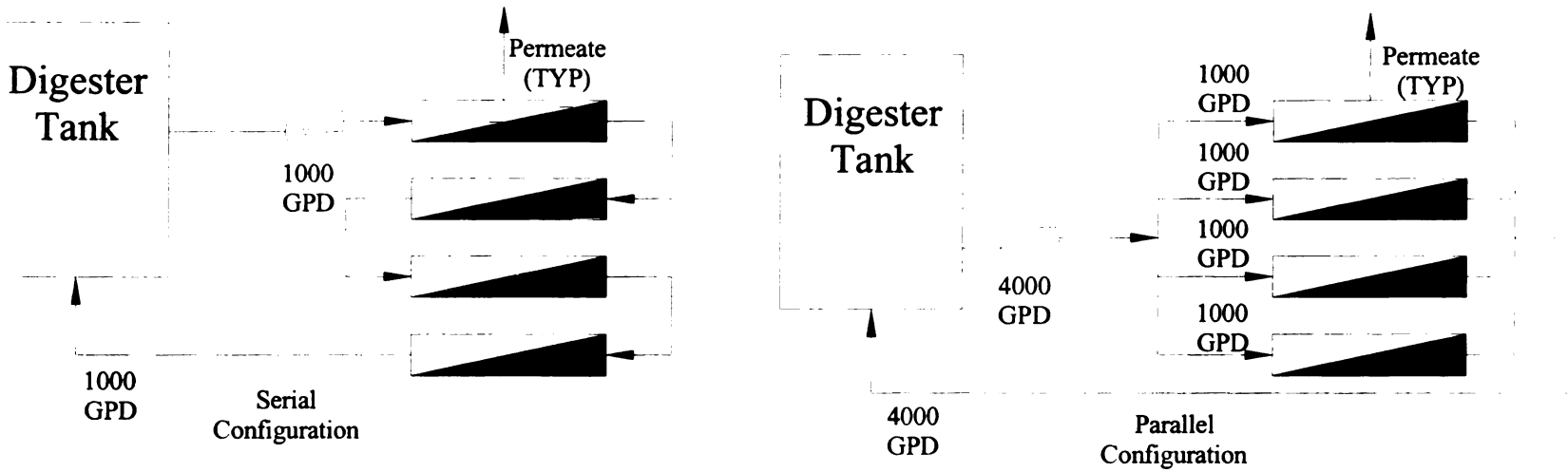
4.1 INTRODUCTION

AnMBRs have been reported to provide robust treatment at high organic loading rates. However, other researchers have found reductions in microbial activity, reportedly due to the shearing impact of the pump/membrane system. A determining factor appears to relate cycle time, a measurement of pumping frequency, to microbial activity. Higher cycle times have been reported to reduce microbial activity. This relationship and advantages and disadvantages of AnMBRs are discussed in detail in Chapter 2, Section 7.

A typical HRT for a complete mix, manure-based digester system is in the range of 10 to 30 days with dairy manure as the substrate (Hills 1979; Oliver, Pain et al. 1986; Summers, Hobson et al. 1987; Pain, Phillips et al. 1988; Ghaly and Echiegu 1992; Vogel 2003). Considering average flux rates identified from this research (Chapter 3, Tables 3.3 and 3.5) and typical HRTs discussed, the estimated cycle time for a manure-based AnMBR is in the range of 4 to 30 cycles per 24 hour period (Appendix D). The cycle time depends on the total system volume and the configuration of the membranes. By way of example, if the cross flow circulation rate through an AnMBR equals 7,500 LPM (10,800,000 LPD) and the digester tank volume equals 400,000 liters, then the cycle time equals 27 cycles/day (10,800,000 L/day ÷ 400,000 liters). If all other parameters remain equal, one way to decrease the cycle time is to increase the size of the digester tank. For example, if the digester tank volume were increased to 800,000 liters, the cycle time for the above example becomes 14 cycles/day. The obvious disadvantage is the increased

capital cost. In an optimum configuration, the tank size will be maintained at the least possible volume. However, as volume decreases, cycle time also increases.

An alternative is to change the membrane configuration. Figure 4.1 illustrates the cycle time differences that result if the membranes are placed in a serial configuration. Placing 4 membranes in series, rather than in parallel, results in a cycle time of approximately ¼ of a parallel configuration. A serial configuration, however, results in a significant increase in pressure to maintain the desired cross-flow velocity through the membrane and creating a more complex design. System pressure limitations also dictate the number of membrane modules that can be placed in series.



Note: Results in a cycle time $\frac{1}{4}$ of that in parallel

Figure 4.1 Comparison of membrane elements connected in a serial versus a parallel configuration

The UF membranes used in this research were full-size and designed for industrial applications. Timers started and stopped pumps in order to achieve target operating conditions. A series of tests were performed at various cycle times, digester VS concentrations (biomass concentrations), cross-flow velocities and membrane configurations to determine the implications of these parameters on system design with the goal of maximizing methane production. Three distinct phases of experimentation were undertaken as discussed in the following subsection.

Phase 1 compared the methane production of three digester systems identified as single membrane digester (SMD), membrane equivalent digester (MED) and complete mix digester (CMD). The objective of this experiment was to determine if a PVC pipe could be used as a surrogate membrane for future configuration evaluation and to establish a baseline for methane production at a cycle time of 6 and cross-flow velocity of 4.5 m/s. The SMD was operated with 100% of the membrane permeate returned to the digester tank so that it resembled a complete mix digester with HRT equal to SRT. The MED employed a 1750 mm x 13.9 mm diameter PVC pipe to simulate the turbulence and pressure drop of the membrane used for the SMD. All three systems were operated at an approximate cycle time of 6 (the SM and MED = 7/day and the CMD = 5/day).

Phase 2 compared the methane production of three digester systems identified as single membrane AnMBR (SM AnMBR), multi-membrane AnMBR (MM AnMBR) and CMD. The objective of Phase 2 was to determine if there was a methane production advantage between a cycle time of 6 (MM AnMBR) and 27 (SM AnMBR). A second objective of

Phase 2 was to evaluate whether the operational time of the CMD mixing pump affected methane production. The mixing pump was operated 6 times per day x 3 minutes for days 1 through 54 (equivalent cycle time of 5/day) and 4 times per day x 1 minute for days 55 through 92 (equivalent cycle time of 1/day).

Phase 3 compared the methane production of three digester systems identified as SMD, multi-membrane equivalent digester (MMED) and CMD. The SMD was operated with 100% permeate recycle at a cycle time of 27 which enabled comparison with the SM AnMBR of Phase 2. The MMED consisted of four 3,000 mm x 13.9 mm diameter PVC pipes and was operated at a cycle time of 6 to evaluate the pressure and turbulence impact of placing four membrane modules in series and was selected because it represented a probable full-scale configuration. The MMED and the SMD were compared with a control CMD operating with an equivalent cycle time of 1. The objective of Phase 3 was to evaluate the impact of biomass concentration on methane productivity and to assess the difference in methane production between the torturous path of the MM AnMBR compared with the more realistic design of the MMED.

4.2 MATERIALS AND METHODS

4.2.1 General AnMBR Configuration

Two AnMBR systems were operated in the previously described configurations. A schematic of the general AnMBR layout is presented in Figure 4.2.

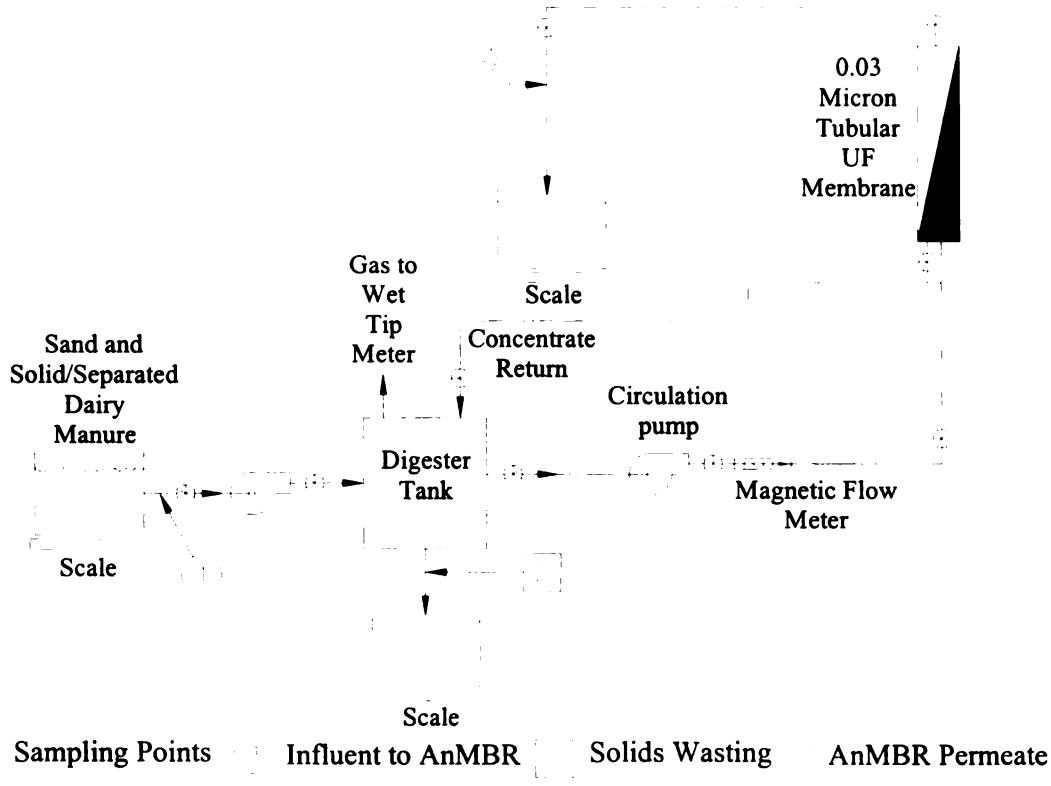


Figure 4.2 General AnMBR layout from which the SM AnMBR and MM AnMBR are derived

4.2.2 Phase 1

Three digester systems operated in this phase: SMD, MED and CMD and each is described in detail below.

SMD - A schematic of the SMD is presented in Figure 4.3. Permeate is returned to the digester. The digester was a 175 cm tall x 30 cm diameter section of schedule 40 PVC pipe with flanged ends. The working volume was 115 liters with approximately 10 cm of headspace for gas collection. A 0.64 cm diameter tube directed the biogas in the headspace to a wet tip meter (Wet Tip Meter Company, Nashville, TN).

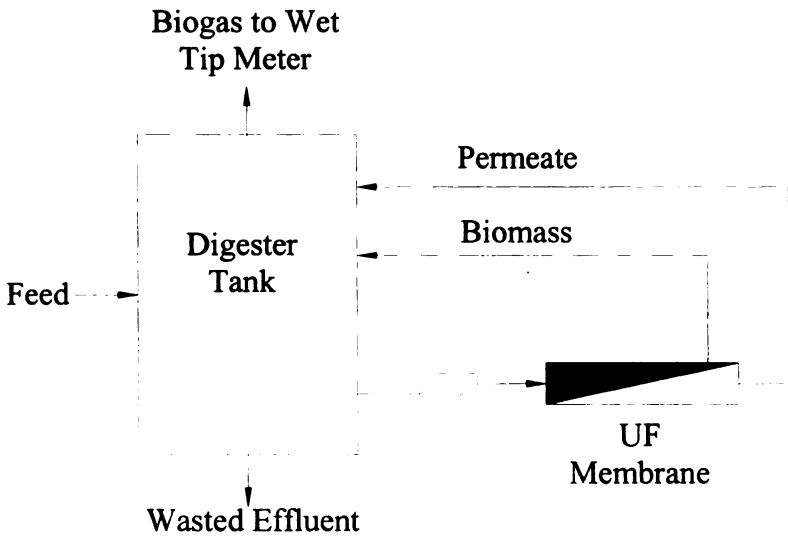


Figure 4.3 SMD operated with single element in a complete mix configuration

Gas samples for GC analysis were collected between the digester and the wet tip meter via a luer-style, 3-way valve. The digester was heated using an external heat blanket and thermostat (BriskHeat, Columbus, OH). Digested manure was removed from the system once per day based on mass and fresh manure was added to the system once per day based on mass. The manure was collected from the Car-Min-Vu Dairy, Williamston, MI one time per week and stored in 5 gallon carboys at room temperature. Carboys were added to a 100-L mixing tank (mixed with submersible pump prior to feeding for approximately 5 minutes). The SMD was operated in a cross-flow configuration and used a 0.03 micron, 14.4 mm diameter, 0.079 m^2 PVDF tubular ultrafiltration product (X-Flow, Inc., Netherlands). A 1.5" self-priming centrifugal pump (AMT Inc., Mansfield, OH) was used to generate a circulation rate of approximately 43 L/min (cross flow velocity = 4.5 m/s).

MED – A diagram of the MED is presented in Figure 4.4. With the exception of a 13.9 mm diameter PVC pipe of 1750 mm in length used as a surrogate for the UF membrane, all other aspects of the digester tank, manure addition and manure source were as described for the SMD of this section.

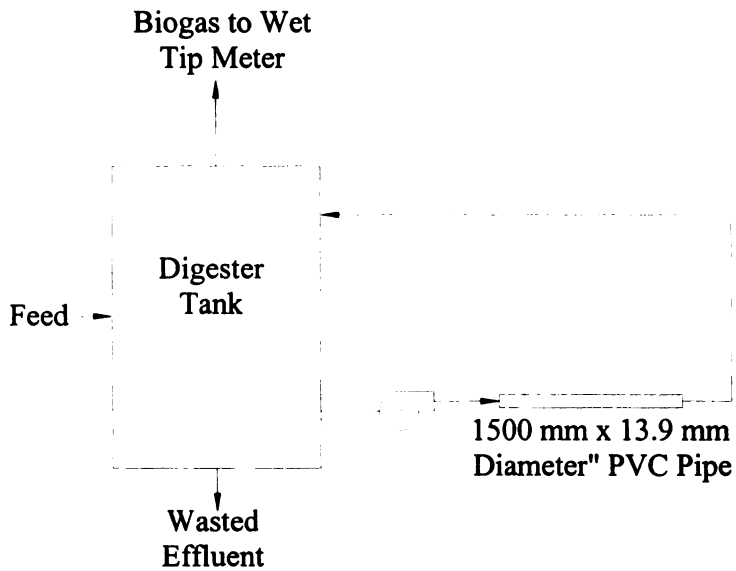


Figure 4.4 MED with 13.9 mm pipe surrogate for membrane

CMD - A CMD was operated as a control to compare performance with the AnMBR systems. Figure 4.5 provides a schematic of the CMD system. The CMD consisted of a 122 cm tall x 55 cm diameter HPDE vessel with a working volume of 166 liters. Mixing was achieved with an AMT 1 x 1.5 centrifugal pump (AMT, Inc. Mansfield, OH), operated 6 times per day for 3 minutes. The circulation rate of the pump was approximately 45 LPM. Digester heating, gas collection, manure addition and manure source as described in for the SMD of this section.

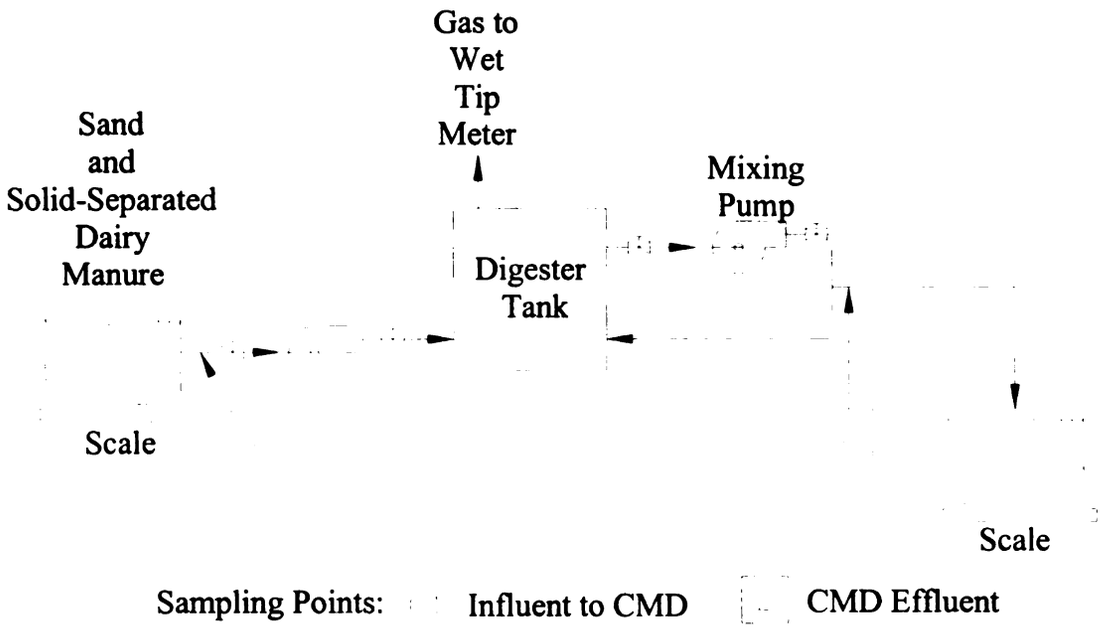


Figure 4.5 CMD system

4.2.3 Phase 2

Three digester systems were operated for this phase of experimentation: SM AnMBR, MM AnMBR and CMD and each is described in detail below.

SM AnMBR – Referencing Figure 4.3, the SM AnMBR was the same as the Phase 1 SMD with the exception that permeate was removed from the system (rather than returned to the digester). The feed rate was set based on a design HRT of 12 days. The quantity wasted equaled the difference between the feed rate and the permeate removal rate.

MM AnMBR - The MED from Phase 1 was replaced with a module containing seven, 14.4 mm diameter x 1750 mm x 0.03 μm pore size PVDF ultrafiltration membranes with a total area of 0.55 m^2, manufactured by X-Flow, Inc. (Netherlands). A schematic is shown in Figure 4.6. The working volume was 119 liters with approximately 10 cm of free board for gas collection (slightly higher than the SM AnMBR due to the additional membranes used in the module). The MM AnMBR was operated in a cross-flow configuration using a Summit 2196LF, 1x1.5x8 centrifugal pump (Summit Pump, Inc., Green Bay, WI) to generate a circulation rate of approximately 28.5 L/min (cross-flow velocity = 2.9 m/s). A manifold was constructed to allow the elements to be operated in series (Figure 4.7) such that the system contained enough membrane surface area to allow for operation at a cycle time of 6. All other aspects of the digester tank, manure addition and manure source as described in Section 4.2.2 for the SMD.

CMD –CMD configuration was identical to that described in Section 4.2.2.

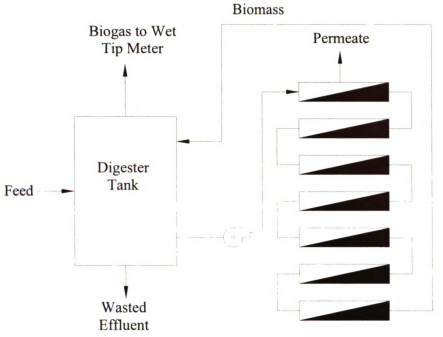


Figure 4.6 MM AnMBR with 7 elements connected in series

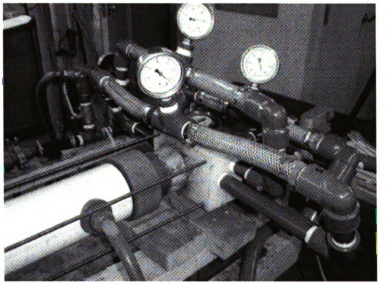


Figure 4.7 MM AnMBR module illustrating manifold

4.2.4 Phase 3

Three digester systems were operated for this phase of experimentation: SMD, MMED and CMD and each is described in detail below.

SMD – The SMD was identical to that described in Section 4.2.2

MMED - The seven element membrane module was replaced with four 3000 mm x 13.9 mm diameter sections of PVC pipe (Figure 4.8). The same Summit centrifugal pump was used to generate a circulation rate of approximately 41.5 L/min (cross-flow velocity = 4.5 m/s) to provide a cycle time of approximately 6. All other aspects of the digester tank, gas collection, manure feeding and manure source are as described in Section 4.2.2.

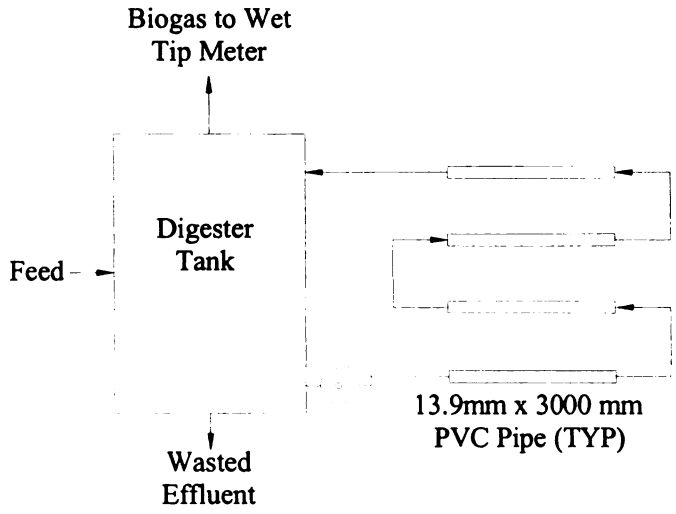


Figure 4.8 MMED operated with four 3000 mm x 13.9 mm diameter PVC pipe

CMD – The CMD was identical to that described in Section 4.2.2

4.2.5 Substrate

The substrate fed to all systems was collected from the Cal-Min-Vu Dairy (Williamston, MI) on a weekly basis. Cal-Min-Vu Dairy employs a McLanahan sand-manure separator followed by a McClanahan *ULTRA* cyclone (McLanahan Corp., Hollidaysburg, PA) for recovery of fine sand particles. The sand-separated manure is further processed through a press screw separator, PSS (Fan Separator, Carol Stream, IL). Liquid manure from the FAN unit was used as the substrate for the pilot-scale experiments outlined in this chapter. Manure was collected on a weekly basis. As with any "real-world" operation, the quality and character exhibited variability depending conditions at the dairy. Table 4.1 summarizes the average feed manure characteristics for Phases 1, 2 and 3.

Table 4.1 Characteristics of substrate for Phase 1

Parameter	Value	Standard Deviation
COD, mg/L	41,800	3,500
TS, %	3.3	0.2
VS, %	2.2	0.1
pH	7.11	0.14
Total alkalinity, mg/L as $CaCO_3$	10,400	1,060
Volatile acids, mg/L as HAc	2,110	417
Bicarbonate alkalinity, mg/L as $CaCO_3$	8,610	1,020

Table 4.2 Characteristics of substrate for Phase 2

Parameter	Value	Standard Deviation
COD, mg/L	52,300	14,800
TS, %	4.0	1.1
VS, %	2.6	0.6
pH	7.02	0.14
Total alkalinity, mg/L as $CaCO_3$	10,600	1,420
Volatile acids, mg/L as HAc	1,810	873
Bicarbonate alkalinity, mg/L as $CaCO_3$	9,100	1,260

Table 4.3 Characteristics of substrate for Phase 3*

Parameter	Value	Standard Deviation
COD, mg/L	42,200	17,500
TS, %	3.9	1.3
VS, %	2.4	0.9
pH	7.03	0.33

*Total alkalinity, volatile acids and bicarbonate alkalinity not analyzed for this phase because systems were stable and test was consistently non-detect

4.2.8 Analytical Methods

General analytical methods used in this chapter were described in Chapter 3.2.2. A GCMS analysis procedure used in this chapter is presented in Appendix E.

4.3 Results and Discussion

4.3.1 Phase 1

This configuration compared the methane production and VS destruction of a single 1750 mm long x 14.4 mm diameter membrane operated under complete permeate recycle conditions (SMD, Figure 4.3) with a 1750 mm x 13.9 mm diameter pipe (MED, Figure

4.4) and a control (CMD, Figure 4.5). Table 4.4 provides a summary of the average performance parameters and Table 4.5 details the water quality results with plus/minus values indicating the standard deviation for each result.

The SMD and MED were operated at a CFV of 4.5 m/s. There is general agreement in the literature that permeate flux increases with increasing cross-flow velocity (see discussion, Chapter 6, Section 3). Most AnMBR work has been conducted at CFVs of 3.0 m/s or less (see Chapter 2, Table 2.2). A higher CFV was selected for this experiment to enable flux rate comparison with a lower CFV used in Phase 2 and to evaluate its impact on methane production. Based on comparing methane production of the SMD and MED with the CMD, there does not appear to be a negative impact on methane production at a CFV as high as 4.5 m/s. In an effort to mirror the operating conditions for the SMD and the MED, the CMD was also operated at a cycle time of approximately 6 and this may have had a negative impact on methane production for the CMD. The mixing rate for the CMD and its impact on gas production is explored further in Phase 2.

Figure 4.9 presents the organic loading rate for VS and COD and Figure 4.10 the feed and effluent VS and COD for all three digesters. Data collection began on the 12th day of operation for VS and on the 16th for COD. There were a limited number of COD sampling events for Phase 1 and, as a result, COD destruction was not presented in Table 4.4.

Table 4.4 Summary of operating data for Phase 1

Parameter	SMD	MED	CMD
Cross-flow velocity, m/s	4.5	4.5	NA
Flux, $L/m^2/hr$	118	Na	NA
Circulation rate, LPM	43	43	45
Transmembrane pressure, kPa	85	87	NA
Membrane entry pressure, kPa	124	124	NA
Avg. TS in Reactor, %	2.6	2.6	2.5
VS destruction, %	27	30	33
L CH_4/kg VS fed/day*	264	268	280
CH_4 concentration, %	72	72	71
Cycle time, day^{-1}	7	7	5
HRT, days	15.3	15.8	14.8
SRT, days	15.3	15.8	14.8

*actual conditions

Table 4.5 Summary of water quality data for Phase 1

Parameter	Influent	SMD	MED	CMD
COD, mg/L	41,800±3,500	30,800±2,940	29,700±4,270	26,800±2,990
TS, %	3.3±0.2	2.6±0.1	2.6±0.2	2.5±0.1
VS, %	2.2±0.1	1.6±0.1	1.5±0.1	1.5±0.1
pH	7.11±0.14	7.71±0.23	7.71±0.23	7.8±0.17

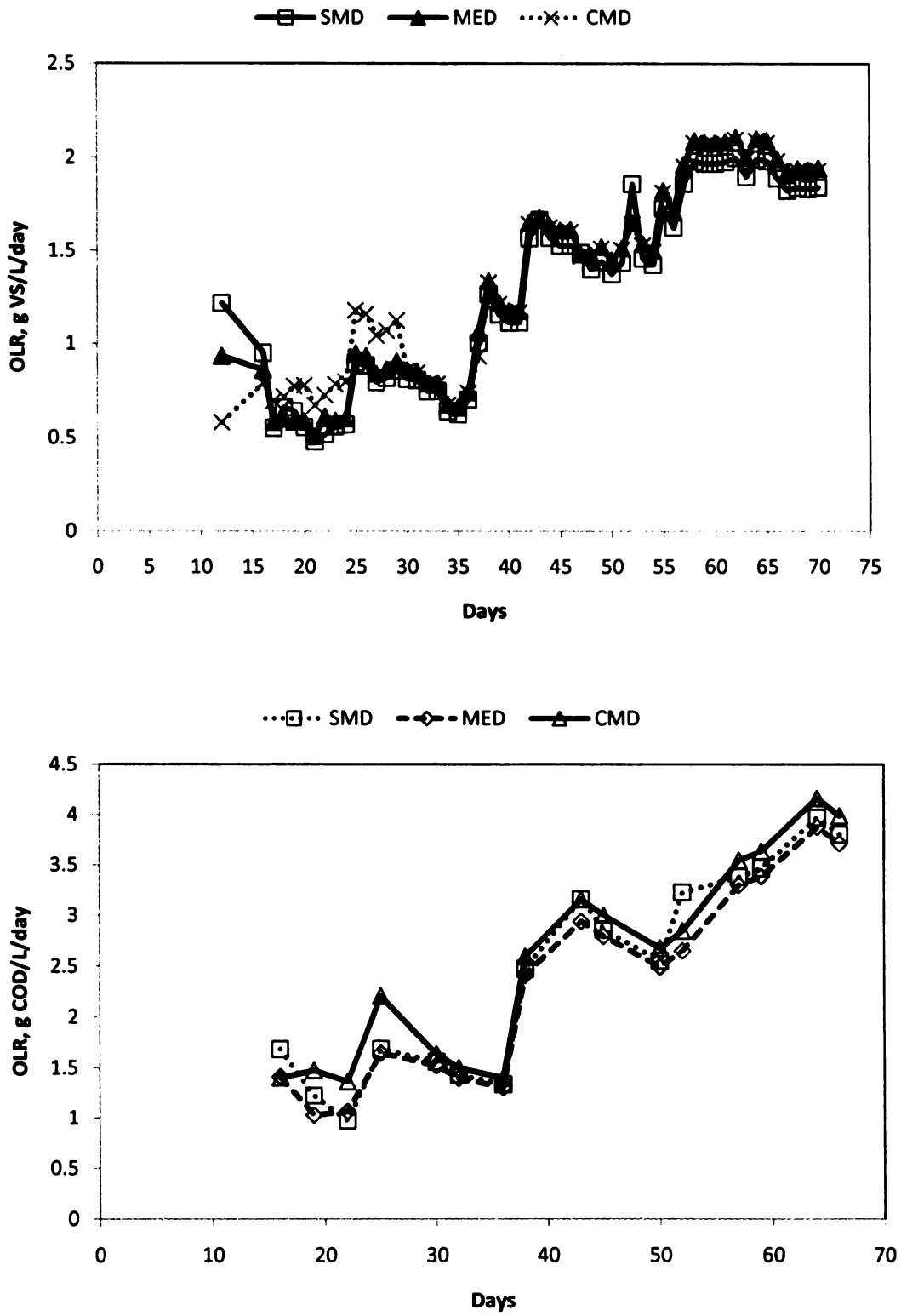


Figure 4.9 Phase 1, organic loading rate, VS and COD

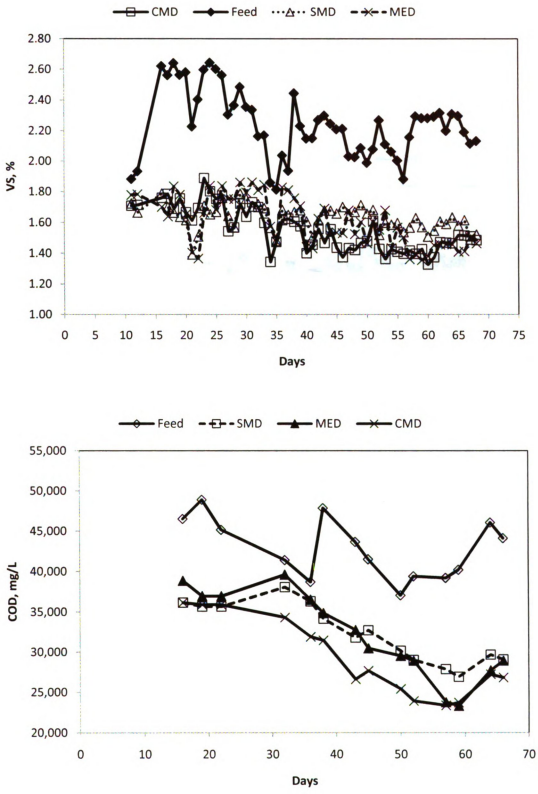


Figure 4.10 Phase 1, COD and VS concentration

The desired operating HRT (11-12 days) was reached around day 57. Figure 4.11 and 4.12 show pH and volatile acid concentration during startup of the three systems in Phase 1.

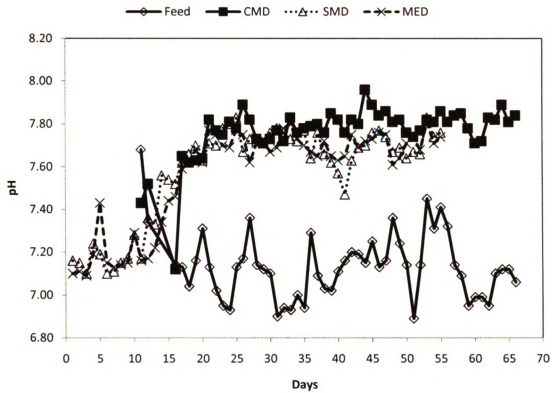


Figure 4.11, Phase 1, pH

Ghaly and Echiegu (1993) reported a total volatile acid concentration of approximately 2,000 mg/L for raw dairy manure with a TS concentration of 3.3% and a volatile acid content of approximately 26 mg/L using a GC method for the digested manure in a continuous mix anaerobic reactor. Hoffmann, Garcia et al. (2008) reported volatile acid concentrations for stable dairy manure digesters in the range of 250 mg/L using a titration procedure for quantification. The systems in the present research were considered stable

when the volatile acid content, as measured via a titration procedure (see Chapter 3, Section 3.2.2 Analytical Methods and Appendix B), was less than 250 mg/L and the pH was consistent and stable. The CMD and the MED were largely stable by day number 40. The SMD was slower to reach stable operation and did not reach a volatile acid concentration of less than 250 mg/L until day 57 (Figure 4.12).

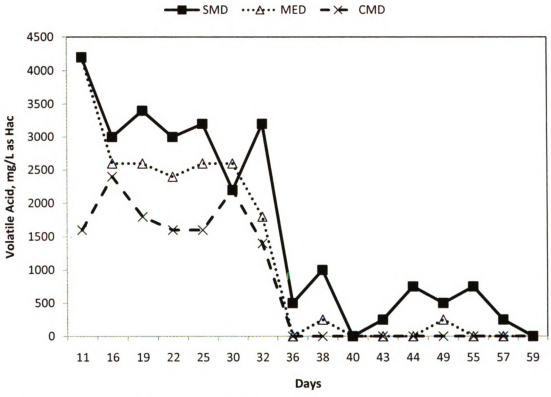


Figure 4.12 Phase 1, volatile acid concentration

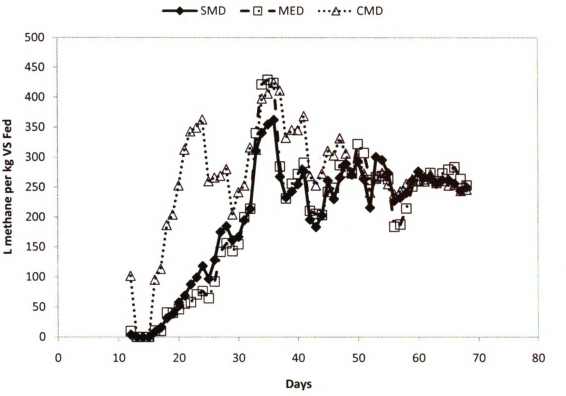


Figure 4.13 Phase 1, methane production

The methane production rate (Figure 4.13) for all three systems stabilized at approximately the same value from day 55 through the end of the experiment with very low volatile acid production and stable pH.

Though the SM system was operated with 100% permeate recycle, its flux rate was still measured on a daily basis and this is presented in Figure 5.14. The flux rate for the SMD was relatively high based on previous work conducted in this research (Chapter 3, Section 3.1 and 3.2, Tables 3.3 and 3.5 and Figures 3.6 and 3.14); however, this test was performed at a digester total solids concentration of 2.6%, much lower than previous work (Figure 4.14). The average flux rate of 118 $L/m^2/hr$ is higher than most other AnMBR work found in the literature. Zitomer, Bachman et al. (2005) also working with

dairy manure, reported flux rates between 40 to 80 L/m^2/hr at an operating CFV of 3.3 m/s and a digester TS concentration of approximately 3%. Pierkeil and Lanting (2005) reported an operating flux of 145 L/m^2/hr with municipal solids as the substrate; however, they were operating at a total solid content of 1% without a reported CFV. Strohwald and Ross (1992) found a linear relationship between CFV and membrane flux for a membrane bioreactor treating brewery effluent. The reported flux for Phase 1 of this research was conducted at a CFV of 4.5 m/s, substantially higher than most reported rates and this likely contributes to the higher flux rate observed in this research compared to flux rates reported in the literature.

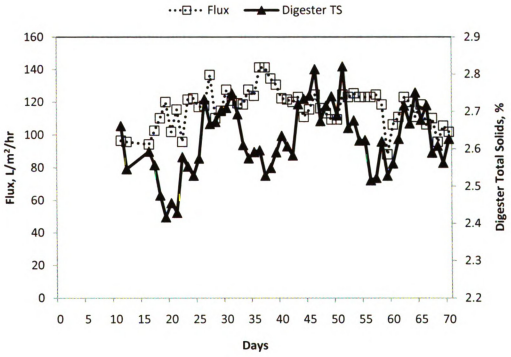


Figure 4.14 Phase 1, SMD flux rate versus digester TS concentration

The following conclusions for Phase 1 follow:

1. Cross-flow velocity as high as 4.5 m/s does not influence methane production when compared with the CMD control mixed 6 times per day x 3 minutes.
2. The 13.9 mm Pipe can be used as a surrogate for a UF membrane under the tested conditions

4.3.2 Phase 2

Testing in Phase 2 evaluated the biogas production differences between two AnMBR systems, one operating at a cycle time of 6 (Figure 4.6) and the other operating at a cycle time of 27 (Figure 4.3, permeate not returned to digester tank) with a control CMD (Figure 4.5). The operating data and water quality data are presented in Tables 4.6-4.9, respectively with plus/minus values indicating the standard deviation for each water quality result.

During days 1-54, the CMD mixing pump was operated 6 times per day x 3 minutes, consistent with its operation for Phase 1. The mixing rate for the CMD was reduced to 4 times per day x 1 minute for days 55-92 in an effort to determine if the higher mixing rate was negatively impacting methane production.

Table 4.6 Summary of operating data for Phase 2

Parameter	SM AnMBR	MM AnMBR	CMD
UF circulation rate, LPM	43	29	NA
Cross-flow Velocity, m/s	4.5	2.9	NA
Flux, $L/m^2/hr$	53	24	NA
Transmembrane Pressure, kPa	86	240	NA
Membrane entry pressure, kPa	124	450	NA
Avg. TS in Reactor, %	4.3	5.0	2.8
VS destruction, %	38	33	33
COD destruction, %	36	34	42
L CH_4/kg VS fed/day*	252	246	247
L CH_4/kg VS fed/day (days 1-54)*	252	NA	245
L CH_4/kg VS fed/day (days 55-92)*	252	NA	251
CH_4 concentration, %	67	67	65
Cycle time, day^{-1}	27	6	1
HRT, days	12	12	12
SRT, days	24	27	11.6

*Actual conditions

COD analysis was conducted approximately 3 times per week while VS analysis was conducted every day. The COD (or VS) destruction was calculated based on the

difference between COD (or VS) added to the digester, COD (or VS) discharged from the digester plus (or minus) any accumulation of COD (or VS) in the digester. The VS destruction for all three systems was similar; however, the COD destruction for the CMD was considerably higher than for the AnMBR digesters. The reported COD destruction for the CMD (42%) appears too high based on comparison to the AnMBR results and methane production and may be a result of the timing of COD analysis. Because VS data was collected every day, it is a better metric for comparison.

The methane production for the CMD, shown in Table 4.6, was very similar for both mixing regiments, indicating the higher rate of mixing during days 1 through 54 did not negatively influence methane production. As a check to estimate if data was skewed by changes in manure fed to the CMD, it was confirmed that the methane production for the SM AnMBR was the same for days 1-54 and 55-92, suggesting consistency in the methane production potential of manure for both periods. The MM AnMBR methane production data was not shown during the two periods of interest because it was still in the start-up phase during days 1 through 15.

Table 4.7 Summary of water quality data for Phase 2 – SM AnMBR

Parameter	Influent	Wasted Effluent	Membrane Permeate
COD, mg/L	52,300±14,800	50,200±7,000	5,260±1,550
TS, %	4.0±1.1	4.3±0.6	0.8±0.1
VS, %	2.6±0.6	2.8±0.4	0.3±0.1
pH	7.02±0.14	7.70±0.06	7.77±0.06
Average fed/removed, kg/day	10.0	4.9	5.1

Table 4.8 Summary of effluent water quality data for Phase 2 – MM AnMBR

Parameter	Influent	Wasted Effluent	Membrane Permeate
COD, mg/L	52,300±14,800	56,500±7,620	5,590±1,950
TS, %	4.0±1.1	5.0±0.7	0.9±0.1
VS, %	2.6±0.6	3.2±0.5	0.3±0.1
pH	7.02±0.14	7.67±0.06	7.75±0.06
Average removed, kg/day	10.1	4.4	5.7

Table 4.9 Summary of effluent water quality data for Phase 2 – CMD

Parameter	Influent	Effluent
COD, mg/L	52,300±14,800	29,900±6,010
TS, %	4.0±1.1	2.8±0.5
VS, %	2.6±0.6	1.7±0.3
pH	7.02±0.14	7.76±0.06
Average removed, kg/day	14.3	14.3

The volatile acid concentration for the SM AnMBR and the CMD remained below the detection limit of the titration procedure used for analysis, consistent with the steady-state condition of Phase 1 (data not shown). The MM AnMBR exhibited a slightly depressed pH and increased volatile acid concentration during the first 14 days of operation (Figure 4.15 and 4.16). GCMS analysis (Appendix E) was conducted for the final day of Phase 2 operation and is presented in Table 4.10.

Table 4.10 Volatile fatty acid data

	Acetic Acid, mg/L	Propionic Acid, mg/L	Butyric Acid, mg/L	Isobutyric Acid, mg/L	Total, mg/L
SM AnMBR	192	85	15	1	**299**
MM AnMBR	182	75	14	0.4	**271**
CMD	199	91	16	38	**344**

Acetate represented the majority of the VFA present in all three digesters. As detailed in Chapter 2, Section 2.3, hydrogen consuming methanogens must work in syntrophic cooperation with fatty acid oxidizing fermenting bacteria to maintain low H_2 concentrations. When H_2 concentrations increase, a shift towards more reduced products such as propionate, as opposed to acetate, occurs (Ianotti 1973; Bryant 1979). The syntrophic relationship requires spatial proximity between the fatty acid oxidizing fermenting bacteria and the hydrogen consuming methanogens (McCarty and Smith 1986). Because a build-up of propionate was not observed in either of the AnMBRs (concentrations are consistent with the CMD control), there is not an apparent break-down in syntrophic activity due to the shearing impact of the pump/membrane system.

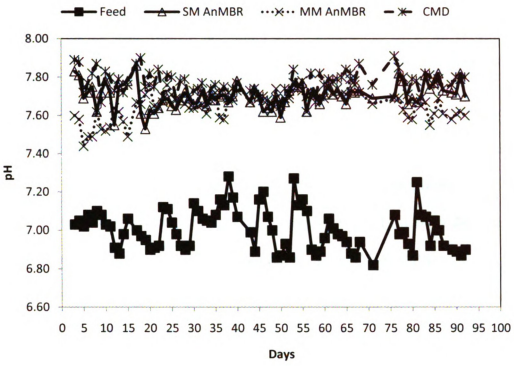


Figure 4.15 Phase 2, pH

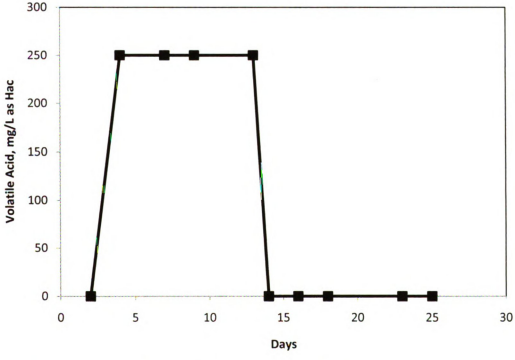


Figure 4.16 Phase 2, volatile acid concentration, MM AnMBR

Figures 4.18 and 4.19 illustrate a steady increase in the VS and COD concentrations respectively for the MM AnMBR beginning at day 1 through 17. At the start-up of the MM AnMBR, the timer operation was set at 2 minutes x 24 times per day. The frequency of this initial setting resulted in a permeate generation rate that approximated the feed rate and, as a result, there was very little biomass wasted from the digester, consequently the digester VS/TS/COD concentration increased at a faster rate than the SM AnMBR. At day 16, the timer operation was adjusted to 1 minute x 24 times per day to more closely approximate the TS concentration of the SM AnMBR.

The volatile acid titration procedure (Appendix B) identifies volatile acids present in a sample; however, at stable operation, the titration procedure was non-detect for the digester systems of this research. GCMS indicated there were volatile acids present, even during stable operation (Table 4.10) suggesting that GCMS is a more accurate method for quantifying volatile acids at low concentration.

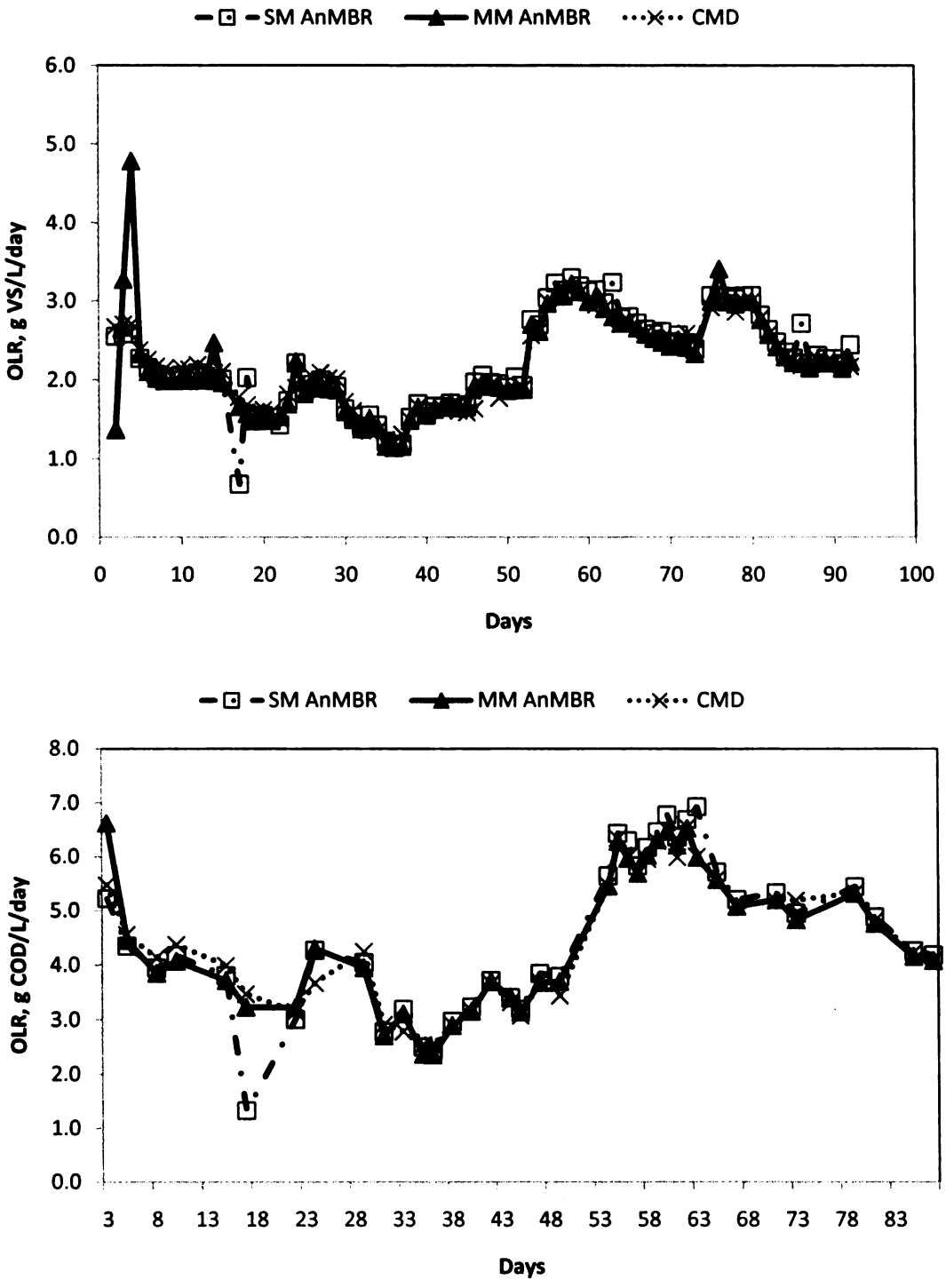


Figure 4.17 Phase 2, organic loading rate, COD and VS

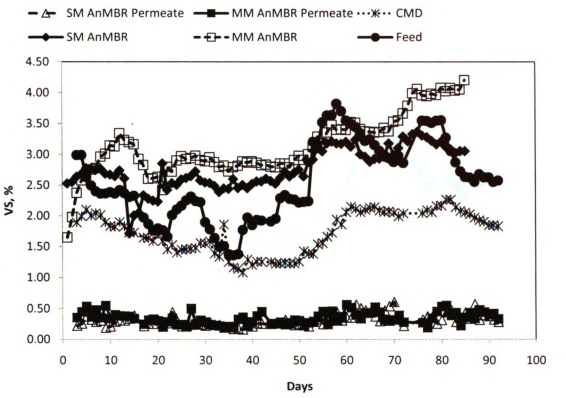


Figure 4.18 Phase 2, VS concentration

Figure 4.17 presents the organic loading rates for VS and COD for all three digesters and shows a significant increase in loading rate at day 50 which resulted in similar increases in effluent VS and COD (Figures 4.18 and 4.19) as well as methane production (Figure 4.22). Volatile acids were not tested during this period; however, pH remained stable (Figure 4.15) indicating the digester systems did not have any problem processing the increased loading rate.

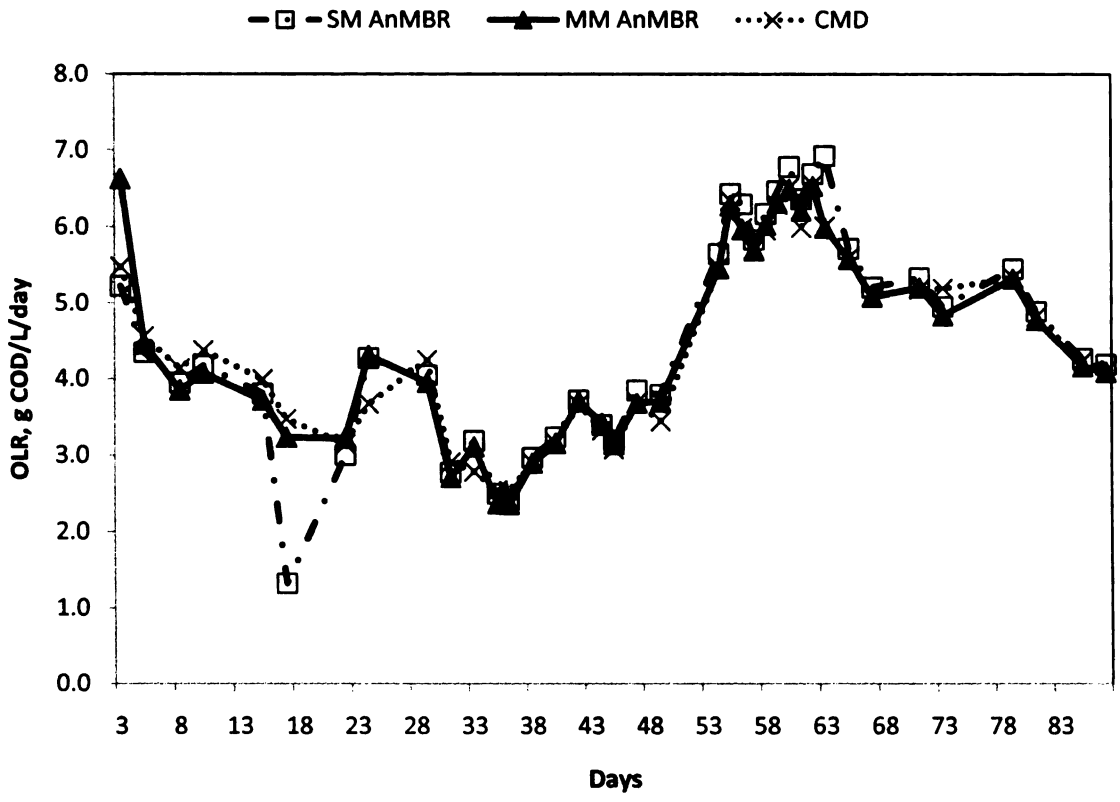


Figure 4.19 Phase 2, COD concentration

The flux data for the SM AnMBR and MM AnMBR are presented in Figures 4.20 and 4.21 respectively. The SM system averaged 53 L/m^2/hr and the MM Membrane averaged 24 L/m^2/hr. Consistent with the previous work presented in Chapter 3, Section 3.1, a declining flux rate follows an increasing digester total solids concentration (Anderson, Saw et al. 1986; Beaubien, Baty et al. 1996; Brockmann and Seyfried 1996). The SM AnMBR system was cleaned on day 16 and there was an immediate increase in flux rate from approximately 33 L/m^2/hr to 104 L/m^2/hr followed by a slow decay in flux rate between days 18-68 when the flux rate was 13 L/m^2/hr and the membrane was

cleaned again. Flux stability and cleaning protocol are discussed in greater detail in Chapter 6, Section 10. A leaking valve on the day 16 cleaning resulted in low pH cleaning solution (approximately pH = 4.5) entering the digester tank. Approximately 25% of the digester volume was displaced with the cleaning solution. This resulted in a reduction in the digester total solids concentration (Figure 4.20) and a reduction in gas production (Figure 4.22). The SM system recovered quickly and within 8 days was producing gas consistent with the CMD. The low pH cleaning solution was acetic acid-based (specific detail is presented in Chapter 6, Section 9).

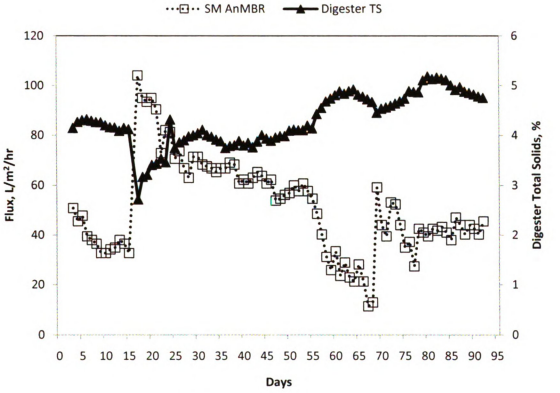


Figure 4.20 Phase 2, SM AnMBR flux rate and digester TS concentration

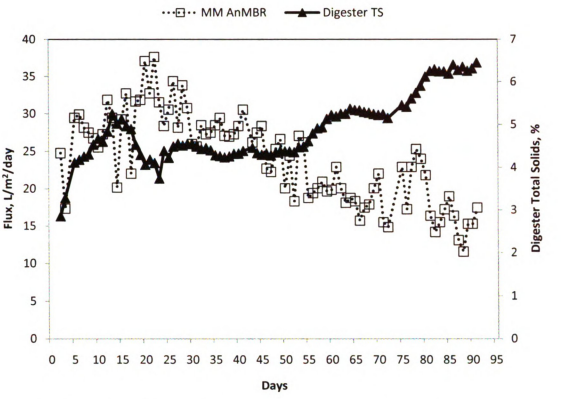


Figure 4.21 Phase 2, MM AnMBR flux rate and digester TS concentration

Figure 4.21 shows an initial increase in flux for the MM AnMBR from day 1 to 22. As detailed above, the initial timer operation was set too high. To counter the very rapid increase in digester TS concentration, the permeate was returned to the digester between days 18 and 22 and the digester contents were wasted consistent with the feed rate causing a decline in the digester TS concentration. At day 23, normal operation was resumed and the general trend of decreasing flux against increasing digester TS also resumed.

The MM AnMBR required approximately 12-15 days of operation to reach a gas production rate consistent with the other two digesters (Figure 4.22). This lag was most

likely due to the change in operating conditions from Phase 1 to Phase 2 and is consistent with the period of volatile acid production identified in Figure 4.16.

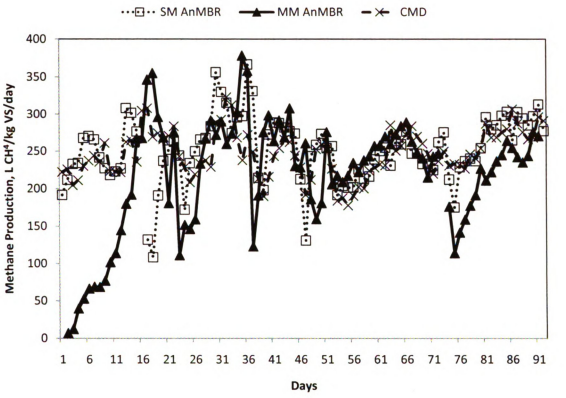


Figure 4.22 Phase 2, methane production

Initially, there appeared to be a significant difference in gas production for the MM AnMBR (data not shown); however, on day 59 it was recognized that the wet tip meter measuring biogas production for the MM AnMBR was double counting gas production. A review of the data indicated the double counting started around Day No. 14. The data was corrected by dividing the meter reading by 2. The MM AnMBR membrane was cleaned at day 74. Following this cleaning, its gas production lagged the other digesters for the balance of the experiment. The MM AnMBR's module that houses its 7

membranes is considerably larger than the SM AnMBR module. When cleaning the membranes, the final step was to flush the membrane with clean water for 15 to 30 minutes (Chapter 6, Section 9 provides membrane cleaning detail). However, due to the greater volume of the MM AnMBR module, there was probably still residual cleaning solution in the module. This may have led to a decline in performance of the MM AnMBR digester.

It was anticipated that at low cycle time, the MM AnMBR (cycle time = 6/day) would outperform the CMD and the SM AnMBR (cycle time = 27/day). However, based on the fact all systems produced equal amounts of methane, the following conclusions were made.

1. The potential advantage of operating at a higher VS concentration for the SM AnMBR is off-set by the impact of a high cycle time (cycle time = 27).

2. The potential advantage gained by operating at a low cycle time and higher VS concentration for the MM AnMBR (cycle time =6) is off-set by the high degree of turbulence in the system due to the torturous path the biomass must negotiate. The torturous path is a design issue unique to the nature of the pilot-scale setup. Figure 5.7 presents a picture of the manifold that was constructed to direct flow in a series fashion through the membrane elements. This issue is further explored in Phase 3.

3. Methane production is equal for both SM AnMBR and MM AnMBR because methane production is independent of the operating conditions of these systems.

4. CMD methane production was independent of the two mixing conditions tested and, referencing the conclusions of Phase 1, methane production is independent of cross-flow velocity at 4.5 m/s or less.

4.3.3 Phase 3

For the final phase of testing, the SM AnMBR was converted back to a SMD (resembling a complete mix digester with the permeate returned to the digester tank) and operated at a cycle time of 27 (consistent with Phase 2 operation of the SM AnMBR). The purpose of this experiment was to evaluate whether the higher biomass concentration of the SM AnMBR provided a methane production advantage. Phase 2 results indicated that that the MM AnMBR, operating at a cycle time of 6/day, did not have a methane production advantage over the CMD or the SM AnMBR (cycle time equaled 27/day). It was theorized that the MM AnMBR may have been negatively impacted by the system design. Therefore, in Phase 3, the MM AnMBR was removed and replaced with four 13.9 mm diameter x 3000 mm PVC pipes (MMED) to mimic a design that might be used in a full-scale application. This design provided a less torturous path for the biomass to negotiate compared to that of the MM AnMBR which, in order to modify a full-scale membrane/module for pilot-scale work, necessitated the use of a manifold to route flow between membranes (Figure 4.7). Table 4.11 presents a summary of the operating data

and Table 4.12 a summary of the water quality data for each of the digesters with plus/minus values indicating the standard deviation for each water quality result.

Table 4.11 Summary of operating data for Phase 3

Parameter[1]	SMD	MMED	CMD
UF circulation rate, LPM	42	44	NA
Cross-flow Velocity, m/s	4.3	3.7	NA
Flux, $L/m^2/hr$	48	NA	NA
Avg. TS in Reactor, %	3.0	3.2	2.5
VS destruction, %	35	38	37
COD destruction[1]	NA	NA	41
L CH_4/kg VS fed/day[2]	275	250	280
CH_4 concentration, %	70	69	67
Cycle time, day^{-1}	27	6	1
HRT, days	12	12	12
SRT, days	12	12	12

[1]COD destruction not reported for SM AnMBR with 100% recycle or Four 3000 mm Pipes due to limited number of COD data points.
[2]Actual conditions

Table 4.12 Summary of water quality data for Phase 3

Parameter	Influent	SMD	MMED	CMD
COD, mg/L	42,200±17,500	32,800±8,850	34,200±10,870	26,200±5,180
TS, %	3.9±1.3	3.0±0.8	3.2±1.0	2.5±0.5
VS, %	2.4±0.9	1.9±0.5	2.0±0.7	1.5±0.3
pH	7.03±0.33	7.67±0.05	7.62±0.07	7.72±0.05

Figure 4.23 presents the COD and VS organic loading rate data and Figure 4.24 presents the feed and effluent COD and VS data for Phase 3. The SMD and MMED were previously operated as AnMBRs. As a result, the initial digester VS and COD concentrations were much higher for these two systems at the start of Phase 3 and required approximately 25 days of operation to reach a point where the VS and COD concentrations for these digesters were consistent with the CMD. Despite the initial VS differences between the systems, the gas production (Figure 4.26) for all three systems was very similar for the duration of the Phase 3 testing. At day 31, there was a spike in the VS and COD concentration of the manure due to farm operations (Figures 4.23 and 4.24) resulting in a decrease in methane production and pH (Figures 4.25and 4.26).

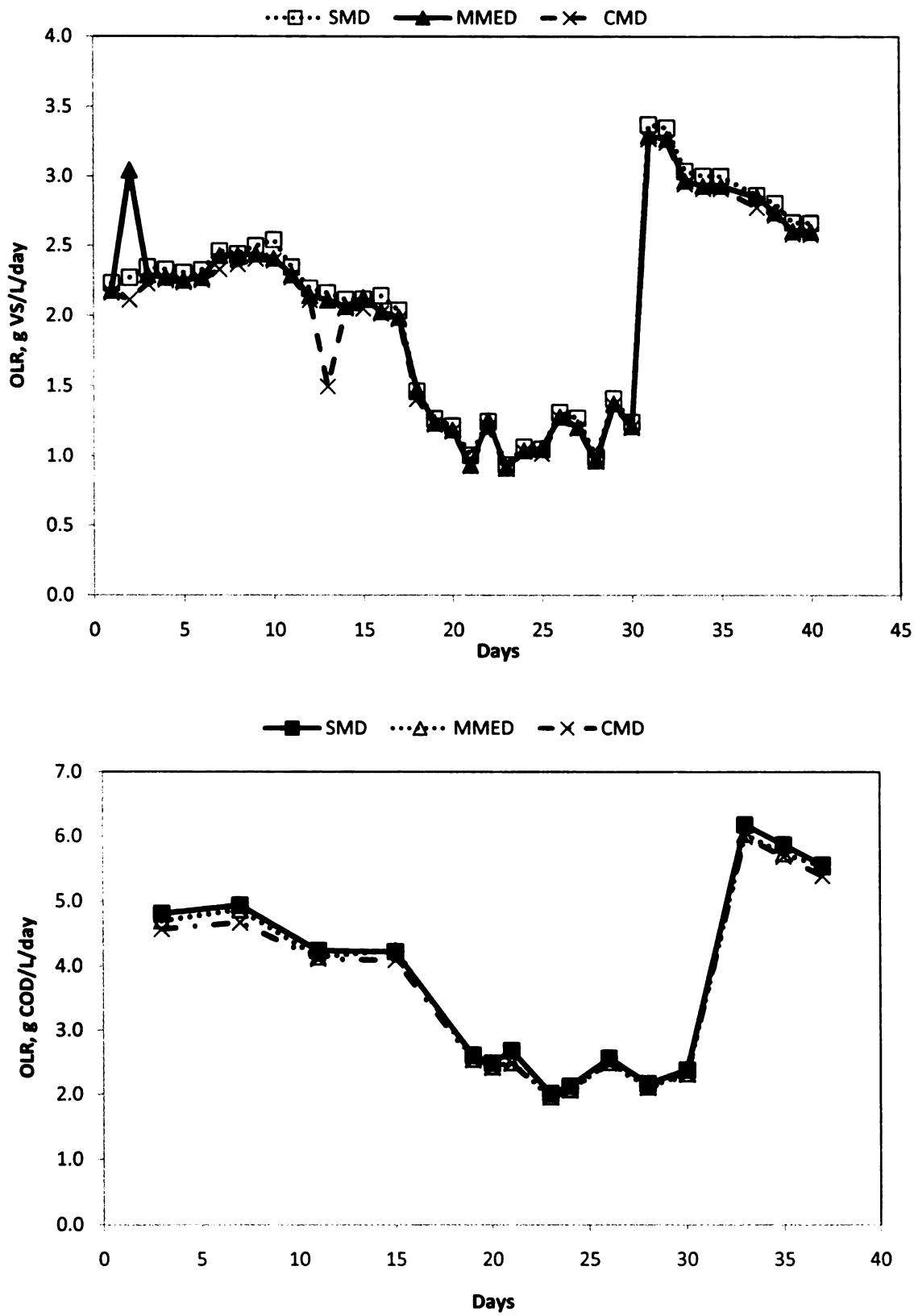
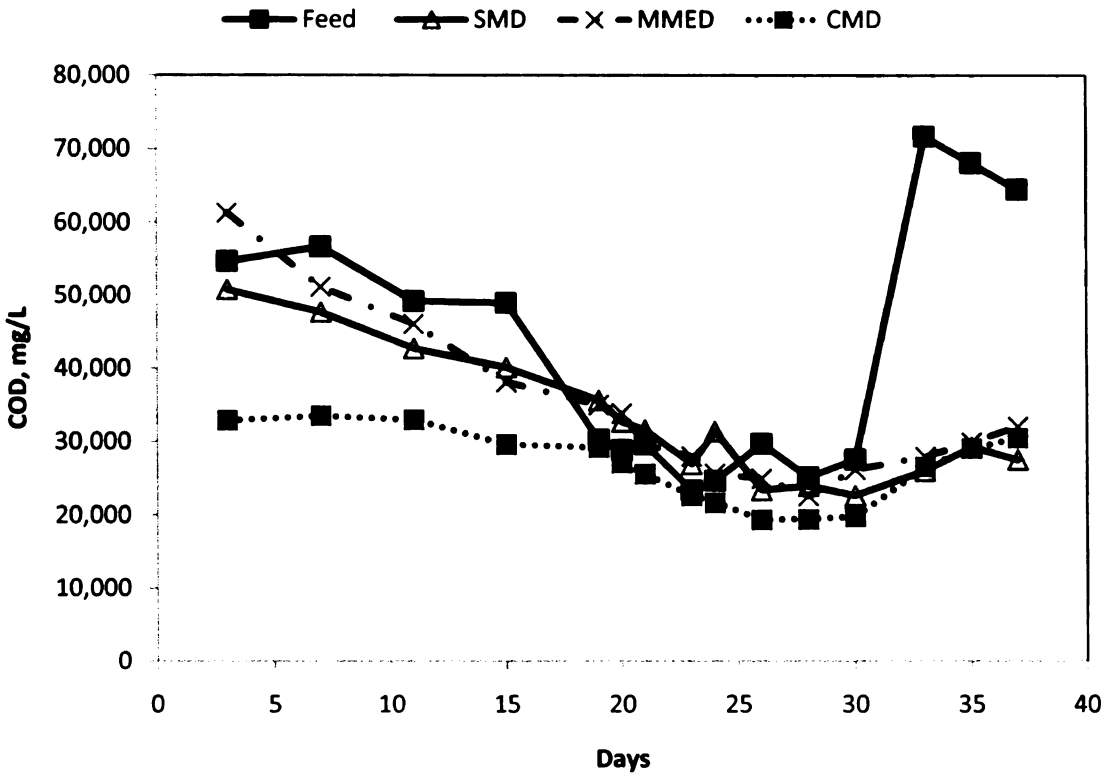


Figure 4.23 Phase 3, organic loading rates for VS and COD



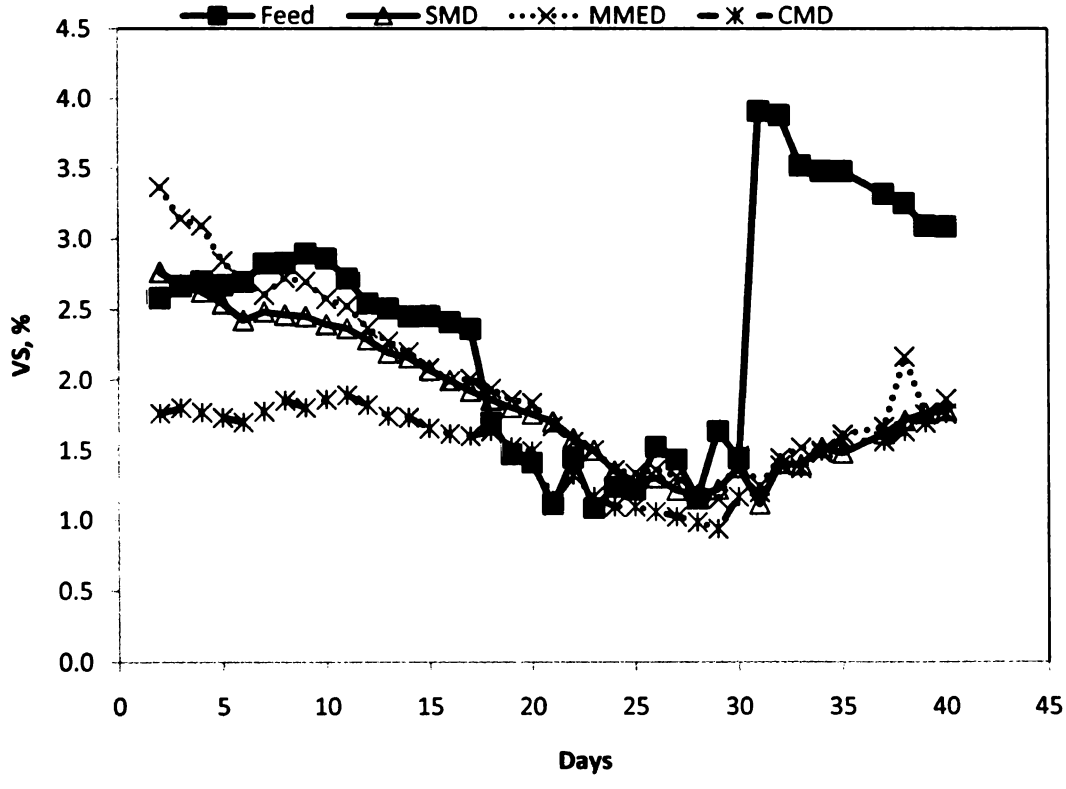


Figure 4.24 Phase 3, COD and VS concentration

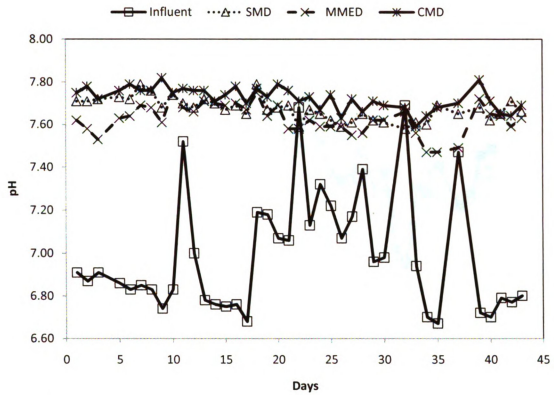


Figure 4.25 Phase 3, pH

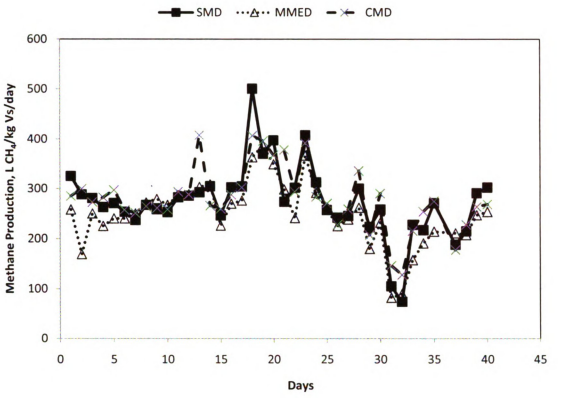


Figure 4.26, Phase 3, methane production

However, both pH and methane production quickly recovered as the systems responded to the change in organic loading. The pH impact on the MMED was more dramatic than for the other two digesters, suggesting this system was not as stable and more prone to an increase in VFA when perturbed, though there is not a clear explanation for this condition.

Tables 4.13-4.15 present acetic, propionic, butyric and isobutyric acid concentrations for the three digester systems as determined via gas chromatography mass spectroscopy (GCMS, protocol outlined in Appendix E). Total acetic, propionic, butyric and isobutyric acid concentrations were similar during the periods tested. The results are very similar to those in Phase 2 and indicate stable operation.

Table 4.13 Volatile fatty acid data, SMD

	Acetic Acid, mg/L	Propionic Acid, mg/L	Butyric Acid, mg/L	Isobutyric Acid, mg/L	Total, mg/L
Day 2	187	82	13	0	282
Day 7	132	47	23	6	208
Day 12	153	53	19	0	225
Average Value	**168**	**67**	**18**	**2**	**254**

Table 4.14 Volatile fatty acid data, MMED

	Acetic Acid, mg/L	Propionic Acid, mg/L	Butyric Acid, mg/L	Isobutyric Acid, mg/L	Total, mg/L
Day 2	208	89	14	0	311
Day 7	169	77	18	36	300
Day 12	200	97	16	43	356
Average Value	**190**	**85**	**16**	**20**	**310**

Table 4.15 Volatile fatty acid data, CMD

	Acetic Acid, mg/L	Propionic Acid, mg/L	Butyric Acid, mg/L	Isobutyric Acid, mg/L	Total, mg/L
Day 2	296	89	14	27	427
Day 7	184	70	14	0	268
Day 12	193	71	13	20	297
Average Value	**218**	**80**	**14**	**22**	**334**

The SMD experiment was designed to examine the impact of operating at a biomass concentration consistent with that of a complete mix digester compared to the elevated biomass concentration and extended SRT of the SM AnMBR of Phase 2. A cycle time of

27 was maintained (as in Phase 2) and the SMD produced 275 L CH_4/kg VS/day. The SM AnMBR of Phase 2 produced 251 L CH_4/kg VS/day. To account for differences in manure gas production potential between Phases 2 and 3, the CMD acted as a control and produced 247 L CH_4/kg VS/day during Phase 2 and 280 L CH_4/kg VS/during phase 3. Based on these methane production rates, there was virtually no difference between the SMD and SM AnMBR.

The MMED lagged the SMD and the CMD by about 10% during this period, a consistent trend beginning on day 74 of Phase 2 (same biomass for MMED and MM AnMBR), when the MM AnMBR membrane was cleaned. A potential reason for the lower than anticipated methane production rate may be a residual effect of this cleaning operation as previously discussed in Section 3.2 of this chapter. However, the operation of all three digesters resulted in similar VS destructions of 35%, 38% and 37% for the SMD, MMED and CMD respectively.

Based on the results of Phase 3, the following conclusions are made:

1. At a HRT of 12 days and a cycle time of 27, the AnMBR configuration that provides for an extended SRT compared to a complete mix configuration (HRT = SRT), did not provide a gas production advantage.

2. There does not appear to be a pronounced advantage or disadvantage to operating with the less turbulent condition of Phase 3 (MMED) compared to the MM AnMBR of Phase 2, suggesting its membrane/manifold configuration did not have a negative impact on gas production.

4.4 Summary

There was not an increase in methane production associated with operating at the higher biomass concentration of the AnMBR system. Nor was there an apparent biogas production difference between an operating cycle time of 27/day and 6/day. All three systems operated at volatile acid concentrations that were not detectable via the titration procedure. A GCMS technique was used to measure the concentrations of acetic, propionic, butyric and Isobutyric acid for Phase 2 and Phase 3 showed all three digesters operated in the range of 200 mg/L to 450 mg/L. Acetic acid was the predominate VFA in all three systems suggesting the systems were stable and that syntrophic activity was not disrupted. Chapter 5, "AnMBR Metabolic Evaluation of Cycle Time", explores the metabolic level interactions in an effort to explain the observed pilot-scale results presented in this chapter.

Chapter 5

AnMBR METABOLIC EVALUATION OF CYCLE TIME

5.1 Introduction

The goal of this phase of the research was to develop a better understanding of the impact of cycle time on microbial activity. Much of this effort focused on three sets of respirometer experiments. These experiments were developed to allow for comparison of biomass from the AnMBRs and the CMD with the objective of using activity measurements to characterize and compare the microbial pathways associated with digester configurations described in Chapter 3 and 4.

Acetate was used as a substrate to evaluate the activity of acetate consuming methanogens by measuring methane production of a known quantity of digester biomass provided with a known quantity of substrate. Referencing the flow of electrons in Figure 2.1, acetate is converted directly to methane via acetate consuming methanogens. James et al. (1990) outlined a methodology for evaluating specific methanogenic activity (SMA) using a Warburg respirometer and sodium acetate as the substrate. The SMA test provides a basis for evaluating the methane generating potential for active biomass. The specific methanogenic activity is estimated from the methane production rate or the substrate depletion rate and the amount of sludge present (Vandenbe.L, Lentz et al. 1974; Owen, Stuckey et al. 1979; Valcke and Verstraete 1983; Dolfing and Bloemen 1985; James, Chernicharo et al. 1990; Soto, Mendez et al. 1993). As previously discussed in in Chapter 2, Section 5, acetate is fermented directly to methane and accounts for approximately 70% of total methane production, therefore, as a test substrate, acetate provides a very good indication of the maximum methane generating potential of a given

biomass. The results of this test are sometimes used to optimize organic loading rate for a faster and more reliable start-up (James, Chernicharo et al. 1990; Soto, Mendez et al. 1993). The SMA test is also used to evaluate ongoing process performance (Soto, Mendez et al. 1993). It provides a maximum gas generation potential against which actual performance can be compared.

Formate was used as a surrogate for hydrogen to assess the metabolic activity of the hydrogentrophic methanogens. Dolfing and Bloemen (1985) illustrated that methanogenic activity on H_2-CO_2 was comparable with the activity on formate. Dolfing and Bloemen (1985) also indicated that the relative contribution of mixed function methanogenic biomass (for example, *methanosarcina* spp) can be estimated by comparing the activities associated with a mixture of formate (as a hydrogen surrogate) and acetate and comparing with formate and acetate individually. The presence of *Methanosarcina* will result in a lower activity on hydrogen plus acetate as compared to the sum of the activities on acetate and formate individually because it has been shown to preferentially degrade hydrogen over acetate at high substrate concentrations (Dolfing and Bloemen 1985).

Propionate was used as a substrate to evaluate the syntrophic activity of the biomass to degrade propionate to acetate, H_2 and CO_2. Referencing Figure 2.1, propionate must first be degraded to acetate, H_2, and CO_2 prior to the occurrence of methanogenesis. As described in Chapter 2, Sections 2.4 and 2.5, propionate degradation is endergonic under standard conditions and requires syntrophic interaction between fatty acid oxidizing bacteria and hydrogen consuming methanogens. An evaluation of the biomass' ability to degrade propionate provides insight relative to the existence of syntrophic cooperation.

It is also of interest to compare the acidogenic activity of the biomass from the CMD and from the AnMBR. Similar approaches are presented in the literature using glucose as the substrate to measure acidogenic activity (Soto, Mendez et al. 1993; GarciaMorales, Nebot et al. 1996). Padmasiri et al. (2007) reported a decrease in methanogenic activity resulting from a build-up of volatile fatty acids and theorized that this was a direct result of an increase in the rate of hydrolysis caused by the high shear environment of the AnMBR, occurring at a rate that exceeded the metabolic capacity of the methanogens. Based on this theory, comparison of the acidogenic activity was undertaken to explore the potential of the AnMBR to select a more robust community of primary fermenters compared to the CMD.

Table 5.1 presents a summary of the substrates with their utility for the respirometer experiments.

Table 5.1 Substrates used for metabolic testing

Substrate	**Objective**
Acetate	Used to measure activity of acetate consuming methanogens
Formate	Used as a hydrogen surrogate to assess hydrogentrophic activity
Formate/Acetate	Methanosarcina will preferentially consume H_2 prior to acetate when both present in high concentrations.
Propionate	Used to compare syntrophic activity
Glucose	Used to compare acidogenic activity

In addition to the respirometer work, most probable number enumeration was conducted to allow for comparison of the viable organisms present in the SM AnMBR, the MM AnMBR and the CMD for Phase 2. Microscopic evaluation was also performed

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for these samples to gain a general sense of the spatial relationship of the organisms of the AnMBRs compared with the CMD.

5.2 Materials and Methods

5.2.1 Experimental Setup

The respirometer setup (Challenge Technology AR-200, Springdale, AZ) consisted of sixteen (16) reaction vessels of 675 ml, each with a working volume of 600 ml. Biogas flow measuring cells were dedicated to each respirometer reaction vessel. Gas flow data was logged automatically using a computer software interface. The reaction vessels were provided with gas-tight screw membrane caps allowing insertion of a needle in the headspace for removal of both gas and liquid from the reaction vessel without impacting ongoing experiments.

5.2.2 Methanogenic Activity Setup

To ensure a reliable estimation of a specific biochemical activity, the biomass should be present in the test bottles such that it is the limiting factor in the reaction to be studied (Dolfing and Bloemen 1985; Chynoweth, Turick et al. 1993). Typical values used in previous work ranged between 0.3 to 2.5 g HAc/g VSS (Valcke and Verstraete 1983; James, Chernicharo et al. 1990; Soto, Mendez et al. 1993), although values ranged as high as 30 g HAc/ g VSS (Dolfing and Bloemen 1985). Soto et al. (1993) proposed a VFA concentration of 2.0 and 0.5 for HAc and HPr respectively.

In this research, approximately 1.2 g acetate, 1.2 g formate, 1.2 g acetate plus formate and 0.3 g propionate were selected for methanogenic testing with the objective of

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achieving substrate to VSS ratios of 0.5 to 1.0 for acetate , formate and the combination of acetate and format and, for propionate, between 0.20 to 0.50. For a given respirometer bottle, the biomass was diluted to a VSS concentration that resulted in approximately 1.2-1.8 g VSS/bottle. Using Table 5.3 as an example, 1.2 g of acetate was added to a respirometer bottle that contained 1.38 g VSS, such that the Ac/VSS ratio became 1.2 g Ac ÷1.38 g VSS = 0.87 for the CMD.

5.2.3 Acidogenic Activity Setup

Glucose was used for acidogenic activity measurement because it is considered as the main intermediate pathway of anaerobic digestion of carbohydrate complex organics (Soto, Mendez et al. 1993). This is also appropriate for manure as cellulose and hemicellulose comprise a significant fraction of dairy manure (Amon 2007) and degrade to glucose. The half-saturation constant for acidogenic bacteria (K_s) is about 0.2 g COD/L (Henze and Harremoes 1983). A glucose concentration of 1.2 g/L was used in this research to ensure the initial substrate concentration was significantly greater than the half-saturation constant as recommended by Soto et al. (1993) and was close to the value of 1.5 g/L used by Soto et al. (1993).

5.2.4 Dilution Media Composition

The composition of the nutrient media solution used in this work is shown in Table 5.2 and was adapted from Garcia-Morales et al. (1996). The media composition recommended by Valcke and Verstraete (1983) was very similar and was also used by James et al. (1990).

Table 5.2 Dilution media composition

Chemical	Acidogenic Activity, g/L	Methanogenic Activity, g/L
Yeast extract	0.2	0.2
$NaHCO_3$	1	NA
K_2HPO_4	NA	1
KH_2PO_4	NA	2.5
NH_4Cl	NA	1
$MgCl_2$	NA	0.1

The acidogenic activity dilution media uses a sodium bicarbonate buffer to maintain an alkaline pH, which is favored by acidogenic bacteria. The Methanogenic activity dilution media uses a phosphate buffer to maintain the pH close to neutrality, which is favored by methanogens. Macro nutrients were provided for methanogenic growth but were not considered necessary for the acidogenic growth.

5.2.5 Operational Procedure

A schematic of the respirometer setup is shown in Figure 5.1. The biogas generated in the respirometer bottle was bubbled through a 1 M solution of potassium hydroxide (KOH) to scrub the CO_2 from the gas prior to measuring the generated volume for the CMD/AnMBR and Phase 2 testing. KOH scrubbing was not used for the Phase 1 test. Instead, biogas was measured directly and gas chromatograph analysis used to determine methane generation rate. A control was run for each digester and the gas generated from the control bottle was subtracted from the gas generated by each of the bottles testing the

various substrates. The operational procedure was adapted from James et al. (1990) and presented below.

1. Determine the volatile suspended solids concentration of the sludge to be analyzed prior to the start of the respirometer study.
2. Prepare the dilution media solution per Table 5.2
3. Dilute inoculum with media solution to desired VSS concentration and introduce into respirometer bottles.
4. Flush respirometer vessel headspace with nitrogen.
5. Seal respirometer vessels and connect to gas measuring cell.
6. Initiate water circulation in water bath and set temperature to 35° C.
7. Activate stir mechanism at a rate of 60 RPM.
8. Add substrate to bottles following an acclimation period of approximately 12 hours.
9. Continuously measure gas production for approximately 100 hours.
10. Measure glucose concentration every 1 to 2 hours for (acidogenic test bottles). The samples for glucose analysis to be removed using a needle/syringe setup via the reaction vessel's membrane cap.
11. Record methane production every 10 minutes.
12. Make periodic measurements of gas using a gas chromatograph to ensure all CO_2 is being removed by the potassium hydroxide.

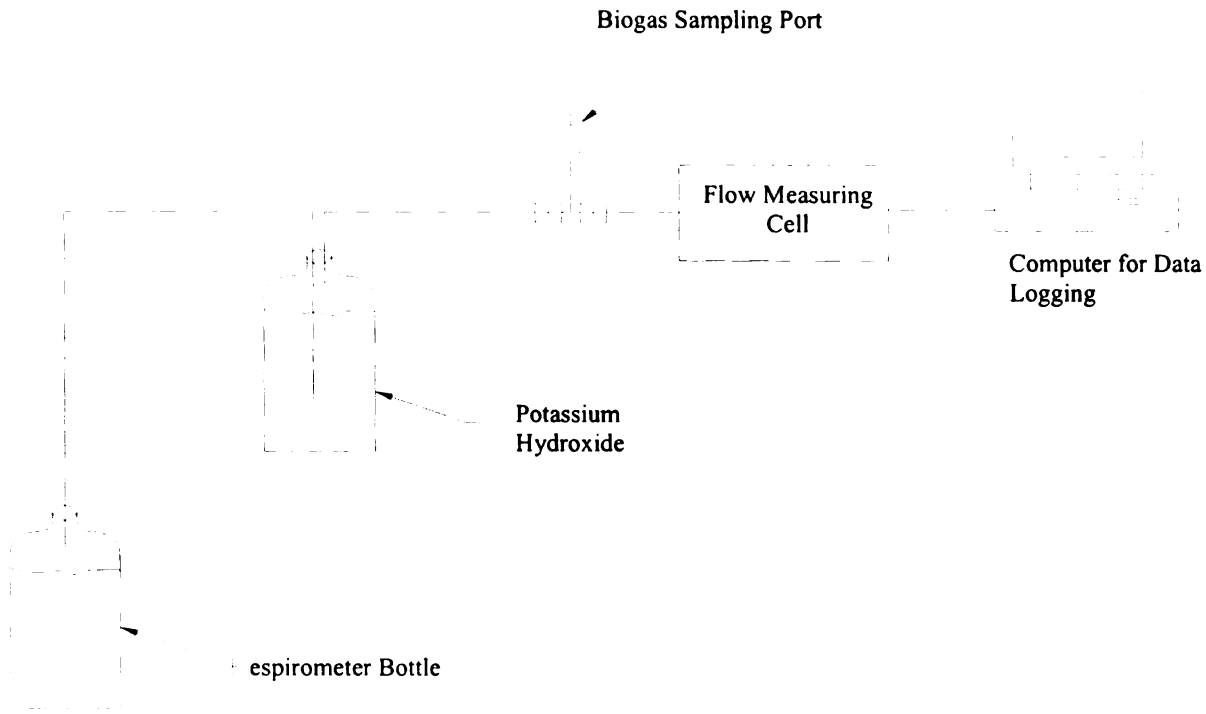


Figure 5.1 Respirometer Setup

5.3 Analytical Methods

5.3.1 General

General analytical methods used in this chapter were described in Chapter 3, Section 2.1. Glucose concentration was determined using a glucose assay kit (Sigma Aldrich, GAGO-20, St. Louis, MS). Most probable number enumeration was used to assess the estimated number of viable cells in the digester biomass and specific detail can be found in Appendix C.

5.3.2 Microscopic Observations

A scanning electron microscope was used to view the biomass from Phase 2 of the research which included the SM AnMBR operated at a cycle time of 27, the MM

AnMBR operated at a cycle time of 6 and the CMD control. Samples were taken from the systems on Day 92 and fixed at 4°C for ½ hour in 4% glutaraldehyde buffered with 0.1 m sodium phosphate at pH 7.4. One drop of 1% poly-L-lysine (Sigma P1399) was placed on a plastic petri dish and a 12 mm round glass coverslip was placed on top of the drop and allowed to stand for 5 minutes. The coverslip was removed and gently washed with several drops of water and drained but not allowed to dry. One drop of the cells fixed in suspension was placed on the side of the coverslip which previously faced down. The suspension was allowed to settle ten minutes before it was gently washed with several drops of distilled water. Next, the coverslip was placed in a graded ethanol series (25%, 50%, 75%, 95%) for five minutes in each with three five minute changes in 100% ethanol (Klomparens, Flegler et al. 1986). The coverslips were mounted with epoxy on aluminum stubs and coated with osmium. The preparatory work described above was conducted by personnel in the Center for Microscopy at Michigan State University. A JEOL 6400 scanning electron microscope in the Center for Microscopy at Michigan State University was used for viewing samples.

5.4 Results and Discussion

Three sets of respirometer experiments were performed on the biomass from various digester configurations of Chapter 3 and 4. The first set was conducted on the biomass from the combined CMD/AnMBR described in Chapter 3, Section 2.5 and referenced in this chapter by the same heading. The second and third set of respirometer experiments were conducted on the biomass from the digester systems detailed in Chapter 4, Section 2.2 and 2.3 and described as Phase 1 and Phase 2.

5.4.1 CMD/AnMBR Respirometer

The CMD/AnMBR respirometer work compared the biomass from the AnMBR to the CMD (outlined in Chapter 3, "Utility of an Anaerobic Membrane Bioreactor"). For this configuration, the pilot CMD effluent was the AnMBR influent (See Chapter 3, Figure 3.3). There was a significant difference in the gas production for the two systems. The CMD averaged 176 L CH_4/kg VS fed/day and the AnMBR averaged 82 L CH_4/Kg VS fed/day (Chapter 3, Table 3.5). This difference is not surprising considering the CMD was converting the readily degradable substrate and its effluent was the AnMBR influent. The respirometer tests provided a basis for evaluation by pairing equal concentrations of biomass from each digester with equal substrate concentrations in the reaction vessels. Table 5.3 outlines the substrate to biomass ratios used for the respirometer experiments described in Section 5.2.2 with results presented in Table 5.4. Figures 5.2-5.5 present the respirometer methanogenic results in graphical format. The CMD biomass produced methane at a higher rate than the AnMBR biomass for all methanogenic substrate. Figure 5.6 presents the glucose consumption data for the AnMBR/CMD system. The acidogenic testing illustrated that the biomass from the AnMBR degraded glucose at a higher rate than the CMD.

Table 5.3 CMD/AnMBR respirometer feed ratios, g substrate/g VSS

Substrate[1]	CMD	AnMBR
Acetate	0.87	0.78
Propionate	0.58	0.52
Formate	0.87	0.78
Acetate and Formate	0.87	0.78

[1]Substrate was sand-separated dairy manure

Table 5.4 Summary of CMD/AnMBR respirometer results, mL CH_4/g VSS/hr

Substrate[1]	CMD	AnMBR
Acetate	2.22	1.81
Formate	4.48	2.59
Acetate + Formate	6.74	2.28
Propionate	0.98	0.24
Cycle time, day^{-1}	NA	56
L CH_4/kg VS/day[2]	176	82

[1]Substrate was sand-separated dairy manure
[2]Actual conditions

The acetate activity of the CMD was similar to that for the AnMBR.

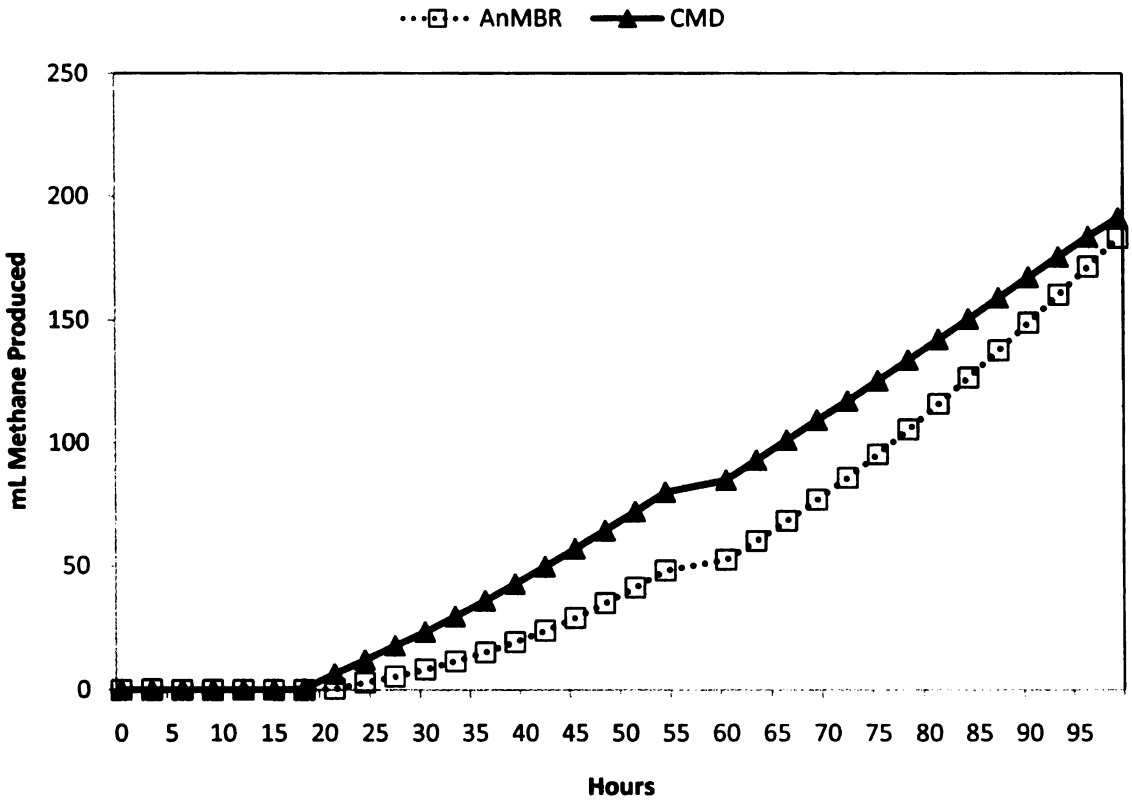


Figure 5.2 CMD/AnMBR, acetate

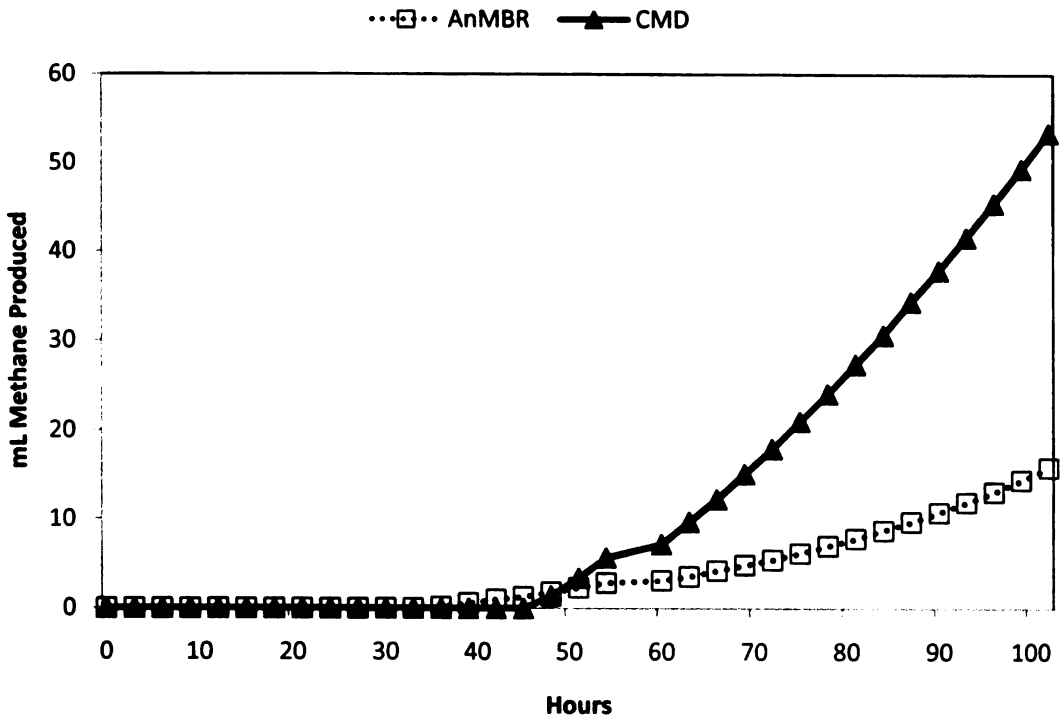


Figure 5.3 CMD/AnMBR, propionate

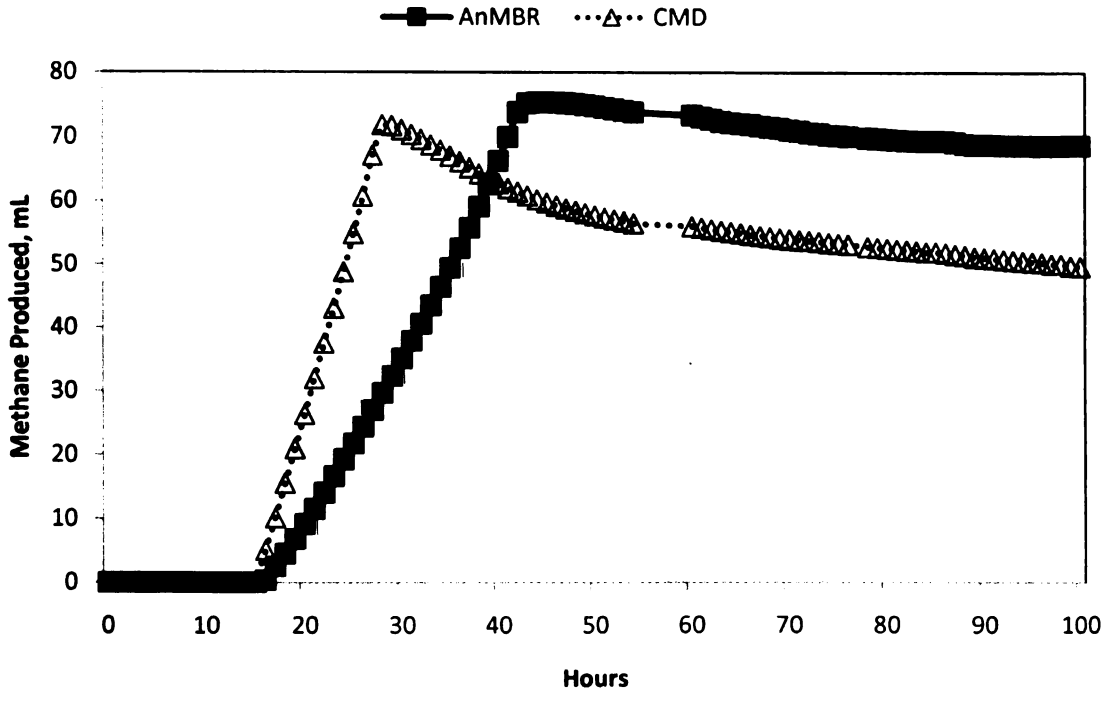


Figure 5.4 CMD/AnMBR, formate

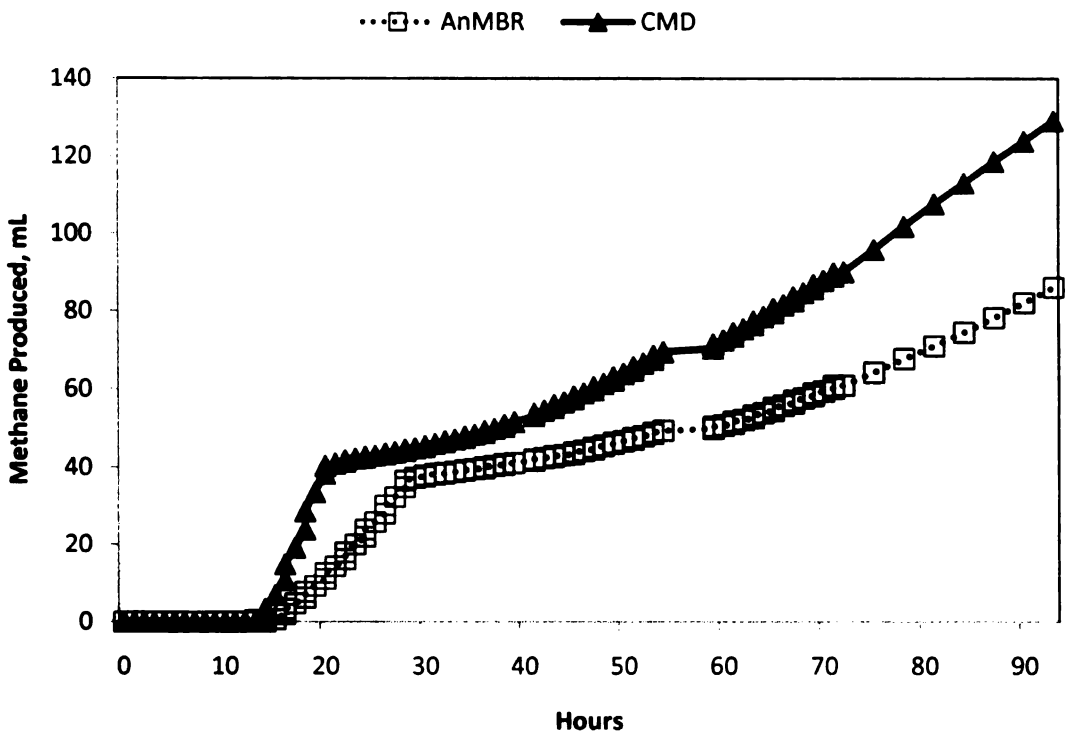


Figure 5.5 CMD/AnMBR, acetate + formate

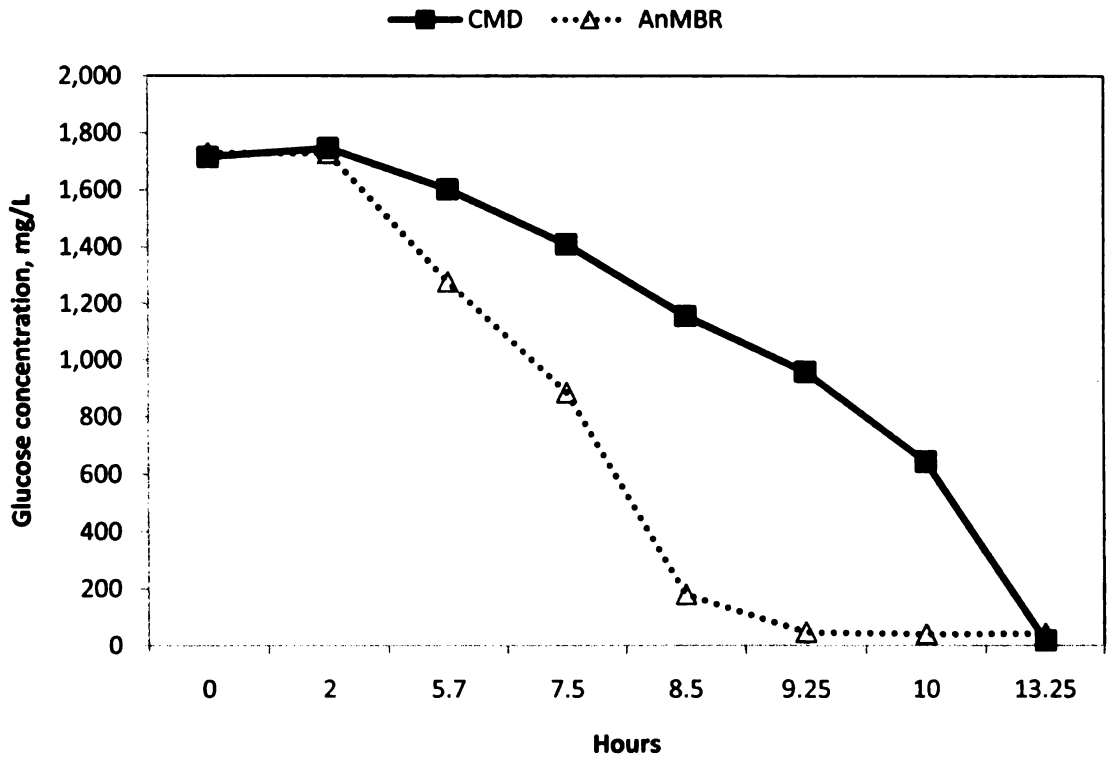


Figure 5.6 CMD/AnMBR glucose consumption per mass VSS

However, of particular note, there was a significant difference between the rate of methane production for the CMD on propionate compared to the AnMBR (Figure 5.3). Propionate requires a syntrophic interaction between acetogenic bacteria and hydrogentrophic methanogens (Kaspar and Wuhrmann 1978; Boone and Bryant 1980; McCarty and Smith 1986). The syntrophic interaction appears to be significantly greater for the CMD. This suggests the high shear environment of the AnMBR negatively impacted the juxtaposition between the acetogenic bacteria and the hydrogentrophic methanogens. The AnMBR also exhibited a lower activity for formate, consistent with a breakdown in syntrophic interaction (Figure 5.4). Lastly, referencing Table 5.4, the activity of formate and hydrogen individually are approximately equal to the activity for formate and hydrogen combined, suggesting that *Methanosarcina* like organisms did not make a significant contribution to the overall acetate consuming methanogenic population present for the CMD (Dolfing and Bloemen 1985). Following this same logic, it appears that *Methanosarcina* like organisms are present in the AnMBR. *Methanosarcina* cells grow as cocci whereas *Methanosaeta* cells grow as long filaments in anaerobic biomass (Hoffmann, Garcia et al. 2008). Due to their morphology, the *Methanosarcina* cells are likely to experience a competitive advantage in a high shear environment. Referencing Chapter 3, Figure 3.13, the volatile acid concentration of the AnMBR (CMD effluent was AnMBR influent) effluent averaged 232 mg/L as HAc as determined via a titration procedure (Appendix B). It is generally accepted that under conditions of high acetate concentration, *Methanosarcina* spp. will outcompete *Methanosaeta* (McMahon, Stroot et al. 2001; Hoffmann, Garcia et al. 2008). During this same period, the CMD exhibited a volatile acid concentration of zero (VFA method

located in Appendix B), suggesting that the AnMBR environment was more selective towards *Methanosarcina* spp.

Figure 5.6 illustrates the acidogenic activity for the two digester systems as determined by the rate of glucose consumption. This rate was markedly higher for the AnMBR compared to the CMD. This finding was also observed at the time of the methanogenic testing (data not shown). To confirm, fresh biomass samples were taken from each of the digesters and re-tested. The results (shown in Figure 5.6) were consistent with the initial testing. It is not clear why the AnMBR biomass exhibited a higher acidogenic activity than the CMD. Padmasiri et al. (2007) suggested the high shear environment of an AnMBR treating swine waste increased the rate of hydrolysis, thus increasing the rate of fermentation. Considering this theory, the AnMBR may have selected for a more robust acidogenic population due to an increased rate of hydrolysis compared to that of the CMD.

5.4.2 Phase 1 Respirometer

Respirometer experimentation was completed for the biomass for the Phase 1 digester systems. In place of CO_2 scrubbing, methane content was measured via gas chromatograph. As described in detail in Chapter 4, Section 2.2, the digesters are referenced as SMD, MED and CMD. All three systems were operated at approximately the same cycle time of 6. The CMD was mixed with a centrifugal pump that was energized on the same schedule as the circulation pumps for the SMD and MED. Figure 4.13 illustrates that the methane production for the SMD and MED lagged the CMD at

startup; however, all three systems produced methane at nearly the same rate once the SMD and MED reached stable operation.

Tables 5.5 and 5.6 present the respirometer feed ratios and results for Phase 1 respectively. Figures 5.7-5.10 present the respirometer methanogenic results in graphical format for the SMD, MED and the control CMD and Figure 5.11 presents the glucose consumption data for this period.

Table 5.5 Phase 1 respirometer feed ratios, g substrate/g VSS

Substrate[1]	CMD	SMD	MED
Acetate	0.70	0.73	0.83
Propionate	0.18	0.18	0.21
Formate	0.70	0.73	0.83
Acetate and Formate	0.70	0.73	0.83

[1]**Substrate was sand and solid-liquid separated dairy manure**

Table 5.6 Summary of Phase 1 respirometer results, mL CH_4/g VSS/hr

Substrate[1]	CMD	SMD	MED
Acetate	1.53	1.49	1.50
Formate	3.57	3.10	3.70
Acetate + Formate	2.94	2.37	2.80
Propionate	0.25	0.36	0.34
Cycle time, day^{-1}	5	7	7
L CH_4/kg VS/day[2]	280	264	268

[1]**Substrate was sand and solid-liquid separated dairy manure**
[2]**Actual conditions**

The results for the Phase 1 respirometer show nearly equal activity for the biomass from each of the digester systems and approximate the findings for the CMD/AnMBR

respirometer work with the exception of the activity for each of the digesters on propionate.

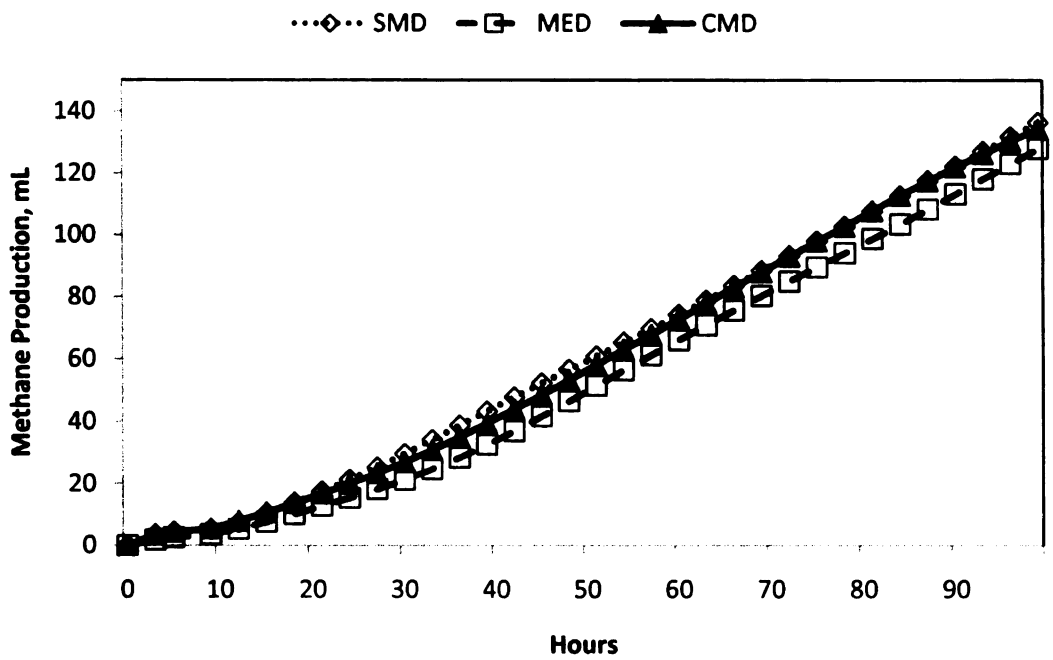


Figure 5.7, Phase 1, acetate

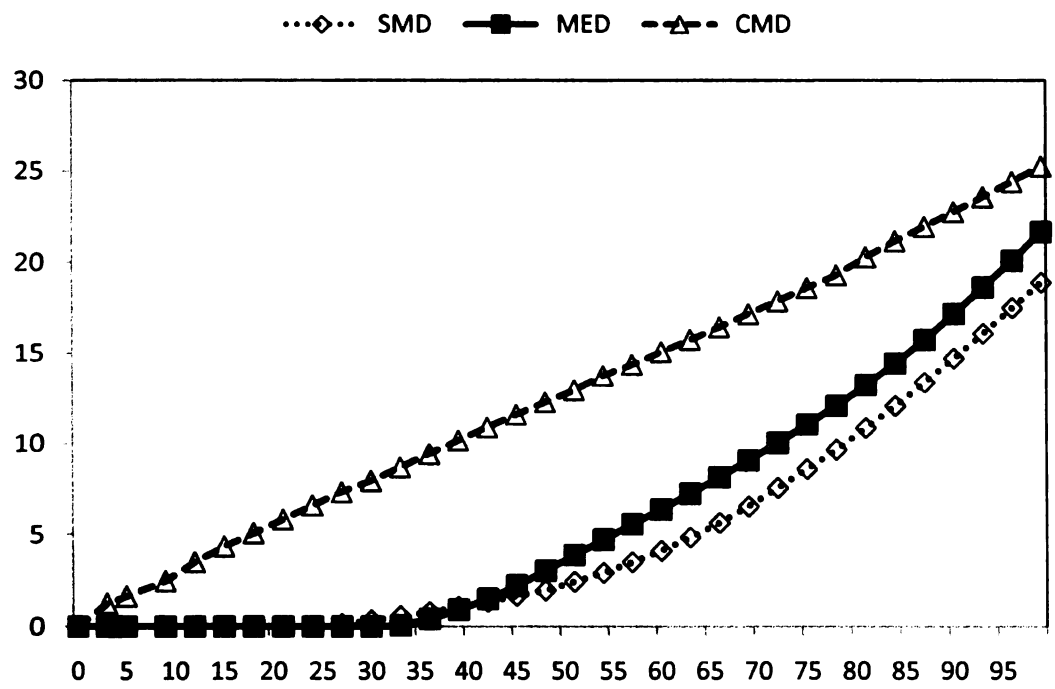


Figure 5.8 Phase 1, propionate

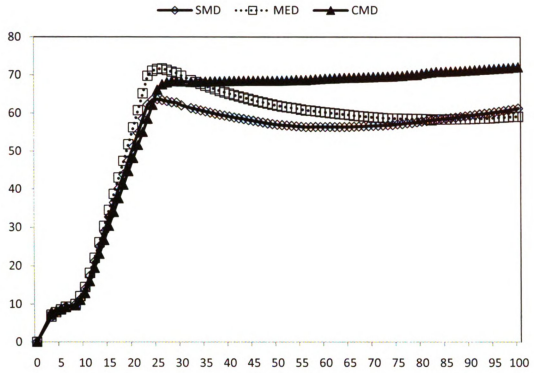


Figure 5.9 Phase 1, formate

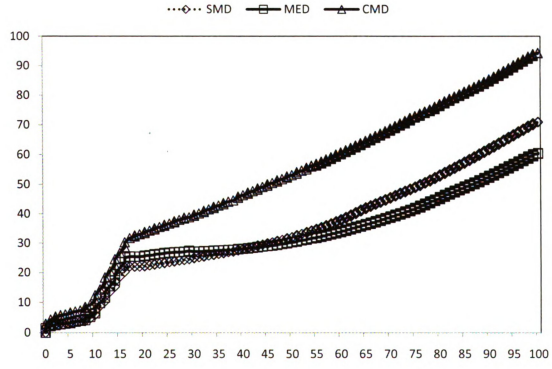


Figure 5.10 Phase 1, acetate + formate

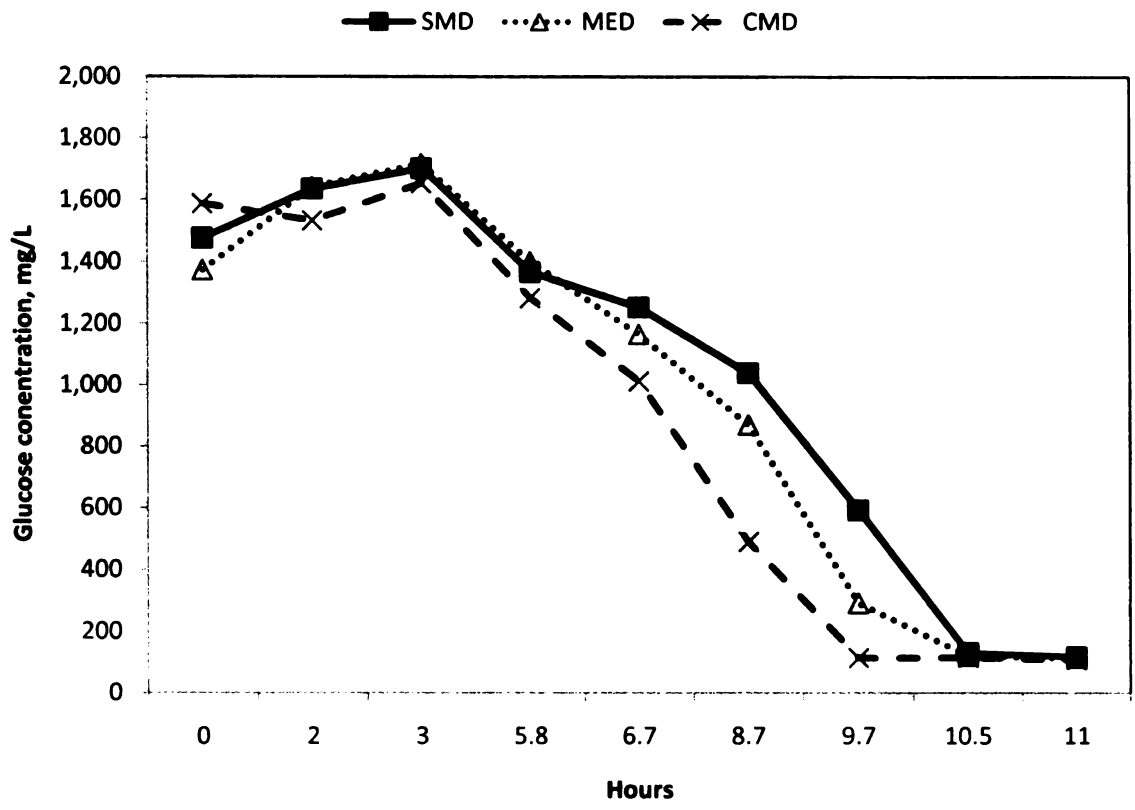


Figure 5.11 Phase 1, glucose consumption per mass VSS

The resulting activity on propionate was similar for the SMD and MED; however, the CMD curve for propionate was very different in appearance and not consistent with earlier (or later data) (Figure 5.8). GC analysis resulted in a methane concentration of 38% for the SMD and the MED control bottles; however, the methane concentration for the CMD control bottle was 25% and this likely skewed the propionate curve. If the methane content for the CMD control was assumed to approximate the other respirometer bottles, the CMD would have looked similar to the SMD and MED curves (data not shown). Further, none of the Phase 1 systems performed as well on propionate as did the CMD in the CMD/AnMBR work. This suggests a disruption of the syntrophic interaction occurred for all three digesters of Phase 1.

The measured activity on formate+acetate (Figure 5.10) was less than on formate alone for all three digesters, indicating that *Methanosarcina* like organisms made a significant contribution to the methanogenic population (Dolfing and Bloemen 1985). All three digesters were in a startup mode during much of Phase 1 and exhibited significant volatile acid concentrations through day 36 (Figure 4.12). The SMD and the MED had low but measureable volatile acid until Day 55 (MED) and day 59 (SMD). *Methanosarcina* has been shown to exhibit high growth rates at elevated acetate concentrations, while *Methanosaeta*, with its higher affinity for acetate, results in a competitive advantage at low acetate concentrations (McMahon, Stroot et al. 2001; Hoffmann, Garcia et al. 2008). One explanation for the apparent contribution of *Methanosarcina* like organisms to the methanogenic structure could be driven by the initial elevated concentrations of volatile acid of which acetate was likely a significant contributor.

The rate of glucose consumption (Figure 5.11) was very similar for all three digesters suggesting there was not a discernable difference with respect to the acidogenic activity of the systems.

5.4.3 Phase 2 Respirometer

The biomass from the SM AnMBR, the MM AnMBR and CMD were compared using the substrate feed ratios shown in Table 5.7. CO_2 scrubbing was used for the Phase 2 respirometer work. The SM AnMBR was operated at a cycle time of 27, the MM AnMBR was operated at a cycle time 6 and the CMD was operated at a low level of mixing equivalent to a cycle time of 1/day (the mixing pump was operated for 1 minute x 4 times per day).

Table 5.8 provides a summary of the methanogenic activity for each system. Figures 5.12 - 5.15 present the respirometer methanogenic results in graphical formate for the SM AnMBR, the MM Membrane AnMBR and the control CMD and Figure 5.16 presents the glucose consumption data for this period. The methane production for the pilot systems was nearly equal over the course of Phase 2. Despite this, there were significant differences in metabolic activity for these systems.

Table 5.7 Phase 2 respirometer feed ratios, g substrate/g VSS

Substrate[1]	CMD	SM AnMBR	MM AnMBR
Acetate	0.72	0.93	0.76
Propionate	0.21	0.23	0.19
Formate	0.71	1.0	0.80
Acetate and Formate	0.80	0.95	0.68

[1]**Substrate was sand and solid-liquid separated dairy manure**

Table 5.8 Summary of Phase 2 respirometer results, mL CH_4/g VSS/hr

Substrate[1]	CMD	SM AnMBR	MM AnMBR
Acetate	1.54	2.18	2.98
Formate	4.95	3.58	1.81
Acetate + Formate	3.61	2.63	1.74
Propionate	0.91	0.24	0.0
Cycle time, day^{-1}	--	27	6
L CH_4/kg VS/day[2]	247	251	246

[1]**Substrate was sand-separated solid-liquid separated dairy manure**
[2]**Actual conditions**

The CMD results were similar with those of previous experiments and the CMD activity on propionate returned to a level similar to that of the CMD in the CMD/AnMBR respirometer experiment. In Phase 1, the CMD contents were circulated through a mixing pump at a rate consistent with the two AnMBR systems. For Phase 2, the CMD pumping rate was reduced to a low rate of mixing. The gentle mixing of the CMD in Phase 2 may explain the increase in the propionate activity compared to Phase 1.

Both the SM AnMBR and the MM AnMBR exhibited increased acetate activity compared to the Phase 1 results and the CMD (Figure 5.12 and Tables 5.4 and 5.7). One possible explanation may be that the SM AnMBR was operated at a higher cycle time than in Phase 1. In a similar fashion, the MM AnMBR was operated at a cycle time of 6; however, due to the design of the system, the biomass was pumped through a series of 7 elements connected by common manifolds (Figure 4.7) causing the biomass to travel a much more torturous path than in Phase 1. As previously discussed, *Methanosarcina*, due to its morphology, has an advantage in higher shear environments and, as also previously discussed, *Methanosarcina* is particularly effective at higher acetate

concentrations, such as in the respirometer bottles. This may explain the increase in acetate activity for the SM AnMBR and the MM AnMBR compared to the CMD and the Phase 1 results.

As described above, the MM AnMBR design caused the biomass to travel a torturous path and may have negated the benefit of operating at a lower cycle time.

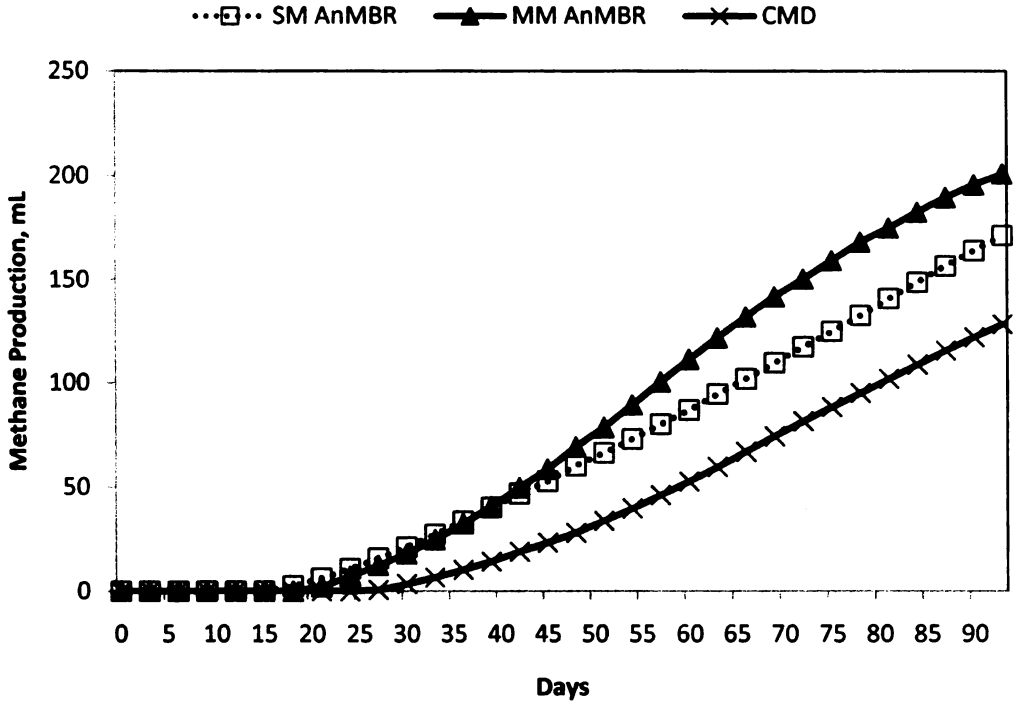


Figure 5.12 Phase 2, acetate

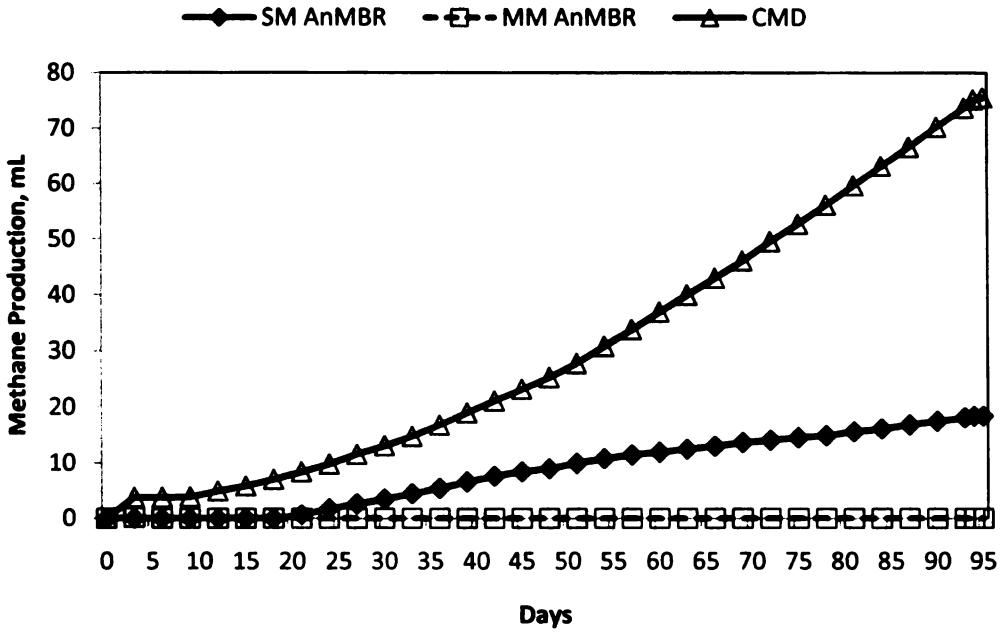


Figure 5.13 Phase 2, propionate

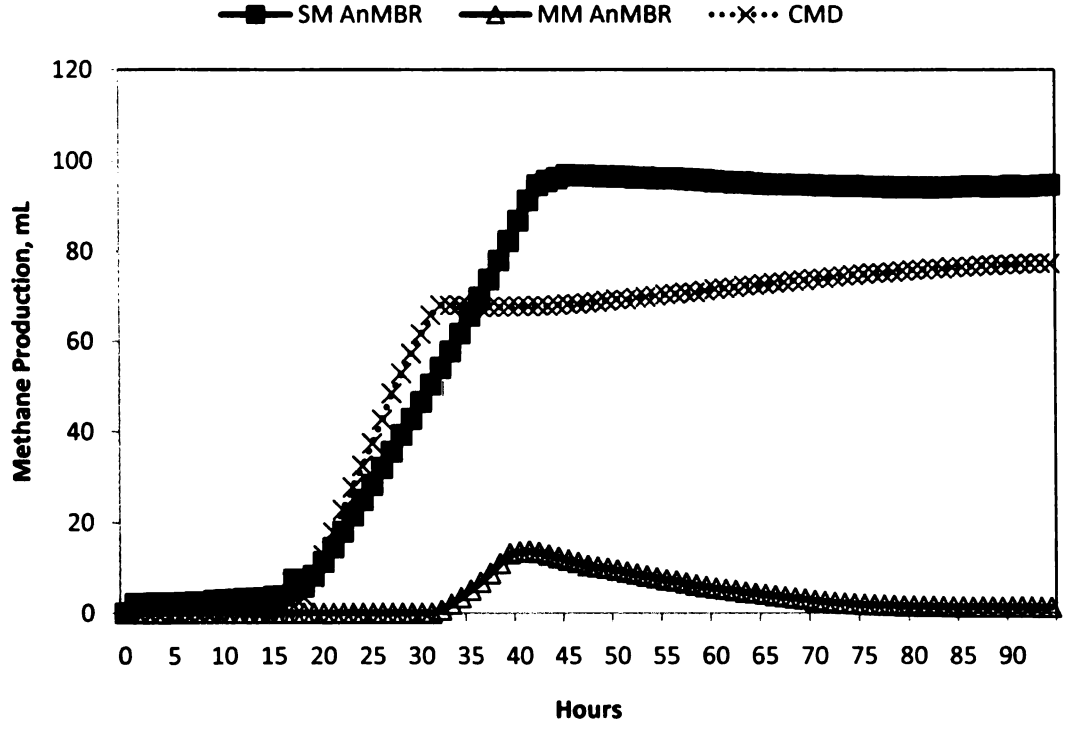


Figure 5.14 Phase 2, formate

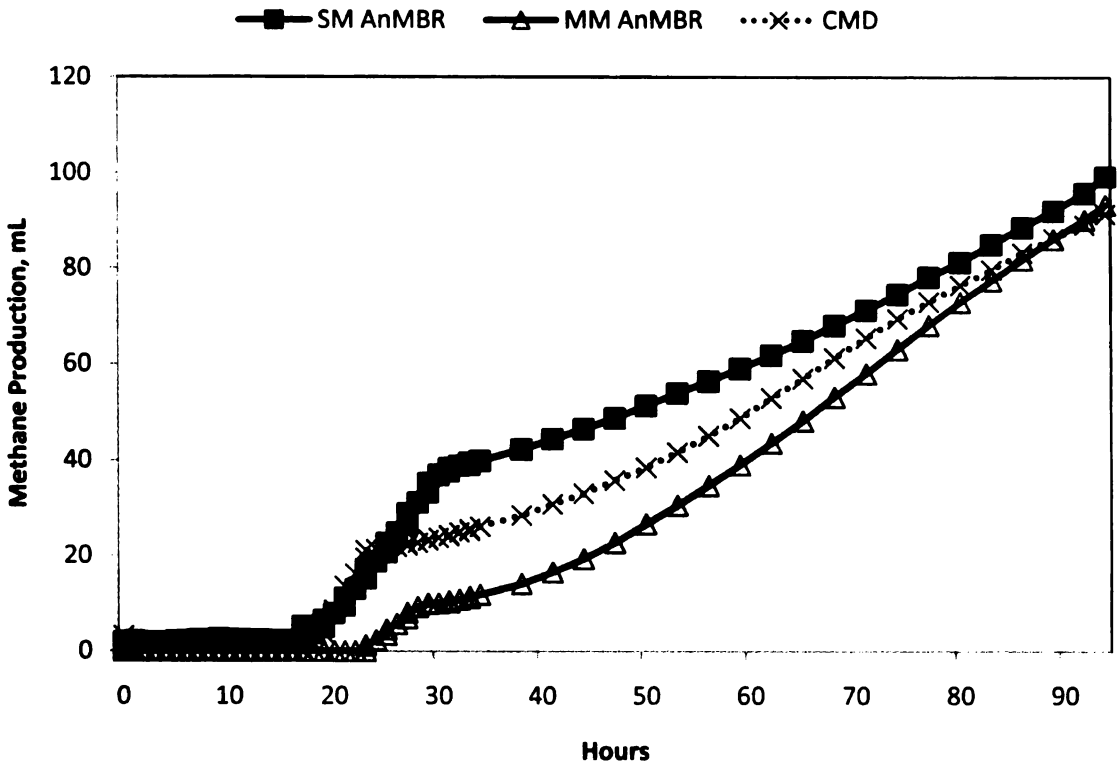


Figure 5.15 Phase 2, acetate + formate

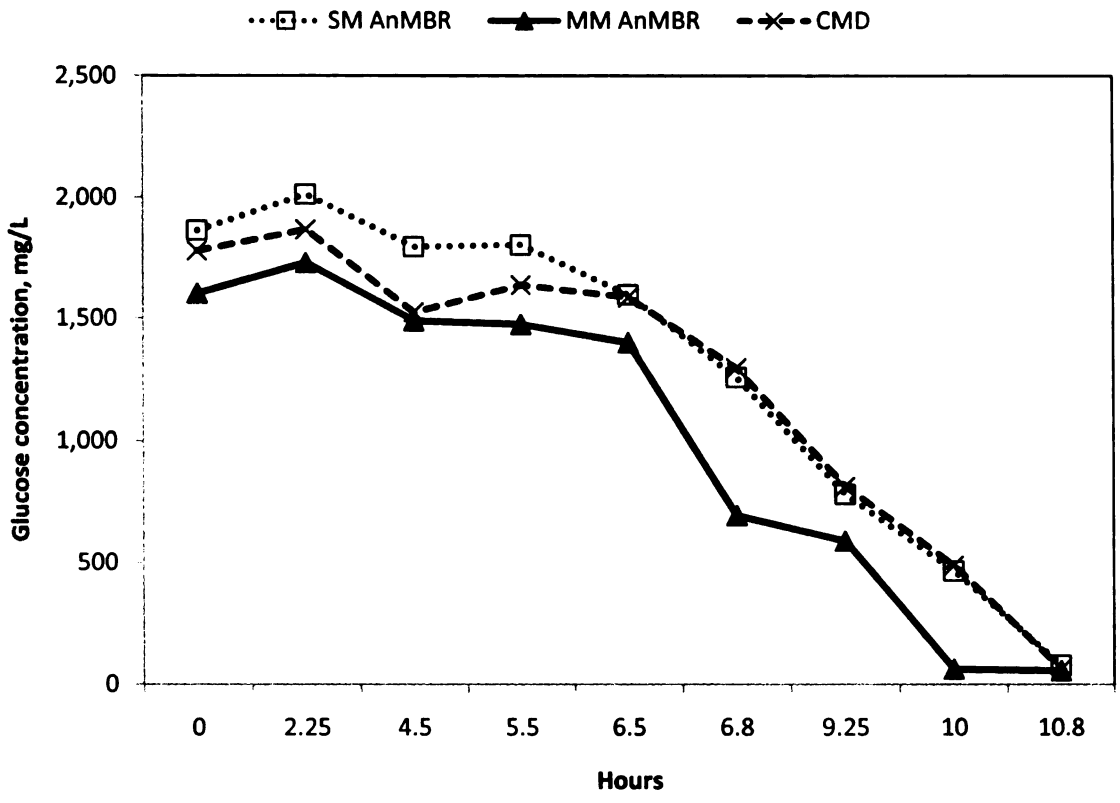


Figure 5.16 Phase 2, glucose consumption per mass VSS

This is likely reflected in the fact that the MM AnMBR exhibited no activity on propionate (Figure 5.13) and little activity on formate (Figure 5.14). The SM AnMBR activity on propionate was less than Phase 1, indicating the increase in cycle time from 6 to 27 had a slight impact on syntrophic activity.

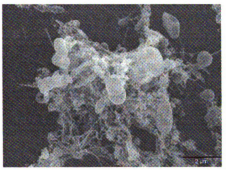
All of three of the digester systems exhibited lower activity for the combination of acetate+formate compared to formate alone (Figure 5.15), suggesting that *Methanosarcina* like organisms comprised an important component of the methanogenic community (Dolfing and Bloemen 1984).

The glucose consumption test used to measure the acidogenic conversion rate (conducted at approximately the same VSS concentration for each digester system)

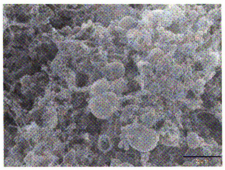
produced similar results for all three digesters (Figure 5.16). In practice, the pilot AnMBRs were operated at a higher VSS concentration than the CMD. Considering this, if provided with a greater concentration of readily fermentable substrate, they should produce more methane than the CMD. Since this did not occur, it appears that hydrolysis could be limiting the rate of substrate conversion.

5.4.4 Microscopy

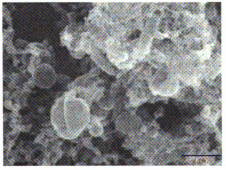
Biomass from each of the three digesters at day 92 of Phase 2 (see Chapter 4, Section 3.2 for specific operating detail) was viewed using a scanning electron microscope. A representative image from each of the digester systems is presented in Figure 5.17.



(A)



(C)



(B)

Figure 5.17 SEM Images, (A) CMD, (B) SM AnMBR, (C) MM AnMBR

Significant groupings of organisms were observed for all three digester systems, suggesting the potential for spatial proximity of hydrogen consuming methanogens and fatty acid oxidizing bacteria remained intact.

5.4.5 Most Probable Number

Samples were collected from the Phase 2 digester experiment at day 92 (see Chapter 4, Section 3.2 specific operating detail) for most probable number evaluation of cell viability. Because hydraulic retention time is decoupled from solids retention time, theoretically, the AnMBR systems are expected to have higher concentrations of viable cells than the CMD. The results are presented in Table 6.8. Surprisingly, there was very little difference between the three systems with respect to the predicted number of viable cells.

Table 5.9 MPN results after 144 hours of incubation, estimated using 5-tube serial dilution

	CMD	SM AnMBR	MM AnMBR
MPN x 10^8/mL	2.4	2.4	5.0
Lower 95% Confidence limits ($x10^8$/mL)	1.0	1.0	2.0
Upper 95% Confidence limits ($x10^8$/mL)	9.4	9.4	20.0
VSS, mg/L	15,267	16,250	29,833
VS, mg/L	19,700	31,600	39,600

5.5 Summary

A theory in previous anaerobic membrane bioreactor research suggests that the shearing impact of the pump/membrane system acts to degrade the juxtaposition of the syntrophic relationship (Brockmann 1995; Brockmann and Seyfried 1996; Brockmann and Seyfried 1997; Ghyoot and Verstraete 1997; Stroot, McMahon et al. 2001). The present research does indicate a reduction in the syntrophic interaction. However, with the exception of the result for the MM AnMBR operating at a cycle time of 6, the respirometer experiments suggest that the syntrophic relationship is still present and functioning, although at a reduced efficiency as compared to the CMD. Kasper and Wuhrmann (1978) reported that propionate-degrading systems were saturated to only 10 to 15% and hydrogen removal was less than 1% of the maximum possible rate. This indicates that, provided the juxtaposition of acetogenic bacteria and hydrogentrophic methanogens remains somewhat intact, there is significant excess capacity available to process hydrogen in anaerobic systems. The SEM photos for the SM AnMBR, the MM AnMBR and the CMD provide evidence that the membrane systems maintain a degree of biomass agglomeration (Figure 5.17) suggesting that hydrogen producers and hydrogen consumers are able to maintain spatial proximity to each other.

Padmasiri et al. (2007) reported a reduction in microbial activity for an anaerobic membrane bioreactor treating swine waste and proposed that the high velocity environment promotes (due to the pumping action) an increase in the rate of hydrolysis leading to a buildup of fermentation intermediates and ultimately a depression in methanogenic activity. Padmasiri's research was conducted at organic loading rates between 1 and 3 g VS/L/day with unstable operation at loading rates greater than 3 g

VS/L/day characterized by VFA excursions greater than 4,000 mg/L. For each of the operating conditions evaluated in the present research, the AnMBRs did not experience a build-up of VFA. The AnMBR from the CMD/AnMBR research was operated at a cycle time of 56 with an average volatile acid concentration of 232 mg/L as HAc (Chapter 3, Figure 3.13, titration method outlined in Appendix B). The SM AnMBR and the MM AnMBR operated at cycle times of 27 and 6 respectively and, at steady-state, there was no volatile acid recognized by the titration technique of Appendix B. However, acetic, propionic, butyric and Isobutyric acids were measured for the SM AnMBR and MM AnMBR using a GCMS procedure (Appendix E) and resulted in a total VFA concentration of 299, 271 mg/L respectively (Chapter 4, Table 4.10). Provided hydrogen consuming methanogens maintain a sufficiently low H_2 concentration, the fermentation pathway results in the production of acetate, formate and H_2. In the absence of these scavengers, fermentation will proceed independent of methanogenic activity in the direction of high molecular weight VFA (Hungate 1975; Bryant 1979). Neither the SM AnMBR or the MM AnMBR (or the CMD) exhibited high concentrations of propionate, butyrate or isobutyrate for Phase 2 of the research (Chapter 4, Table 4.10) suggesting hydrogen was maintained at a sufficiently low concentration to avoid a build-up of higher molecular weight VFA. This is consistent with the findings of Beaubien et al. (1996) who found that the high shear stress generated by the operating condition of a membrane bioreactor did not induce a significant reduction on methanogenic specific activity at organic loading rates (0.8-0.9 kg COD/kg VSS/day), similar to loading rates in the present research.

Despite variations in metabolic activity for the three digesters in Phase 2, the methane production was nearly the same for each system. This suggests that, though respirometer testing indicated an apparent degradation of syntrophic activity, there remained an adequate hydrogen consuming population capable of metabolizing H_2 such that the fatty acid oxidizing bacteria (acetogens) were not inhibited.

One possible theory for why the AnMBRs did not exhibit greater gas production compared to the CMD is thought to be related to the rate of hydrolysis. Theoretically, the longer SRT of the AnMBR provides a higher concentration of viable cells and a corresponding increase in the rate of hydrolysis. However, if the rate of hydrolysis is not influenced by the function of the AnMBR, it stands to reason that the number of viable cells in the AnMBR will not differ significantly from the CMD, as was observed for the MPN testing (Table 5.8). Considering that all three systems of Phase 2 produced methane equally (Chapter 4, Figure 4.22) without VFA build-up (Chapter 4, Table 4.10), provides support for the theory that hydrolysis is controlling the production of fermentation pre-cursors and thus limiting the growth potential for the downstream anaerobic consortium. Furthermore, the glucose consumption test illustrated that all three systems (SM AnMBR, MM AnMBR and CMD) operated at approximately the same acidogenic rate per mass VSS (Figure 5.16), indicating that fermentation (of hydrolysis breakdown products) is not the "bottleneck" in the process.

Apparently the AnMBR, despite the longer SRT, is not able to affect a higher rate of hydrolysis than the CMD system. Munch et al. (1999) proposed a kinetic expression to describe the hydrolysis rate as the ratio of:

$$\frac{\text{Particulate concentration} \quad x \quad \text{Hydrolytic enzyme concentration}}{\text{Acidogenic bacteria concentration}}$$

This indicates that the rate of hydrolysis is reduced at high biomass concentrations. Myint et al. (2007) theorized that that the reduction in the rate of hydrolysis at high biomass concentrations is likely due to limited surface area causing mass transfer limitations of the hydrolytic enzyme. The theory suggests that hydrolysis is limited by the particle surface area occupied by organisms secreting hydrolytic enzymes and, once the surfaces are completely occupied, the maximum hydrolysis rate is defined and additional organisms cannot influence this rate. Methane production and most probable number of viable cells for the Phase 2 SM AnMBR, MM AnMBR and the CMD suggest that the available sites for particulate occupation are exhausted at the biomass concentration found in the CMD. Therefore, the increased VSS concentration of the AnMBR does not affect a higher rate of hydrolysis and, as a result, the viable cell population reflects the substrate concentration available for metabolism.

Chapter 6

AnMBR DESIGN CONSIDERATIONS

6.1 Introduction

The purposed of this chapter is to bring together all of the AnMBR operational data, as well as a qualitative understanding of the associated microbiology, so that design considerations can be formulated for the treatment of liquid dairy manure. General guidelines associated with the impact of cycle time, cross flow velocity and membrane configuration, operating pressure, TS concentration and flux rate, HRT and SRT, pump selection, membrane pore size, membrane pore size, and membrane cleaning are presented. The recommendations are a combination of specific findings of this research with consideration for the previous work conducted by others in related areas.

6.2 Cycle Time

There is much research in support of the AnMBR as an effective, high rate process capable of producing excellent effluent quality while providing a very high level of organic conversion. However, other research suggests consideration for microbial inhibition due to the shearing impacts associated with turbulent transport of biomass through the membrane system or other high shear applications (discussed in detail in Chapter 2, Section 7).

Based on the findings presented in Chapters 5 and 6, recommended cycle times are between 6/day and 27/day. Cycle time is the starting point for AnMBR design, providing

an engineering benchmark against which the other design parameters must fit. Therefore, knowledge of acceptable cycle time limits is critical.

6.3 Cross Flow Velocity and Membrane Configuration

Cross-flow velocity (CFV) and total solid content were identified in this research as the most important factors in optimizing permeate flux for liquid dairy manure. Baker et al. (1985) observed higher permeate flux rates at higher cross-flow velocities for a mineral slurry. They reported that permeate flux was proportional to cross-flow velocity raised to the power of 0.6. Fane and Dell (1987) found that initial flux declines were proportional to the cross-flow velocity raised to the power of 1.0; however, long-term steady-state fluxes were proportional to cross-flow velocity to the power of 2.4 for bacterial suspensions and, when fouled, the membrane exhibited negligible flux increases with increasing cross-flow velocity. According to Fane and Dell (1987), increasing the cross-flow velocity has the effect of decreasing the degree of polarization by increasing mass transfer and other back-transport mechanisms.

Though it is clear that higher flux rates occur with higher CVFs, there is also a corresponding increase in pressure drop resulting in higher energy costs. Alternatively, operation at a lower CFV requires less energy but results in a larger membrane surface area to obtain the same permeate generation rate. The cost of a membrane system is linear based on the membrane surface area requirement (determined according to the design flux rate). Consequently, a life cycle analysis is needed to find the optimum CFV to flux to energy relationship. Because all wastes are unique, prior to selection of a

design CFV, flux testing at various CFVs is suggested. Table 6.1 presents flux and related operating conditions for the systems evaluated in the present research.

Table 6.1 Flux summary

Digester Description	Flux, L/m^2/hr	TS, %	CFV, m/s	TMP, kPa
AnMBR comparison	43	10.3	3.4	100
CMD/AnMBR	34	5.7	3.6	100
Phase 1 Single Membrane	118	2.6	4.5	86
Phase 2, Single Membrane AnMBR	53	4.9	4.5	86
Phase 2, 7-Element AnMBR	24	5.0	2.9	240

Selection of the membrane configuration is closely aligned with CFV and is based on balancing the desired cycle time with system energy and capital cost constraints. Phase 2 (Chapter 4, Section 3.2) compared the MM AnMBR operated at a CFV of 2.9 m/s with the SM AnMBR operated at a CFV of 4.5 m/s and it was shown that that higher CFV generated a flux rate that was approximately twice that of the lower CFV (Chapter 4, Section 3.2 and table 4.6).

Equation 6.1, the Darcy Equation, states that head loss (or ΔP) is proportional to the velocity squared.

$$H_L = f * \frac{L}{D} * \frac{v^2}{2g} \qquad (6.1)$$

The following discussion is provided to illustrate that the Darcy relationship is valid for this manure pumping application. During Phase 3, the SM AnMBR CFV was

reduced from 4.5 m/s to 3.5 m/s for a period of approximately 4 days and a summary is presented in Table 6.2.

Table 6.2, Phase 3 CFV comparison

CFV, m/s	Flux, $L/m^2/hr$	ΔP	CFV^2
4.5	51	76	20.25
3.5	31	49	12.25
Ratio of Change		**1.6**	**1.7**

According to the Darcy relationship, the ratio of ΔP should equate to the ratio of the CFV^2 and, in fact, the actual conditions are consistent with predicted expectation, as illustrated in Table 6.2. Table 6.3, data from Phase 2, compares the flux rate and cross-flow velocity of the SM AnMBR and the MM AnMBR. Due to the manifold configuration of the MM AnMBR (discussed in Chapter 4, Section 2.3), an accurate measurement of the ΔP per element was not possible. However, considering the Darcy relationship, the calculated ΔP is shown in Table 6.3.

Table 6.3, Phase 2 comparison of SM AnMBR and MM AnMBR

CFV, m/s	Flux, $L/m^2/hr$	ΔP	CFV^2
4.5	53	76	20.25
2.9	24	32*	8.41

*Calculated ΔP per element

The power required for pumping relationship is shown in Equation 6.2.

$$P_h = q \cdot \rho \cdot g \cdot h \div 3.6 \times 10^6 \qquad (6.2)$$

Where,

P_h = power (kW).
q = flow capacity (m^3/hr).
ρ = density of fluid (kg/m^3).
g = gravity (9.8 m/s^2).
h = differential pressure head, (m).

Considering this relationship, the energy input difference between a CFV of 4.5 m/s and a CFV of 2.9 m/s can be directly compared based on the fact that power required for pumping is proportional to flow rate x ΔP. Operating at a CFV of 2.9 m/s provides a tremendous energy advantage compared to 4.5 m/s.

The findings of Chapter 4 suggest that the operating conditions of the MM AnMBR did not negatively influence gas production and also indicated flux was stable at 2.9 m/s. Research presented in Chapter 4 also suggests there is negligible difference between gas production at a cycle times between 6 and 27 (Table 4.6) and that operation at CFVs as high as 4.5 m/s does not negatively impact methane production (Table 4.6). Therefore, it is recommended that membranes be configured based on ease of design and operation within the general framework of a maximum pump discharge pressure of 480 kPa (Table 4.6) and a maximum cycle time of 27/day with the CFV selected to balance the capital cost versus operating cost objectives of the project.

6.4 Operating Pressure

At operating pressures between 180 and 200 kPa and greater, Ghyoot and Verstraete (1997) found flux to be independent of pressure for sludge concentrations between 6.0 and 25.0 g TS/l. Strohwald and Ross (1992) found that membrane flux was independent of operating pressure above 260 kPa and a cross-flow velocity of 1.9 m/s. Beaubien et al. (1996) referenced Equation 6.3 from (Cheryan 1986):

$$J = \frac{\Delta P_t}{\mu(R'_m + ß\Delta P_t)} \qquad (6.3)$$

Where,

J = permeate flux (μm/s)
ΔP_t = Applied transmembrane pressure
μ = Permeate viscosity
R'_m = Resistance comprised of membrane-solute interactions presumed unaffected by operating parameters
$ß\Delta P_t$ = Resistance related to operating conditions

Based on Equation 6.3, two regions of interest can be identified, a low pressure region where the hydraulic resistance of the membrane dictates the flux rate ($R' >> ß\Delta P_t$) and a high pressure region where flux is controlled by the operating conditions of the system ($ß\Delta Pt >> R'_m$). The experimental work of Beaubien et al. (1996) showed two distinct zones that depended on operating pressure. At operating pressures less than 80 kPa, permeate rate was largely dependent on applied pressure and suspended solids concentration. At operating pressures above 100 kPa, flux is largely pressure independent and permeate flux was directly proportional to cross-flow velocity. Fugere et al. (2005) reported similar results indicating that flux was relatively pressure independent above 100 kPa; however, they noted that at pressures between 150 and 300

kPa, the rate of flux change with increasing cross-flow velocity was less than at pressures between 50 and 100 kPa.

Identification of optimum operating pressure was not a goal of the present research; however, based on the results of previous research, and considering the necessary transmembrane pressure (TMP) for a dairy manure AnMBR, the flux will most likely be pressure independent. As such, it is recommended that design be based on the minimum pressure drop (based on membrane configuration) to achieve the design cross flow velocity.

6.5 Total Solids Concentration and Flux Rate

Digester TS concentration was used in this research as a benchmark to compare digester flux conditions; however, it is common in the research to also reference total suspended solids (TSS), mixed liquor total suspended solids (MLTSS),VSS or mixed liquor volatile suspended solids (MLVSS). TS and VS were commonly used throughout this research because they are accurate and easy to determine and were analyzed every day. TSS/VSS analysis is considerably more time consuming to analyze. Figure 6.1 illustrates that TS tracked closely with (TSS) for this research. A similar relationship held for the comparison of VS to VSS (data not shown).

Ross et al. (1992) found a constant flux up to a suspended solids concentration of 40 g/L, after which fluxed decreased rapidly for a maize-processing effluent. Berube et al. (2006) indicated that Saw et al. (1985) reported a log-linear decrease in the steady-state permeate flux with an increase in the concentration of suspended solids for digested sludge. Kitamura et al. (1996) theorized that a decrease in membrane performance was

due to fouling caused by an increase in viscosity of the sludge at higher suspended solid concentrations.

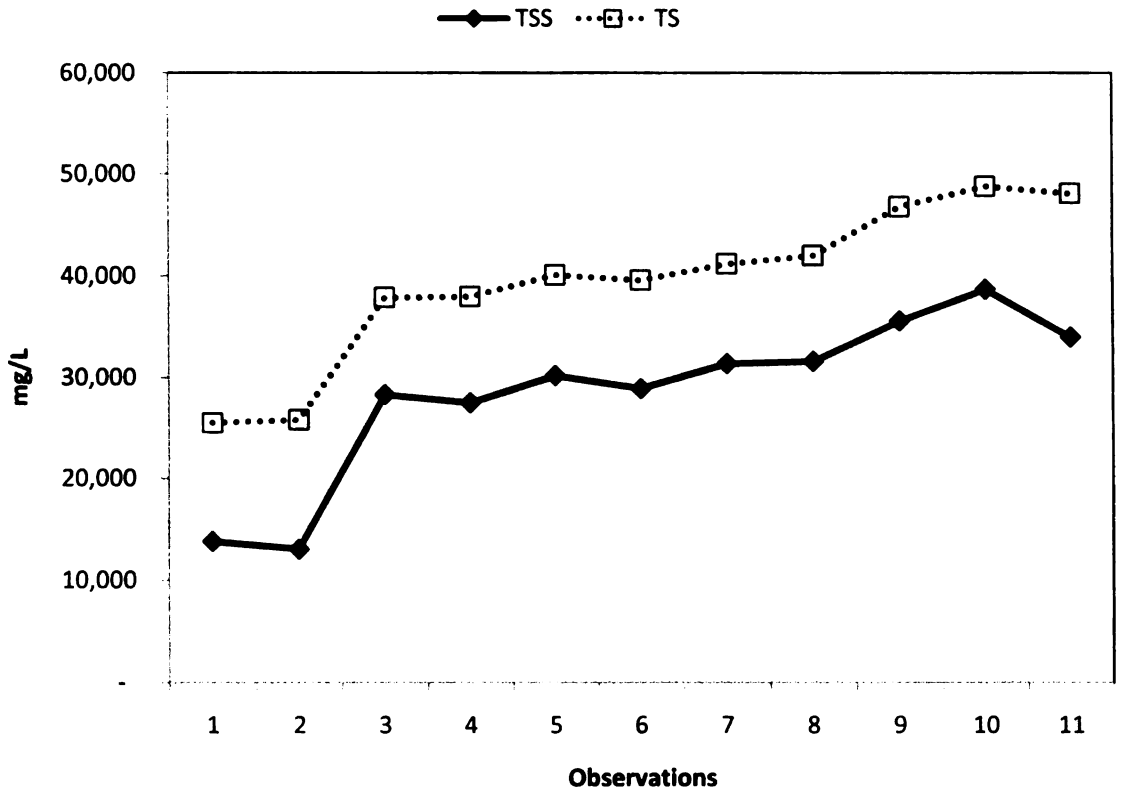


Figure 6.1 TSS versus digester TS concentration

In general, based on the findings of this and previous research, there is a direct relationship between digester TS concentration and membrane flux rate between 2-10% (Figure 3.6, Figures 4.20 and 4.21). This is consistent with the findings of (Li 1985; Beaubien, Baty et al. 1996; Madaeni 1997). The initial research that compared the AnMBR to the CMD resulted in relatively high flux rates despite a very high digester TS

concentration (Table 3.3 and Figure 3.6). This is likely the result of the type of manure used for this experiment. The manure was sand-separated and still contained large pieces if undigested fiber. The AnMBR of the CMD/AnMBR system was fed the digested CMD effluent (feedstock to the CMD was sand-separated manure), resulting in a homogeneous feedstock. Sand and solid-liquid separated manure was used as the feedstock for Phases 1, 2 and 3 and, due to the solid-liquid separation process, provided a consistent feedstock devoid of large particles. Madaeini (1997) indicated that smaller particles sizes lead to lower flux rates and this appears to be consistent with the outcome of these experiments.

Flux rates trend down as TS concentration increases. However, from a design perspective, it is the SRT, combined with the starting TS concentration of the wastewater, that will define the digester TS operating concentration. Section 6.6 provides recommendations relative to the design condition based on the findings of this research.

6.6 HRT and SRT

The experiments of the present research were conducted at HRTs of 12-20 days. Due to the extended SRT that can be achieved with the AnMBR, HRTs lower than 12 days are certainly possible. Dugba and Zhang (1999) operated two-stage anaerobic sequencing batch reactors at 3 and 6 day HRTs with SRTs between 13-18 days and reported VS destruction of 23-34% for a mesophilic system with organic loading rates of 2-4 g VS/L/day of screened dairy manure. Zhang et al. (1997) operated an anaerobic sequencing batch reactor on swine manure with VS reductions of 55-61% at an HRT of 3 days and organic loading rates of 1.0-5.5 g VS/L/day. Padmasiri et al. (2007) operated a

mesophilic AnMBR treating swine manure at a HRT of 6 days and organic loading rates of 1.0-3.0 g VS/L/day (VS destruction not reported).

The selected OLR, in conjunction with the characteristics of the wastewater, define the HRT (Equation 6.4). Digester volume equals the required treatment volume per day multiplied by the HRT.

$$HRT = \frac{VS_{concentration}}{OLR_{VS}} \quad (6.4)$$

Based on the combination of digester volume, design SRT and design digester TS (or VS, TSS, VSS) operating concentration, the digester wasting rate can be calculated. A typical goal in the operation of an AnMBR is to maximize permeate production (and minimize the total volume that is wasted from the digester) and this is accomplished by operating the digester at the highest possible TS (or VS, TSS, VSS) concentration. Equation 6.5 is used to calculate the required digester wasting rate.

$$\text{Wasting rate, mass dry solids/day} = \frac{\text{Total mass dry solids in digester}}{SRT} \quad (6.5)$$

The present research was conducted at OLRs of 1.0-4.0 g VS/L/day (with an average of 2.2 g VS/L/day). Based on the present and related research, OLR rates of 2.0 – 4.0 g VS/L/day are suggested for sand and solid-separated dairy manure with digester TS operating concentrations of approximately 1.2 to 2.5 times the feed TS concentration and a design SRT in the range of 20-30 days. Digester TS concentrations of 10-11% are

attainable; however, specific work related to determining the maximum TS concentration in relationship to flux rate was not evaluated in this research.

6.7 Pump Selection

Kim et al. (2001) concluded that the activity of microorganisms was damaged more severely and the microbial flocs more easily destroyed with a positive displacement pump compared to a centrifugal pump. He et al. (2005) reported that the mechanical shearing impact of the pump used in an AnMBR for the treatment of food waste negatively impacted the microbial activity of the system, particularly the methanogens and further suggested the selection of a low shearing pump. Choo and Lee (1996) theorized that the low viable suspended biomass concentration in the bioreactor (of an AnMBR) treating alcohol-distillery wastewater was due to cell lysis caused by mechanical sheer stress from the positive displacement recirculation pump, noting that the cells moved from the bioreactor to the surface of the membrane.

A centrifugal pump was used in the present research. SEM images from the three digesters used in this research (Figure 5.17) indicate that microbial flocs are intact. Metabolic testing suggests that the syntrophic interaction, though reduced compared to a minimally mixed CMD, was intact (Chapter 5, Section 5). Positive displacement pumps operate with close tolerances creating a greater potential for floc disintegration Madaeni (1997) reported flux decline with smaller particles. Considering these outcomes, it stands to reason that a centrifugal pump is the most appropriate selection for system design.

6.8 Membrane pore size

Choo and Lee (1996) identified that the fouling tendency was at a minimum for a membrane pore size of 0.1 μm for bacterial cells isolated from an anaerobic digester system but also suggested that the size of the influent solid content was important when selecting membrane pore size. Their line of reasoning followed that macrosolutes smaller than the pore size of the membrane will easily pass through the membrane while colloids that are considerably larger than the more size, will tend to remain on the membrane surface but not penetrate the pores deeply and are thus easily swept away due to the affect of CFV. Madaeni (1997) reported flux decline with smaller particles. Chang et al. (2002) reported that that Shimizu et al. (1990) correlated flux with the pore size for methanogenic wastes and illustrated that membranes with pore sizes in the range of 0.05-0.2 μm produced the maximum flux among membranes ranging from 0.01-1.6 μm.

Pore size may be an important factor with regard to nutrient and pathogen/virus retention. For example, the majority of the phosphorus content in raw and anaerobically digested swine manure is linked to particles larger than 0.45 microns (Masse, Masse et al. 2005). The present research was conducted with a 0.03 μm membrane. Total phosphorus reduction was 96%. Vogel et al. (Vogel 2003), operating a thermophilic AnMBR on dairy manure, also reported a 96% reduction of total phosphorus using a membrane with pore openings from 0.005 to 0.1 μm.

Wong et al. (Wong, Xagoraraki et al. 2009) evaluated the removal efficiency of the CMD/AnMBR system from Chapter 3, Section 3.2 for E. *coli*, enterococci, C. *perfringens* and coliphage with total $\log_{10}$ removals of 1.5, 1.2, 0.1 and 0.5 respectively

for the CMD and the 5.2, 6.1, 6.4 and 3.7 respectively for the AnMBR. The vast majority of the removal was attributed to the AnMBR. The lowest removal efficiency was for coliphage. This finding was not surprising considering viruses are typically smaller than bacteria and can be as small as 0.01 μm. Nevertheless, the removal efficiency for the coliphage was still 99.96% (3.7 $\log_{10}$ removal). Wong et al. (Wong, Xagoraraki et al. 2009) also evaluated the AnMBR independent of the CMD. This analysis illustrated that the AnMBR, in a stand-alone capacity, was capable of achieving the same total pathogen and virus removal rates attributed to the combined CMD/AnMBR system.

Considering the findings of this and other research, the optimum membrane pore size appears to be in the range of 0.03 and 0.1 μm. For future design, in the absence of new information, a membrane pore size of 0.03 μm is recommended.

6.9 Cleaning Protocol

Vogel et al. (2003) used a caustic cleaning solution (3.5% NaOH) followed by a water rinse and subsequent treatment with 3% phosphoric acid for a ceramic membrane used in a dairy manure AnMBR. Zhang et al. (2007) was able to recover 44% of the original clean water flux through a membrane used in a swine manure AnMBR cleaning with EDTA at pH 2 and NaOH at pH 10. In addition, slightly better cleaning efficiency was reported using HNO_3 with the best results for both the EDTA and HNO_3 occurring at 50°C. Zhang (2007) also concluded that the irreversible portion of the fouling (that which could not be recovered with chemical cleaning) was most likely due to a rapid process that cannot be avoided by weekly chemical cleaning using HNO_3.

Several cleaning procedures were tested in present research. All included NaOH and a citric acid cleaner at various pH levels and soaking times and some also included soaking the membrane overnight in a 500 PPM bleach solution. Ultimately a consistent approach that incorporated only NaOH and citric acid cleaner were used for membrane cleaning. The cleaning was accomplished at temperatures of approximately 10°C to 25°C. The cleaning procedure included the following.

1. Isolate membrane from the digester.
2. Pump clean water from the CIP tank through the membrane to flush membrane and dispose of this material.
3. Re-fill CIP tank with clean water, add NaOH to pH of 11.0 and then circulate through the membrane returning to the CIP tank for approximately 30-45 minutes.
4. Add citric acid cleaner (used Citrajet® low foaming cleaner) to pH of 4.0 and circulate for approximately 30-45 minutes.
5. Increase pH to 7.0 with NaOH.
6. Begin steady addition of clean water to CIP tank and direct effluent from membrane to disposal for approximately 15-30 minutes.

6.10 Summary

Considering the findings of this research and incorporating the findings of previous researchers, Table 6.4 provides recommended design condition for AnMBR for the treatment of liquid dairy manure.

Table 6.4 Design Consideration for AnMBR System

Design Parameter	Recommended Value
Cycle Time	<27
Cross Flow Velocity	Up to 4.5 m/s
Operating Pressure	As dictated by CFV membrane geometry but less than 480 kPa
OLR	2.0 – 4.0 g VS/L/day
SRT	20-30 days
Digester TS, %	<10%
Membrane Pore Size	0.03 μm
Pump Selection	Centrifugal
Membrane cleaning	See Section 6.9

Chapter 7

ENGINEERING SIGNIFICANCE AND FUTURE WORK

The objective of this research was the development of a design approach for an anaerobic membrane bioreactor for the treatment of liquid dairy manure. Evaluation of cycle time at the pilot-scale and the metabolic level were central to this effort. A summary of the basic findings of this research and recommended future work follows.

7.1 Summary of Research Findings

A summary of the findings of this research include the following.

1. Cycle times greater than 86/day negatively impacted AnMBR performance as measured by methane production.
2. Cycle times less than 27 do not negatively impact AnMBR performance as measured by methane production.
3. The pump/membrane system of the AnMBR impacted syntrophic activity; however, the biomass still exhibited approximately 35% of the activity on high concentrations of propionate compared to a control CMD.
4. SEM imaging of the biomass indicated large groupings of microorganisms and this supports the notion that the juxtaposition between acetogens and hydrogentrophic methanogens stayed intact.
5. For the tested conditions, biomass concentration (or SRT) did not affect AnMBR performance. The pilot-scale gas production was equal when the AnMBR was operated at a VS concentration of 2.6% compared to less than 1.9% (when the all

permeate was returned to the digester tank such that the AnMBR was operated in a complete mix configuration).

6. A high CFV up to 4.5 m/s did not affect AnMBR performance as measured by methane production.
7. The Increased biomass concentration of the AnMBR apparently did not increase the rate of hydrolysis; hence, under the conditions tested, AnMBR performance mirrored that of the control CMD.

Appendix D outlines an example case using typical operating conditions from this research. The example case illustrates the SRT calculation and relates SRT to HRT and digester volume and the impact of membrane configuration on cycle time. Finally, the example outlines the energy implications of the AnMBR system.

7.2 Future Work

Based on the AnMBR research conducted in support of this research, there are numerous areas that are deserving of future effort.

7.2.1 Increased OLR

A finding of this research was that the estimated number of viable cells in the AnMBRs was very similar to the number of viable cells in the CMD. This outcome was observed despite the difference in biomass concentration between the CMD and the AnMBR. Based on the pH and VFA data, all of the digesters functioned at low VFA concentration suggesting there was not excess fermentation intermediates available for consumption. It

was theorized that, despite the higher biomass concentration, the AnMBRs did not promote a higher level of hydrolysis due to mass transfer limitations. As a result, the concentration of fermentable substrate was likely very similar for the AnMBRs as it was for the CMD. This theory suggests that without a higher concentration of fermentable substrate, the advantage of operating at a higher biomass concentration is negated.

One recommended course of action is to achieve steady-state operation for the AnMBR systems and the CMD and then increase the OLR in a stepwise fashion through the addition of a readily fermentable substrate (i.e. a substrate that does not require hydrolysis such as glucose) to evaluate the effect on gas production and digester stability. This would model the effect of adding an additional substrate such as ethanol plant syrup to a manure-based digester.

A second recommendation is to increase the OLR by decreasing the HRT. The rate of gas production of the AnMBR should follow the rate of hydrolysis. If the rate of hydrolysis is significantly impacted by the reduction in HRT, gas production will be effected. Theoretically, the CMD should become unstable due to washout of acetotrophic methanogens while the AnMBR should remain stable even at HRTs (potentially in the range of 3 to 6 days).

7.2.2 Temperature Impact on Flux Rate

Ross et al. (1990) showed a flux rate increase of 2% for each 1°C increase in operating temperature. (Ross, Barnard et al. 1990). Preez et al. (2005) reported that thermophilic fluxes, on average, were 29% higher than the Mesophilic fluxes that were measured in comparison research.

Under thermophilic conditions, microbes exhibit 2-3 times higher maximum specific growth rates compared to mesophilic microbes (Mladenovska and Ahring 2000). As a result, the organic loading potentials of thermophilic anaerobic reactors are substantially higher with improved process economy (Ahn and Forster 2002; Chackhiani, Dabert et al. 2004).

The thermophilic process is reported to be less stable to environmental changes than the mesophilic process (Yu and Fang 2001). In general, methanogenic diversity (for 15 full-scale biogas plants operating under either mesophilic or thermophilic with either manure or sludge as feedstock) was broader in plants operating at mesophilic temperatures (Karakashev, Batstone et al. 2005).

Though thermophilic operation is believed to be less stable, high rate processes, such as the AnMBR, maintain the advantage of long SRT (higher biomass concentrations) and this may improve the stability of the operation. Further, because the capital costs are driven by membrane flux rates, the potential to improve these rates with thermophilic operating conditions deserves evaluation.

7.2.3 Flux Recovery with Cleaning

A cleaning protocol was developed in this research that used NaOH circulated through the membrane at pH of 11.0 for 30-45 minutes followed by the circulation of a citric acid cleaner at pH 4.5 for 30-45 minutes followed by NaOH neutralization and freshwater rinsing. Excellent results were obtained with this cleaning protocol and it could be accomplished very quickly with minimal effort; however, quantification of flux recovery was not conducted. It is recommended that experiments be performed with new membranes to evaluate the flux recovery that can be obtained. Lastly, based on the work of Zhang et al. (2007), experimentation with cleaning at much higher temperatures than used in this research is also suggested.

APPENDIX A

Wong, K., Xagoraraki, I., Wallace, J., Bickert, W., Srinivasan, S., Rose, J.B. (2009). Removal of Viruses and Indicators by Anaerobic Membrane Bioreactor Treating Animal Waste. Journal of Environmental Quality, 38:In Press.

Removal of Viruses and Indicators by Anaerobic Membrane Bioreactor Treating Animal Waste

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Abbreviations: AnMBR, anaerobic membrane bioreactor; BAdV, bovine adenoviruses; BPyV, bovine polymaviruses; CMAD, complete mix anaerobic digester; COD, chemical oxygen demand; MBR, membrane bioreactor; TKN, total Kjeldahl nitrogen; TP, total phosphate; TS, total solids; VS, volatile solids

ABSTRACT

Appropriate treatment of agricultural waste is necessary for the protection of public health in rural areas since land-applied animal manure may transmit zoonotic disease. In this study, we evaluated the potential of using a pilot anaerobic membrane bioreactor (AnMBR) to treat agricultural waste. The AnMBR system, following a conventional complete mix anaerobic digester (CMAD), was able to achieve high removals of both biological and chemical agents. The mean $\log_{10}$ removals of *E. coli*, enterococci, *C. perfringens* and coliphage by the AnMBR were 5.2, 6.1, 6.4 and 3.7, respectively, and for the CMAD were 1.5, 1.2, 0.1, and 0.5, respectively. Compared to other indicators, coliphage was observed most frequently and had the highest concentration in effluent samples. Bovine adenoviruses (BAdV) and bovine polymaviruses (BPyV) were monitored in this study using nested PCR methods. All of the CMAD influent and CMAD effluent samples were found positive for both viruses and three AnMBR effluent samples were found BPyV positive. The mean removals of total Kjeldahl nitrogen (TKN), total phosphate (TP), chemical oxygen demand (COD), total solid (TS) and volatile solid (VS) by the entire system were 31%, 96%, 92%, 82% and 91%, respectively, but there was no removal of ammonium. When the AnMBR was operated independent of the CMAD, AnMBR achieved similar *E. coli* and enterococci removals as the combined CMAD/AnMBR system. The high quality of effluent produced by the pilot AnMBR system in this study demonstrated that such systems can be considered as alternatives for managing animal manure.

Keywords: Agricultural waste, manure, anaerobic membrane bioreactor, pathogen removal, indicators, animal viruses, zoonotic pathogens

INTRODUCTION

With the increase of animal agriculture facilities, there is a growing concern regarding transmission of enteric zoonotic pathogens via food and water. One of the most important sources of microbiological pollution is fecal contamination from storage and management of manure (EPA 2006). *Campylobacter* spp., *Salmonella* spp. (nontyphoid), *Listeria monocytogenes*, *E. coli* O157:H7, *Cryptosporidium parvum*, and *Giardia lamblia* were identified by the Center for Disease Control and Prevention as causative agents most likely originating from farm sources (Gerba and Smith, 2005). Hepatitis E, *Rotavirus, and Saprovirus* have also been documented as zoonotic viruses (Gerba and Smith, 2005; Costantini *et al.* 2007). One of the most severe recent waterborne disease outbreaks occurred in Walkerton, Ontario, in May 2000 and was attributed to farm runoff. Seven people died and over 2,000 were ill as a result of the outbreak (Holme 2003).

Proper treatment of agricultural animal waste and manure should not be overlooked especially in terms of pathogen removal and inactivation. Animal manure is often land applied without prior treatment. Although anaerobic digestion, aerobic digestion, and facultative lagoons are manure treatment alternatives (Johnson *et al.* 2004), these systems do not necessarily remove zoonotic pathogens.

Membrane bioreactor (MBR) systems have become popular in the last couple of decades even with the drawback of high capital investment and maintenance cost. Membranes provide a barrier for the separation of pathogens and contaminants from wastewater and often provide a high quality effluent. To the best of our knowledge, there are currently no published studies evaluating the removal of pathogens and pathogen indicators from animal waste using MBR systems. However, Cicek (2003) proposed that MBR systems have great potential for agricultural waste treatment.

Ottoson *et al.* (2006) compared the removal of indicators, *Giardia* cysts, *Cryptospordium* oocysts and enteric viruses in a municipal wastewater by MBR and conventional treatment. Virus genomes were removed equally by conventional and MBR treatment. However, MBR treatment removed microbial indicators more efficiently than conventional treatment. There are also a number of published studies focusing on the removal of MS2 coliphage and coliphage T4 by MBR systems and all of these studies

demonstrated high removals of coliphage (Shang *et al.* 2005; Lv *et al.* 2005; Zheng *et al.* 2005; Comerton *et al.* 2005; Ahn *et al.* 2001; Ueda and Horan 2000).

Anaerobic membrane bioreactors (AnMBR) have been reported to generate high quality effluent (Fuchs et al., 2003; Fakhru'l-Razi, 1994). However, no study has been conducted on the removal of biological agents in animal waste by AnMBR. The main objective of this study was to evaluate the removal of pathogen indicators and animal pathogen viruses from agriculture waste using a pilot AnMBR following a conventional anaerobic CMAD digester. The removals attributed by CMAD and AnMBR were compared. Removals of *E. coli* and enterococci were evaluated when AnMBR was operated independent of the CMAD. Finally, the removals of important chemical parameters are also presented in this study.

METHODS

CMAD and AnMBR Pilot Systems

The experiments were conducted in a pilot unit located at Michigan State University (Figure 1). Sand-separated dairy manure was first treated by a 100-L complete mix anaerobic digester (CMAD) and the effluent from the CMAD digester was further treated by 100-L AnMBR. The AnMBR was operated in a cross-flow configuration. The system employed a centrifugal pump capable of approximately 35 L/min at 200 kPa. The membrane was a 0.03 micron, 14.4 mm diameter, 0.126 m^2 PVDF tubular product manufactured by X-Flow, Inc. (Netherlands).

During the operating period, the CMAD was fed sand-separated dairy manure at an average organic loading rate of 3.3 g (VS)/L-day. The effluent from the CMAD was fed to the AnMBR , which resulted in an average organic loading rate of 2.4 g (VS)/L-day. The permeate generation rate and pump circulation rate were 64 ml/min and 35 L/min, respectively. The hydraulic retention time for both CMAD and AnMBR was 9 days. The combined system hydraulic retention time was 18 days. The AnMBR solids retention time averaged 28 days during the period of study. The system was operated under mesophilic conditions.

Sand-separated dairy manure from Green Meadow Farms (Elsie, MI), a commercially operating dairy farm, was the substrate for the CMAD. The effluent from the CMAD was the substrate for the

AnMBR. The manure was pre-treated at the farm via sand separation. This process was essentially a grit separator, where recycled water was added and the sand was settled from the manure.

Sampling and Sample Preparation

This study was conducted from February to April and June to August 2007. During the first period, the AnMBR was operated independent of the CMAD (AnMBR system alone, flow bypassed CMAD). A total of seven sampling events were conducted during the period. The sampling took place in approximately one-week intervals. There were two sampling points: influent, and AnMBR effluent (points 1 and 3 as shown in Figure 1). From June to August (second sampling period), the pilot unit was operated as a combined CMAD/AnMBR system. There were three sampling points: CMAD influent, CMAD effluent/AnMBR influent (referenced throughout as CMAD effluent) and AnMBR effluent (points 1, 2, 3 as shown in Figure 1). A total of eight sampling events were conducted during this period and the sampling took place in approximately 1 week internals. Only *E. coli* and enterococci were monitored during the first period. Six chemical parameters, four microbial indicators, and two animal enteric viruses were monitored during the second period.

Both the CMAD influent and CMAD effluent were grab samples. Due to large volumes needed for microbiological analysis and low AnMBR permeate generation rates, the AnMBR effluent was collected as a 24 hr composite sample. All of the samples were collected in sterilized disposable containers. Once the samples were collected, they were placed in an ice-chest and transferred to the Water Quality Laboratory at Michigan State University within 2 hours. All samples were stored in a 4°C refrigerator upon arrival to the laboratory and were analyzed the day of collection. Any repeated testing was done the following day.

Due to the low concentration of viruses in the AnMBR effluent, effluent samples were concentrated in order to achieve a larger equivalent volume during the PCR reaction. The concentration method used in this study was developed by Haramoto *et al* (2005) except Amicon Ultra (Millipore, Billerica MA) was used to concentrate the NaOH eluent instead of Centriprep YM-50. The final volume of concentrated eluent was around 140 µl and was stored at -80 °C for DNA extraction. The literature reported virus recovery

percentage for this method was 56%±32%. The equivalent volume of AnMBR effluent for each PCR reaction was about 20ml.

Indicator Analysis

E. coli, Enterococci, and *C. perfringens* were the bacterial indicators and somatic coliphage was the viral indicator monitored in this study. Membrane filtration (MF) technique was used for the detection of indicator bacteria. *E. coli* and Enterococci were analyzed by EPA 1603 and 1600, respectively and the analytical procedure used for *C. perfringens* was adopted by Bisson and Cabelli (1979). The CMAD influent and CMAD effluent samples were first diluted 10, 100 and 1000 fold with phosphate buffer water. Then, 1.0 ml of each dilution was aliquoted for MF. The reported concentration was calculated from the dilution that gave the most statistically accurate result (20 to 100 cfu per plate). For the AnMBR effluent, 1L of sample volume was analyzed.

After filtration, the membranes were placed on agar media for growing *E. coli*, Enterococci, and *C. perfringens*, respectively. The incubation temperature for *E. coli* was at 35°C for 2.0±0.5 hours and 44.5°C for 22.0±1.0 hours. Enterococci and *C. perfringens* were incubated for 24±2.0 hours at 41 and 45°C, respectively.

Somatic coliphage was analyzed according to EPA 1602 single agar layer method. The host culture was *E. coli* CN13. Similar to bacterial indicator analysis, 10, 100 and 1000 fold dilutions were analyzed for the influent and digester samples. The volume of AnMBR effluent sample analyzed was 10 ml. The incubation procedure for somatic coliphage was 37.0°C for 24 ±2.0 hours. The dilution that gave the most statistical accurate result, 20 to 200 pfu per plate, was used for calculating the reported concentration.

Molecular Analysis

A stool extraction kit (Qiagen, Valencia, CA) was used for DNA extraction in this study. After extraction, the DNA samples were stored in a -20 °C freezer before PCR analysis. The PCR reactions were run in an iCycler thermal cycler (Bio-Rad, Hercules, CA). The two PCR assays for the detection of bovine adenoviruses and polyomaviruses were selected from the nested PCR method published by Hundesa *et al.*

(2006). For adenovirus assay, the first round PCR primers were 5'-GRT GGT CIY TRG ATR TRA TGGA-3' (forward primer) and 5'-AAG YCT RTC ATC YCC DGG CCA-3' (reverse primer). The nested primers were 5'-ATT CAR GTW CCW CAR AAR TTT TTTGC-3' (forward primer) and 5'-CCW GAA TAH RIA AAR TTK GGA TC-3' (reverse primer). The PCR cycles were increased to 40 instead of the 30 cycles in the published method for increasing the sensitivity of detection.

For the polyomavirus assay, the first round PCR primers were 5'- GGTA TTC GCC CTC TGC TGG TCA AG-3'(forward primer) and 5'- GCT GGC AAT GGG GTA TGG GTT CT-3' (reverse primer). The nested primers were 5'- ATT TCA AAG CCC CCT ATC ATC-3' (forward primer) and 5'- GCC TAC GCC ATT CTC ATC AAG-3'(reverse primer). After amplification, selected positive samples were sent for nucleotide sequencing to confirm whether the bands were indeed the amplification product of BAdV and BPyV. Due to a strong non-target band in BAdV PCR product, MinElute Gel Extraction Kit (Qiagen, Valencia, CA) was used to purify the target band for sequencing. No non-target band was observed in BPyV PCR product; therefore, PCR product was purified by QIAquick PCR Purification Kit (Qiagen, Valencia, CA) before sequencing. All of the sequencing was performed at the Research Technology Support Facility, Michigan State University. The sequence results were blasted using http://www.ncbi.nlm.nih.gov/BLAST/.

Physical and Chemical Analysis

Total solids (TS) and volatile solids (VS) were measured according to AWWA Standard Methods 2540 B and 2540 E, respectively. Chemical oxygen demand (COD) was evaluated using Hach (Loveland, Colorado) high range COD test kits. Total Kjeldahl nitrogen (TKN), ammonium nitrogen and total phosphate (TP) were conducted according to "Recommended Methods of Manure Analysis", Bulletin A3769, University of Wisconsin Extension (2003).

Data Analysis

The concentrations of microbial indicators were described using $\log_{10}$cfu/L. To determine significant differences of microbial and chemical concentration between three sampling locations, analysis

of variance (ANOVA) single test was performed using Microsoft Excel program. The p-values less than 0.05 indicated significant difference.

RESULTS and DISCUSSION

Water Quality in the Combined CMAD/AnMBR System

The samples taken from June to August 2007 were analyzed for TKN, Ammonium, TP, COD, TS, and VS. Number of samples, average concentrations, standard deviations, and removal percentage are summarized in Table 1. More than 80 percent removal of TP, COD, TS and VS was achieved. COD removal in this study is similar to COD removals observed in MBR studies treating other types of waste (Cicek 2003). No ammonium removal was observed by the AnMBR. This may be due to the fact that ammonium remained soluble throughout the entire system (typically ammonium concentration increases during the anaerobic process as protein is degraded). On the other hand, phosphate tends to adhere to the solid particles, which explains why the removal of TP by AnMBR was much higher than the removal of TKN and ammonium.

The p-values obtained from ANOVA test showed there were significant differences between CMAD effluent and AnMBR effluent in all chemical parameters except for ammonium (data not shown). In this case, the significant differences demonstrate effective reduction of chemical parameters in AnMBR effluent. CMAD treatment alone could significantly lower the level of COD, TS and VS, but not TKN and TP.

Microbial Indicator Concentrations and Removals in the Combined CMAD/AnMBR System

The microbiological indicator data collected from June to August 2007 are summarized in Table 2. The mean and standard deviation were calculated based on all samples (for the samples that tested negative, the analytical detection limit was used in the calculations) Enterococci had the highest mean concentration in both CMAD influent and CMAD effluent samples, but coliphage had the highest mean concentration and occurrence in the AnMBR effluent. Two, three and none of the AnMBR effluent samples were tested positive for *E. coli*, enterococci, and *C. perfringens,* respectively. The mean values for the *E. coli* and enterococci in AnMBR samples were 0.31 and 0.51 $\log_{10}$cfu/L, respectively. The occurrence of *E. coli* and

enterococci in the AnMBR effluent was likely due to passage of bacteria through membranes, which had been documented in the literature (Delebecque *et al.* 2006). Five out of eight AnMBR samples were positive for coliphage and the mean level of all samples was 2.47 $\log_{10}$pfu/L. Coliphage had the highest occurrence frequency and concentration in AnMBR effluent as expected since viruses are generally much smaller than bacteria and the diameter of viruses could be as small as 0.01 μm.

The $\log_{10}$ removals of indicators by the CMAD and CMAD/AnMBR are illustrated in Figure 2. The error bars in the figure represent the standard deviation between different sampling events. The $\log_{10}$ removals of *E. coli*, enterococci, *C. perfringens* and coliphage by AnMBR and CMAD were 5.2, 6.1, 6.4, 3.7 and 1.5, 1.2, 0.1, and 0.5, respectively. The total $\log_{10}$ removals of *E. coli*, enterococci, *C. perfringens* and coliphage by the entire system were therefore 6.7, 7.3, 6.5 and 4.2, respectively. These results demonstrated that most of the overall removals were attributed to the AnMBR. One possible explanation for the low removal efficiency of *C. perfringens* by the CMAD may be attributed to its tendency to exist in the spore form under natural environmental conditions. The CMAD influent was the natural raw manure and spores are known as extremely resistant to treatment processes. *C. perfringens* has been used as a surrogate organism for parasites (e.g. *Cryptospordium oocyst*) (Yates, 2007).

Significant differences between the microbial concentrations at the three sampling points were analyzed by the ANOVA single test. Significant reductions were observed for all indicator concentrations between the CMAD effluent and the AnMBR effluent, as indicated by p-values in the range of 10^{-7} to 10^{-12} (data not shown). Although a significant reduction in *E. coli*, enterococci and coliphage were also observed after CMAD treatment, the extent of this initial reduction was far less than that attributed to AnMBR treatment (Figure 2).

Coliphage removals observed in this study were compared with removals stated in the literature using domestic wastewater. Lv *et al.* (2005), Zheng *et al.* (2005), Oota *et al.* (2005) and Ahn *et al.* (2001) reported 98-100%, 90%, 100% and 100% of coliphage removals by an MBR, respectively. 2.3, 5.3, and 5.9 $\log_{10}$ removals were observed in other studies (Ottoson *et al.* 2006; Comerton *et al.* 2005 and Ueda and Horan 2000). All of these studies evaluated the performance of MBR systems in treating domestic

wastewater. These results were similar to our findings for coliphage removal by AnMBR (99.96% and 3.7 $\log_{10}$ removal).

Comparison of the Microbial Removals by the AnMBR System alone to the Combined CMAD/AnMBR System

From February to April 2007, the AnMBR system was operated independent of the CMAD. The $\log_{10}$ removals of *E. coli* and enterococci by this system were 6.9 and 7.3. Figure 3 illustrates the comparison of *E. coli* and enterococci removals by the AnMBR system alone to the combined CMAD/AnMBR system. Results showed there was no difference in the removals of *E. coli* and enterococci between these two systems. These results indicate that an AnMBR could achieve similar microbial removal performance if challenged with the same feedstock received by the CMAD. However, we'd like to note that coupling a CMAD with an AnMBR may provide an economic advantage due to the reduced TS concentration of the substrate fed to the AnMBR since membrane bioreactor flux rates decline with increasing total solids concentration (Anderson *et al.* 1986; Beaubien *et al.* 1996; Ross *et. al.*, 1990) and the flux rate directly impacts the energy required to operate the circulation pump.

Removal of Bovine Polyomaviruses and Adenoviruses in the Combined CMAD/AnMBR System

The number of measurements and occurrence frequency of animal enteric viruses in the samples are summarized in Table 3. Eight samples for each sampling location were tested for bovine polyomaviruses (BPyV) and bovine adenoviruses (BAdV). All of the CMAD influent and CMAD effluent samples were BPyV and BAdV positive. None of the AnMBR effluent samples tested positive for BAdV but there were three BPyV positive samples.

Interestingly, more samples also tested BPyV positive than BAdV by Hundesa *et al.* (2006), when slaughterhouse wastewater and river water were tested. In their study, BAdV was detected in only one sample but twenty-two samples were BPyV positive. These results may suggest the higher prevalence of BPyV than BAdV in animal waste. Also, Polyomaviruses (35-40nm) are roughly half the size of the adenoviruses (60-90nm) (Hurault de Ligny et al 2000, Thomas 2004); the size difference between these two viruses could be one of the factors explaining the fact that only BPyV were detected in AnMBR

effluent. However, this study used the same PCR methods as Hundesa *et al.* (2006). Similarities in the proportion of virus types may also be attributable to the differences in the BPyV and BAdV PCR method sensitivities.

In order to confirm the PCR results, seven BAdV positive samples selected from CMAD influent and CMAD effluent and seven BPyV positive samples selected from all three locations were sent for sequencing. The sequencing results showed all seven samples tested BPyV positive were 100% similar to the nucleotide sequence of BPyV in the genebank. For BAdV, two samples were 85% to 86% similar to BAdV type 2 and five samples were 99 to 100% similar to BAdV type 7.

CONCLUSIONS

The removal of pathogenic indicators and animal viruses from agricultural waste by an AnMBR pilot system was evaluated. The mean $\log_{10}$ removals of *E. coli*, enterococci, *C. perfringens* and coliphage by both CMAD and AnMBR were 6.7, 7.3, 6.5 and 4.2, respectively but most of the removals were attributed to the AnMBR. Three AnMBR effluent samples tested BPyV positive but none tested BAdV positive. The indicator that was found most frequently and had the highest concentration in the AnMBR effluent was coliphage. This suggests that coliphage or viruses would be suitable indicators for evaluating the AnMBR performance. The AnMBR also demonstrated significant removal of TKN, TP, COD, TS, and VS. Overall, the CMAD digester, which is one of the suggested practices of treating animal wastes, had much lower removals of indicators and animal viruses as well as physical and chemical parameters compared to the AnMBR system. This study demonstrates that AnMBR systems could be considered as an alternative treatment for animal waste especially when high removal of zoonotic pathogens is required. The economic feasibility of such systems may be increased if a consortium of community users and farmers could support the use of membrane systems for the co-treatment of human and animal waste.

ACKNOWLEDGMENTS

We thank Dr. Phanikumar Mantha for his advice on statistical analysis.

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TABLES and FIGURES

Table 1. Water quality in the combined CMAD/AnMBR system.

		CMAD Influent		CMAD Effluent		AnMBR Effluent		Removal by Entire System	
	n	Mean	SD	*Mean*	SD	Mean	SD	Mean (%)	SD
TKN (mg/L)	7	2,120	203	2,160	126	1,440	84	31.32	8.64
Ammonium nitrogen (mg/L)	7	1,240	469	1,300	91	1,330	89	-16.55	31.89
TP (mg/L)	7	343	28	317	78	14	5.3	95.84	1.52
COD (mg/L)	7	44,900	12,100	31,800	6,560	3,440	705	92.03	1.78
TS (%)	7	4.54	0.69	3.36	0.64	0.80	0.15	81.71	5.25
VS (%)	7	2.97	0.32	2.21	0.45	0.27	0.05	90.66	2.35

N, number of measurements; SD, standard deviation; COD, chemical oxygen demand; TKN, total Kjeldahl nitrogen; TP, total phosphate; TS, total solids; VS, volatile solids.

Table 2. Pathogen indicators occurrence in the combined CMAD/AnMBR system.

	CMAD Influent			CMAD effluent			AnMBR effluent		
	Mean	SD	F	Mean	SD	F	Mean	SD	F
E. coli (log_{10}cfu/L)	7.01	0.51	8/8	6.46	0.76	8/8	0.31	0.58	2/8
Enterococci (log_{10}cfu/L)	7.87	0.92	8/8	6.71	1.18	7/8	0.51	1.02	3/8
C. perfingens (log_{10}cfu/L)	6.51	0.47	8/8	6.39	0.81	8/8	ND	-	0/8
Coliphage (log_{10}pfu/L)	6.64	0.54	8/8	6.14	0.37	8/8	2.47	0.43	5/8

F, fraction of positive samples; ND, none detected.

Table 3. Animal enteric virus occurrence in the combined CMAD/AnMBR system.

Animal Viruses	CMAD Influent	CMAD Effluent	AnMBR Effluent
Bovine Adenoviruses	8/8	8/8	0/8
Bovine Polyomaviruses	8/8	8/8	3/8

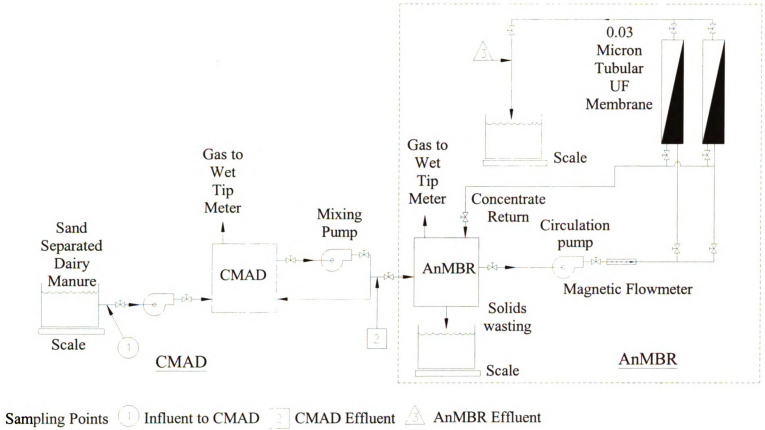


Figure 1. The pilot-scale system that treats animal waste. From February to April flow bypassed CMAD. Sampling was done in points 1 and 3. From June to August flow went through the whole systems. Sampling was done in points 1, 2, and 3. AnMBR, anaerobic membrane bioreactor; CMAD, complete mix anaerobic digester.

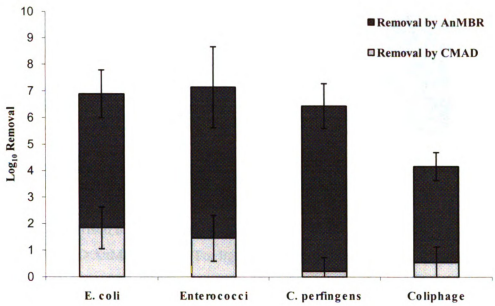


Figure 2. Log_{10} removal of Indicators by the combined CMAD/AnMBR system. Sampling period from June to August.

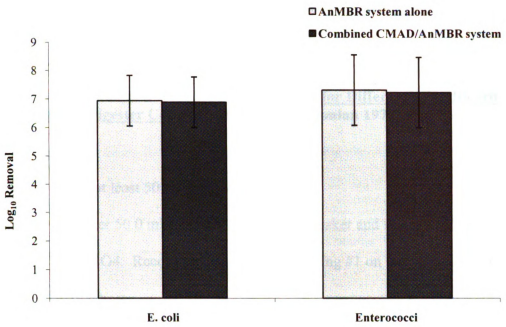


Figure 3. Comparison of $\log_{10}$ removals of *E. coli* and enterococci by the AnMBR system and the combined CMAD/AnMBR system. For light bars: sampling occurred from February to April; for dark bars: sampling occurred from June to August.

APPENDIX B

Volatile Fatty Acid Procedure

The following is adopted from "A Direct Method for Differentiating Bicarbonate and Acetate in Digester Control", (Obrien and Donlan 1977).

1. Filter at least 50 ml of Reactor Liquid.
2. Transfer 50.0 ml of filtrate into 150 ml beaker and titrate to pH 3.3 with 0.10 N H2SO4. Record reading in ml. as reading #1 on Volatile Acids Analysis Worksheet.
3. Cover Sample beaker with 65 mm watch glass and bring to boil for 60-90 sec.
4. Cool to room temperature and rinse watch glass into beaker with distilled water.
5. Titrate sample to pH 4.0 exactly using 0.050 N NaOH. Record volume as #2 on work sheet.
6. Continue titration to pH 5.1 with 0.050 N NaOH. Record volume as #3 on worksheet.

Calculations:

Total Alkalinity:	(TA) as mg/l CaCO3 = (#1) x 100
Volatile Acids:	(VA) as mg/l Acetic = (#3 - #2) x 100
Bicarbonate Alkalinity:	as mg/l CaCO3 = (TA) – 0.83 x VA

APPENDIX C

Most Probable Number Methodology

Non-Selective Medium for Growth off Cecal and Manure Anaerobic Bacteria (Adapted from Caldwell and Bryant (1966).

Substrate	%	Contribution to 300 mL medium solution
Glucose	0.05	0.15 g
Cellobiose	0.05	0.15 g
Soluble starch	0.05	0.15 g
Xylose	0.05	0.15 g
Trypticase	0.2	0.6 g
Yeast extract	0.2	0.6 g
Mineral #1	3.75	11.25 ml
Mineral #2	3.75	11.25 ml
Rumen fluid[1]	20.0	60 ml
Resazurin	0.1 ml/100 ml	0.3 ml

[1]Rumen fluid is clarified by centrifugation at 15,000 x g for 20 minutes and autoclaved at 120°C at 15 psi for sterilization prior to use in medium. Keep refrigerated until use.

Add enough distilled water to bring all of the above ingredients to a volume of 300 ml, taking into account addition of the sodium bicarbonate and cysteine-sulfide solution volumes (21 ml) below later.

Bring medium to gradual boil in a 500 ml round bottom flask (or 1000 ml Erlenmeyer flask) under CO_2, until steam evolves and the medium changes to a reddish color. Cool under ice to the touch and, while continuing to flush the flask with CO_2, add:

- Sodium bicarbonate (8%) solution, 5 ml/100 ml for a total of 15.0 ml
- Cysteine-sulfide (2.5% solution) 2 ml/100 ml for a total of 6.0 ml

Bubble CO_2 into the medium for a few minutes after adding the sodium bicarbonate and cysteine-sulfide solution, then flush headspace of the flask with CO_2 while tubing into Hungate tubes (9 ml per tube) with the tubes also under CO_2. Avoid blowing bubbles into the tube during the pipetting process. Crimp the lids down tightly with a crimper and autoclave the tubes at 120°C for 20 minutes at 15 psi.

Mineral Solution #1: 0.6% K_2HPO_4

Mineral Solution #2: To 100 ml distilled water add:

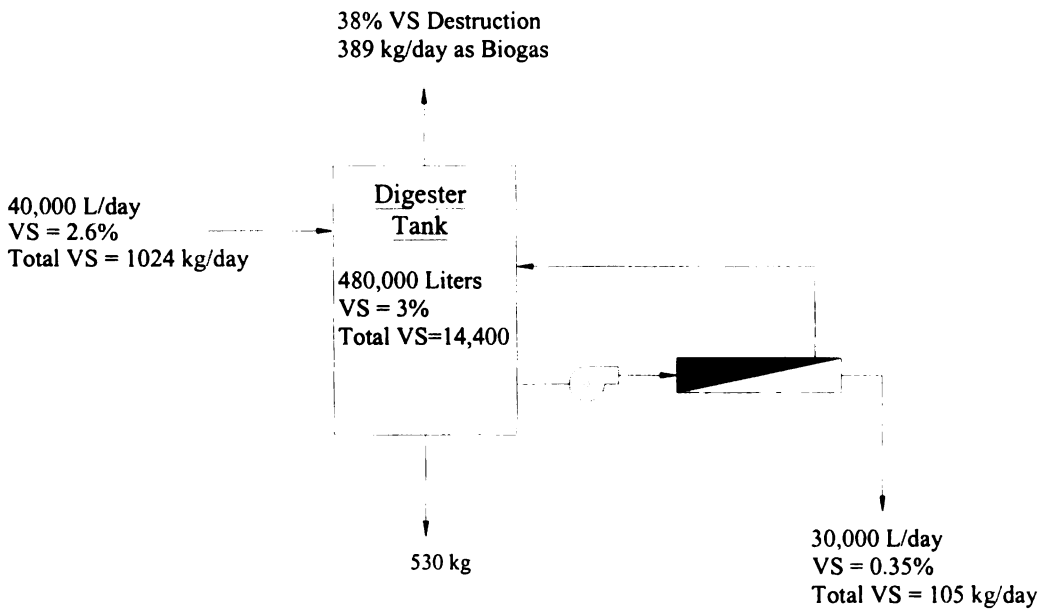
Compound	**Mass, g**
KH_2PO_4	0.6
$(NH_4)_2SO_4$	0.6
NaCl	1.2
$MgSO_4 \cdot 7H_2O$	0.25
$CaCl_2 \cdot 2H_2O$	0.16

APPENDIX D

Example AnMBR Analysis

Operating Conditions:

- SRT = 27 days
- HRT = 12 days
- VS destruction = 38%
- Digester operating concentration = 3% VS
- Membrane flux rate = 40 L/m^2/hr = 960 L/m^2/day
- 14.4 mm diameter membrane x 6000 mm, surface area per module = 1.89 m^2
- Cross flow velocity = 4.5 m/s, required flow rate per module = 310 L/minute
- Desire 75% recovery of influent as UF permeate (40,000 L/day x 75% = 30,000 L/day)
- Pressure drop per module = 100 kPa



SRT Calculation

The SRT can be set to a defined value based on HRT or based on digester operating VS concentration. In the example above, SRT = 14,400 kg ÷ 530 kg = 27 days. If the HRT were decreased from 12 days to 6 days with all else remaining constant, the SRT would decrease to 13.5 days. Alternatively, if a HRT of 6 days is desired with a corresponding

SRT of 27 days, this condition requires that the digester be operated at a VS concentration of 6%. There will be an energy penalty for operating at the higher VS concentration.

Cycle Time Calculation

Required Membrane Surface Area
30,000 L/day ÷ 960L/m^2·d = 31.25 m^2, require 31.25 m^2 ÷ 1.89 m^2 = 16 modules

Membrane Configuration
Assume modules operated in parallel, 310 L/min x 16 modules = 7,142,400 L/day

Cycle time = 7,142,400 L/day ÷ 480,000 L = 15 cycles/day

If modules are placed so that there are 8 sets of 2 in series, the cycle time would be decreased to 7.5 cycles per day.

Energy Calculations (based on 14.4 mm x 6,000 mm module):

- Pressure drop/module at approximately 3% VS (approximately 5% TS) = 100 kPa
- Assume with other system losses, the design pressure = 200 kPa (20.4 m H2O @ 20°C)
- Pump efficiency = 83%
- Motor efficiency = 92%
- Flow rate through membrane system, 7,142,400 L/day (297.6 m^3/hr)
- Liquid density = 1000 kg/m^3
- Methane production rate, 261 L CH_4/kg VS actual conditions
- NIST standard conditions, T= 20°C, P = 101.325 kPa
- Methane production at STP = 232 L CH_4/kg VS
- 34.6 MJ/m^3 methane (assumes LHV)

$$P_h = q \cdot \rho \cdot g \cdot h \div 3.6x10^6$$

Where,

P_h = power (kW)
q = flow capacity (m^3/hr)
ρ = density of fluid (kg/m^3)
g = gravity (9.8 m/s^2)
h = differential pressure head, (m)

P_h = 297.6 m^3/hr x 1000 kg/m^3 x 9.8 m/s^2 x 20.4 m ÷ $3.6x10^6$ = 16.5 kW

Total power required = 16.5 kW ÷ 0.83 (pump eff.) ÷ 0.82 (motor eff.) = 21.6 kW

Comparison of potential energy from biogas:

1024 kg VS fed x 232 L CH_4/kg VS fed = 237,568 L CH_4 produced per day

Converting to electricity equivalent and assuming 35% conversion efficiency

34.6 MJ/m^3 x 237.568 m^3 = 8220 MJ

8,220 MJ x 0.2778 kW-hr/MJ = 22,283 kW-hr

2,283 kW-hr ÷24 hr/day x 35% (conversion to electricity efficiency) = 33.3 kW

APPENDIX E
Gas Chromatograph Mass Spectroscopy Protocol

Instrument

Agilent 5973 inert mass selective detector with autosampler

Column

30 meter DBWAX, 0.25 mm inner diameter x 0.25 μm film thickness

Temperature Program

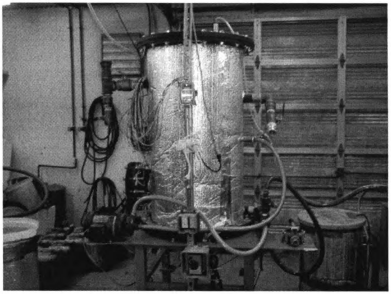
50°C increasing at 20°C per minute to 120°C where held for 5 minutes, followed by 2°C increase each minute to 130°C, followed by 40°C increase per minute to 240°C. The total run time for the heating routine was 16.25 minutes. Total volume was 1 μl injected in a splitless mode and a constant flow of 1.5 mL per minute, scanning masses 10 through 300.

Quantification

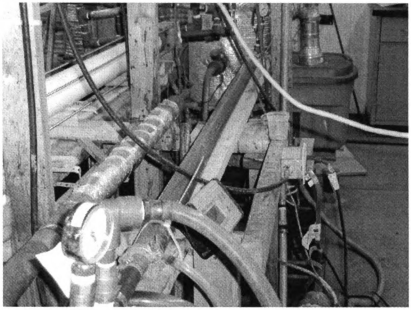
Extracted ion chromatograms of a m/z (mass-to-charge-ratio) indicative for each compound (acetic, propionic, butyric and Isobutyric acids) were integrated and the areas entered into a Microsoft Excel program using a standard curve of 0 through 500 ng/μl or μg/mL.

Appendix F

Photographs of Pilot Digesters



Complete Mix Digester



View of SM AnMBR (grey tube is the single membrane housing)

View of identical digester tanks, the tank to the left was used for MED, MMED and MM AnMBR, the tank to the right for SMD and SM AnMBR

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