Dairy Waste Treatment Utilizing a Two-Phase Anaerobic Digestion System: Evaluation of Parallel Reactor Configuration and Mixing Intensity

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Abstract

The U.S. dairy industry produces roughly 500 billion pounds of manure per year on a wet slurry mass basis, most of which is collected, stored, and applied to cropland. Biodegradation of this manure produces offensive odors and large quantities of methane, a potent greenhouse gas. Anaerobic digestion is a proven treatment method addressing both issues, but economic considerations have prevented its widespread implementation. In an effort to improve the profitability of this process, our research group at the University of Idaho is evaluating a variation of two-phase anaerobic digestion, in which a portion of the organic acids produced during fermentation are used to produce bioplastics, while the remaining material is anaerobically digested to generate methane. This research focused on optimization of the anaerobic digestion component of this system, and specifically on assessing the impact of mixing intensity on digester stability and the potential of a parallel digester configuration to increase methane production.

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Dedication

I would like to thank my amazing wife, Dr. Maia Clay, and my parents, Donald and Edwina Stowe, for their love, support, and encouragement during the completion of this work.

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Chapter 1 Introduction

1.1 Waste Management in the U.S. Dairy Industry

As of the 2007 agricultural census, the dairy herd in the United States consisted of 71,510 dairy operations housing 9.158 million cows producing an estimated 500 billion pounds of manure yearly on a wet slurry mass basis (Betts and Ling 2009). Traditionally, dairy manure has been collected, stored, and applied to fields to enhance forage crop production, as manure contains high levels of nitrogen, phosphorus, and other nutrients required for plant growth (USDA-NRCS 2012). This approach is becoming increasingly problematic due to concerns regarding climate change caused by greenhouse gas (GHG) accumulation, as microbial metabolism of land applied manure releases significant amounts of methane and nitrous oxide to the atmosphere (EPA 2014). Both of these gases are more effective at trapping heat than carbon dioxide, with 1 unit of methane and nitrous oxide having the same warming potential as 21 and 310 units of carbon dioxide, respectively (EPA 2014). In the United States as of 2012, GHG emissions from dairy manure management accounted for 6.3% and enteric fermentation for 6.7% of total biogenic agricultural non-carbon dioxide GHG emissions. Biogenic agricultural non-carbon dioxide emissions accounted for 8.1% of total U.S. GHG production, and while it would appear dairy GHG production is a relatively insignificant source, it is still one of the largest industrial generators of GHGs (EPA 2014). Although some dairy GHG emissions will be offset by growth of cattle forage crops, a carbon balance calculation based on dairy farms in Pennsylvania showed that a moderately sized farm was still a net emitter of about 447 metric tons of carbon dioxide equivalents, even after carbon uptake by crop growth was accounted for (Chianese et al. 2009).

Beyond GHG production, problems associated with conventional storage and land application practices include emission of unpleasant odors, and the fact precautions must be taken to avoid cross-contamination of crops with pathogenic organisms present in land-applied manure (Sahlstrom 2003). These problems have been exacerbated in recent years by the trend of increasing consolidation of the U.S. dairy industry; between 1989 and 2009 the size of the nation's dairy herd shrank by 7% while the number of dairy operations decreased

by 67%. In 2008, 730 dairy operations housed 2,000 or more cows, a size category that was not included in U.S. agricultural statistics until 1998. Public opposition to large operations based on offensive odors, waste management, and environmental concerns has made it increasingly difficult for dairy operators to build new facilities or expand existing ones (Sanders et al. 2010).

Anaerobic digestion (AD) is an established waste treatment technology addressing many of the above concerns that has found increasing acceptance in recent years. The process leverages an interdependent consortium of anaerobic bacteria to break down complex organic wastes and produce a biogas consisting primarily of methane and carbon dioxide. The reactions are the same as would take place during the degradation of manure in a natural system, but a bioreactor is used to accelerate the process rates and capture the biogas. The methane that is produced can be combusted to generate heat and electricity, which reduces GHG emissions through conversion of methane to carbon dioxide, and by reducing the need for electricity generation using fossil fuels. Digestion also provides pathogen reduction and can be used to produce EPA Class A or B biosolids (EPA 2003). Nitrogen-containing compounds present in the digester feed are broken down to form ammonia, a form of nitrogen readily assimilated by plants, and the compounds responsible for objectionable odors are greatly reduced (Betts and Ling 2009, Weiland 2010). The combination of pathogen and odor reduction, increased nutrient availability, and reduced GHG emissions make anaerobically digested manure a superior choice to untreated manure for land application. AD installations are feasible at over 8,000 U.S. dairy, swine, and poultry operations, and have the potential to add 1670 megawatts of electrical generation capacity (EPA 2010). In 2009, the approximately 150 anaerobic digesters operating in the U.S. reduced U.S. GHG emissions by 1.1 million metric tons of carbon dioxide, equivalent to taking 218,000 passenger vehicles off the highway or conserving 128 million gallons of gasoline (EPA 2010). Although the number of digesters has been increasing in the U.S. over the past decade, only about 2% of the sites where AD is feasible actually have digesters installed. As a contrast, Germany, one of the world leaders in adoption of AD, was operating 4,000 biogas plants as of 2008 (Weiland 2010).

U.S. farmers avoid anaerobic digesters for a variety of reasons, with high initial cost and low or negative rate of return (ROR) on investment being the most common (Betts and Ling 2009, Faulhaber et al. 2012, Zaks et al. 2011). Poor digester design, lack of operator skill and/or process knowledge, difficulty in obtaining financing, underutilization of co-digestion (i.e. charging a fee to accept and treat an off-site waste stream) or digester byproducts, and problems negotiating electricity purchase agreements have been cited by farmers as well (Betts and Ling 2009). Studies have shown new AD projects will probably require some form of government market support in order to be profitable, such as carbon offset credits, low interest loans, or grants to compensate for low energy prices and the high initial cost of digester facilities (Faulhaber et al. 2012, Zaks et al. 2011). This is the case in Germany, where strong government support for renewable energy has led to high rates of adoption of AD (Zaks et al. 2011), as well as other sources such as solar and wind (Gillis 2014). Formation of farmer co-ops to spread risk, cost, responsibility for operations, and to handle purchase agreement negotiations with electric utilities has been suggested as an alternative or supplement to government price supports (Betts and Ling 2009).

Despite its marginal rate of return, AD is currently the state of the art approach to dairy manure treatment. However, if improvements could be made to the treatment process, both in terms of improving digester methane yield and in developing new technologies capable of producing additional revenue streams, profitability could be realized and the need for government support could be reduced or eliminated. With this goal in mind, the University of Idaho Environmental Engineering Laboratory research group has been exploring the potential for producing useful commodities from waste streams which have traditionally been viewed as end products requiring disposal (Coats et al. 2013). The research conducted to date has focused on the use of dairy manure as the raw material, but the process could be applied to other agricultural waste streams as well. As shown in Figure 1, the primary commodities generated are polyhydroxyalkanoates (PHAs) using mixed microbial cultures (Coats et al. 2007) and methane through anaerobic digestion of residual material not utilized by the PHA process (Coats et al. 2012).

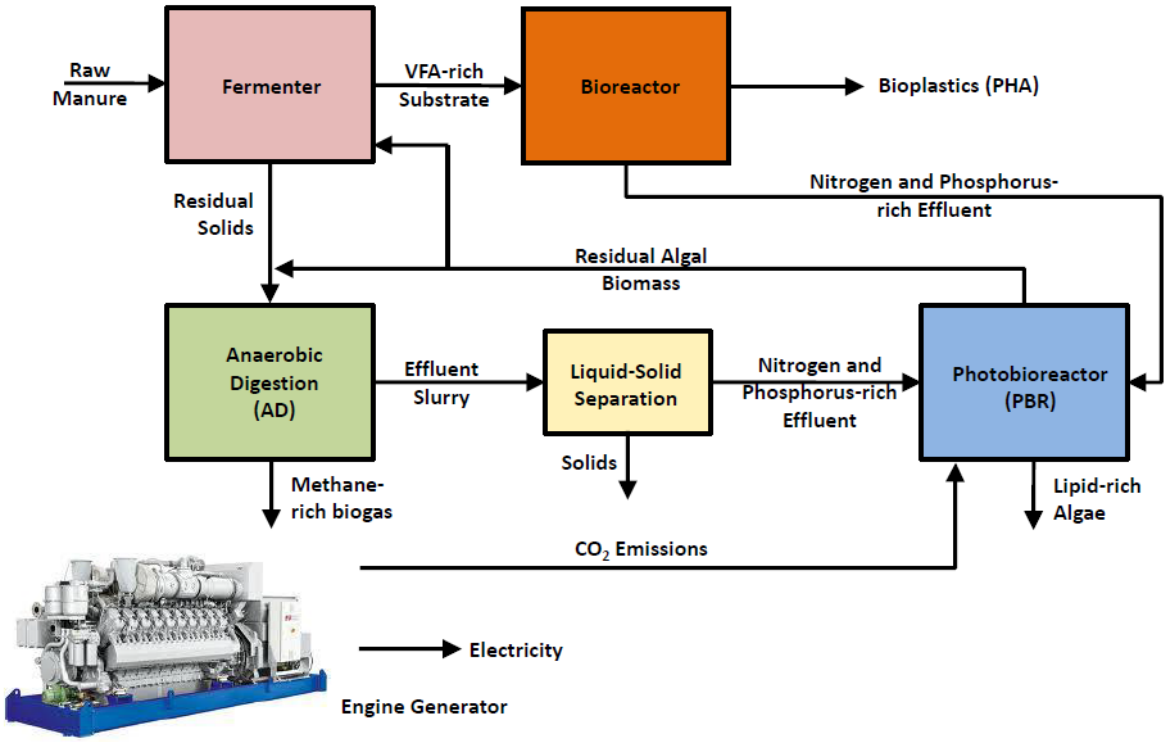

Figure 1. Proposed dairy manure treatment and resource recovery process.

PHA is a biopolymer produced from volatile fatty acids (VFAs) by bacteria for energy and carbon storage, and can be extracted from the cells and refined to form a biodegradable plastic resin. PHA is currently produced using pure microbial cultures and sterile feedstocks (e.g., acetate), but the cost of production has limited it to niche applications. The use of a waste material for PHA production, as we are proposing, has the potential to lower the cost of production sufficiently to make PHA competitive with traditional oil-based plastics. Using our approach, raw manure is fermented to produce VFAs, which are separated from residual solids using liquid/solid separation (e.g. centrifugation, belt filter press, etc.). A portion of the VFA-containing liquid fraction is diverted and used as substrate in bioreactors devoted to PHA production, while the remaining liquid and residual solids are directed to an anaerobic digester to be fermented further and partially converted to methane. Some of the methane is used for digester heating, while the remainder can be used for either heating on-site structures or combusted in an engine generator to produce electricity, which can be sold to the local electric utility and/or used to offset demand at the farm. The nutrient-rich digestate can be land-applied as fertilizer or used to grow algae for biofuel production, and

the solids can be separated and recycled as cattle bedding material. The research presented herein focuses on the anaerobic digestion component of this system, and specifically on improving methane production, the impact of mixing intensity, and collection of performance data which could be used to guide the design of a pilot or full-scale treatment facility.

1.2 The Process of Anaerobic Digestion

As shown in Figure 2 on the following page, the conversion of an organic substrate to volatile fatty acids and ultimately methane and carbon dioxide involves six interdependent microbial-driven processes (Gujer and Zehnder 1983, Kaparaju et al. 2009):

1. Hydrolysis of proteins, carbohydrates and lipids, to form amino acids, sugars and long chain fatty acids (LCFAs)
2. Fermentation of amino acids and sugars to produce volatile fatty acids (VFAs) and CO_2
3. Anaerobic oxidation of LCFAs and alcohols to intermediate VFAs
4. Anaerobic oxidation of VFAs to produce acetate and hydrogen
5. Production of methane and CO_2 from acetate (acetoclastic methanogenesis)
6. Production of methane from hydrogen and CO_2 (hydrogenotrophic methanogenesis)

In addition to these processes, hydrogen and carbon dioxide can be combined by bacteria including *Acetobacterium woodii* and *Clostridium aceticum* to form acetate, as shown in process 5 of Figure 2 (Weiland 2010). This reaction is thought to have little net impact on methane production, as the acetate can be used to produce methane through acetoclastic methanogenesis (Grady et al. 2011). Acetate may also be oxidized to form hydrogen and carbon dioxide, which can then be converted to methane via hydrogenotrophic methanogenesis. This pathway is not shown in Figure 2, and is typically not considered to represent a significant fraction of chemical oxygen demand (COD) flow. However, it has been demonstrated to be active in digesters operated at ammonia concentrations high enough to

induce inhibition of acetoclastic methanogenesis while leaving hydrogenotrophic methanogenesis unaffected, and could therefore become a dominant process when treating waste streams rich in proteins and/or ammonia (Werner et al. 2014).

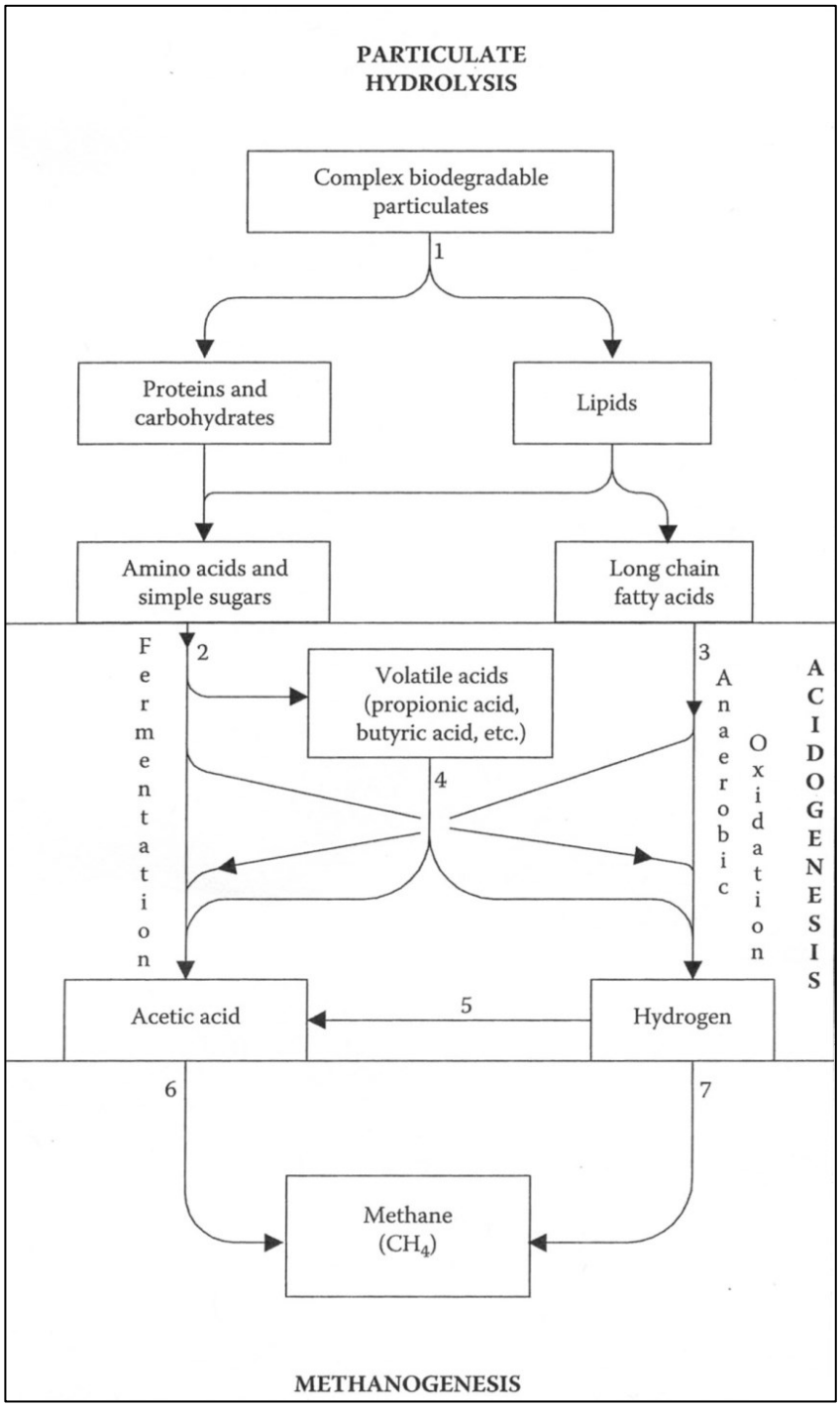

Figure 2. Conversion steps in fermentation and methanogenesis (Grady et al. 2011).

1.3 Hydrolysis

In the hydrolysis step (process 1 of Figure 2), particulate organic material is converted to lower molecular weight water-soluble compounds which can be transported across the cell membrane and metabolized. Members of the prokaryotic domain Bacteria are the organisms responsible for hydrolysis and fermentation, and important groups include *Bacteroides, Clostridia, Bifidobacteria,* and some members of *Porphyromonadaceae*, all of which are obligate anaerobes (Grady et al. 2011, Weiland 2010). Facultative anaerobes including *Streptococci* and *Enterobacteriaceae* may be present as well (Parkin and Owen 1986, Weiland 2010). These organisms may secrete hydrolysis enzymes into bulk solution, or they may attach to particulate material and secrete the enzymes directly to the material surface (Lynd et al. 2002). Not all compounds present in the substrate will be hydrolysable within the solids retention time (SRT) of the reactor due to "structure, inaccessibility, and complex non-hydrolysable linkages" (Parkin and Owen 1986). This undigested fraction can comprise from 35% to 80% of the volatile material present depending on the type of substrate, with higher fractions limiting the ultimate degree of volatile solids (VS) removal that can be obtained. Little stabilization, or reduction in COD, of the substrate is possible if unfavorable conditions for hydrolysis exist in the bioreactor, such as a lack of microbes capable of producing hydrolysis enzymes, inadequate mixing, dilute substrate or low temperature (Parkin and Owen 1986). Hydrolysis is generally modeled as a first order reaction with respect to particulate substrate concentration (Eastman and Ferguson 1981), although Contois models taking into account biomass growth, or two-stage models considering particulate surface colonization by bacteria and subsequent biodegradation of particulate material, have been proposed as more general process representations (Vavilin et al. 2008). Under these models, first order kinetics is a special case, although the first order model should be adjusted under conditions in which the substrate contains a large fraction of non-biodegradable material (Vavilin et al. 2008). The hydrolysis step is typically rate limiting, while acidogenesis (processes 2, 3, and 4 of Figure 2) occurs most quickly (Eastman and Ferguson 1981, Vavilin et al. 1996). Methanogenesis (processes 6 and 7 of Figure 2)

usually proceeds faster than hydrolysis, but could become rate limiting under high organic loading conditions (Vavilin et al. 2008).

1.4 Fermentation and Anaerobic Oxidation (Acidogenesis)

During acidogenesis (processes 2, 3, and 4 of Figure 2), amino acids, LCFAs, and sugars generated by hydrolysis are transported into the bacterial cells and catabolized. The metabolites produced are primarily VFAs including acetate, propionate, butyrate, valerate, and caproate, along with hydrogen, carbon dioxide, and water. Alcohols, principally ethanol, may be produced under acidic conditions. Fermentation reactions involving amino acids and sugars use organic compounds as both oxidizing and reducing agents and consequently there is no change in the net energy level of the substrate (Grady et al. 2011).

Anaerobic oxidation (process 3 of Figure 2) refers to the β-oxidation process in which bacteria sequentially split an acetate molecule and hydrogen from a LCFA. The process continues until the LCFA has been completely broken down, with acetate, propionate (in the case of fatty acids containing an odd number of carbon atoms), hydrogen, and carbon dioxide produced as end products. Anaerobic oxidation reactions take place near thermodynamic equilibrium, and become thermodynamically unfavorable if the partial pressure of hydrogen in the reactor builds to 10^{-4} atmospheres or more (Grady et al. 2011). Inhibition of anaerobic oxidation results in accumulation of VFAs, which will decrease the pH of the reactor if insufficient alkalinity is present to act as a buffer. A syntrophic relationship exists between hydrogenotrophic methanogens and the bacteria responsible for anaerobic oxidation, which prevents hydrogen from accumulating and inhibiting the process (Parkin and Owen 1986).

One advantage of anaerobic treatment is the low cell growth yield of the bacteria involved. Cell yields of bacteria performing carbohydrate fermentations range from 0.10 to 0.17 g cell COD per g substrate COD and 0.06 g/g for amino acid fermentation at neutral pH, or from 0.09-0.15 g/g at a pH of 5.5. Yields are somewhat lower for degradation of VFAs and LCFAs, at 0.04-0.06 g/g (Batstone et al. 2002, Grady et al. 2011). In contrast, the cell yield of aerobic organisms ranges from 0.43 to 0.71 g/g (Tchobanoglous et al. 2003). The optimal pH range

for growth of fermentative organisms has been found to be between 4 and 6.5 (Speece 2008).

1.5 Methanogenesis

During methanogenesis (processes 6 and 7 of Figure 2), the end products of fermentation and anaerobic oxidation are consumed by methanogenic bacteria to form methane. Acetate is split to produce methane and carbon dioxide by acetoclastic methanogens, and carbon dioxide and hydrogen are combined to produce methane and water by hydrogenotrophic methanogens. All methanogens are members of the prokaryotic domain *Archae.* The hydrogen-oxidizing organisms are classified in the orders *Methanobacteriales, Methanococcales,* and *Methanomicrobiales.* These organisms are autotrophic obligate anaerobes limited to using hydrogen or formate as electron donors (Grady et al. 2011). This pathway is responsible for approximately 30% of methane formation, with the acetoclastic route accounting for the other 70% (Gujer and Zehnder 1983). Fewer acetoclastic methanogens have been identified, but all are contained in the families *Methanosarcinaceae* and *Methanosaetaceae* within the order *Methanosarcinales* (Grady et al. 2011). *Methanosarcina* are capable of using a wide array of substrates, including hydrogen, CO_2, carbon monoxide, methanol, methylamines and acetate (Zeikus et al. 1985), while *Methanosaeta* are limited to consumption of acetate (Grady et al. 2011).

Members of *Methanosarcina* grow rapidly and prefer high concentrations of acetate, while members of *Methanosaeta* are more competitive when the acetate concentration is low (Figure 3), meaning the manner in which the digester is operated will determine which group is dominant, although both groups likely will be present to some degree (Speece 2008). Methanogens will grow in a pH range of 6.5 to 8.2 (Speece 2008), with optimal growth occurring between 6.8 and 7.4 (Grady et al. 2011). Acetoclastic methanogens grow more slowly than hydrogenotrophic methanogens, with maximum growth rates for acetoclasts ranging from 0.004 d^{-1} to 0.036 d^{-1} versus 0.02 d^{-1} to 12 d^{-1} for hydrogenotrophs. Cell yields for hydrogenotrophs have been reported from 0.014 g/g to 0.183 g/g, a somewhat wider

range than that of acetoclasts of 0.014 g/g to 0.076 g/g (Batstone et al. 2002), and similar to that of the anaerobic fermentative bacteria.

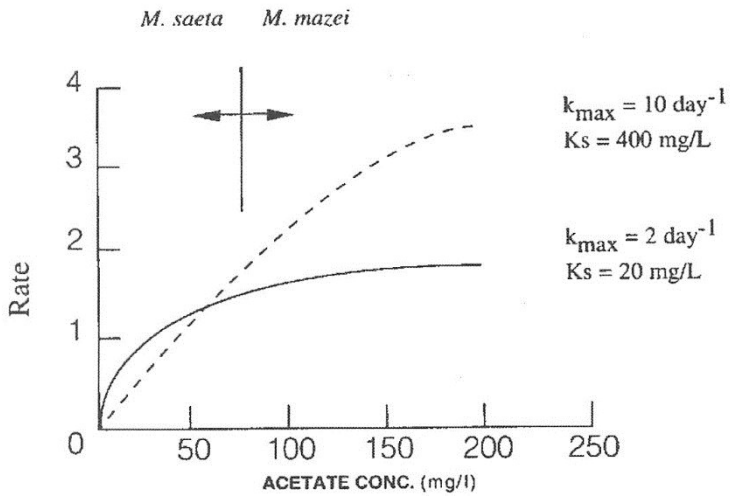

Figure 3. Higher growth rate of *Methanosaeta* vs. *Methanosarcina* (referred to on figure as *M. mazei)* at low acetate concentration (Speece 2008).

Due to the fact optimal pH ranges for fermentative and methanogenic bacteria do not overlap, the digestion process is sometimes split between two reactors to separate the acidogenic step from the methanogenic step, a configuration referred to as phase separation or the two-phase process (Figure 4). The approach takes advantage of the faster growth rate of fermentative microbes in comparison to methanogens, which allows the SRT to be used as a selective mechanism in each reactor. Feed is introduced to the first reactor (the fermenter), which is operated at a retention time of <4 days to prevent significant growth of methanogens. The acidified effluent from the fermenter is fed to the second reactor (the digester), where methanogenesis takes place. VFA production in the first phase results in a low pH conducive to the growth of fermentative bacteria, while production of ammonia by degradation of proteins and the consumption of VFAs in the second phase results in a higher pH suited to methanogenic bacteria (Massey and Pohland 1978, Pohland and Ghosh 1971). This approach has several benefits over conventional single-stage digestion, including increased stability, better effluent quality, and depending on the method of operation, reduced hydraulic retention time (HRT) and increased SRT (Speece 2008).

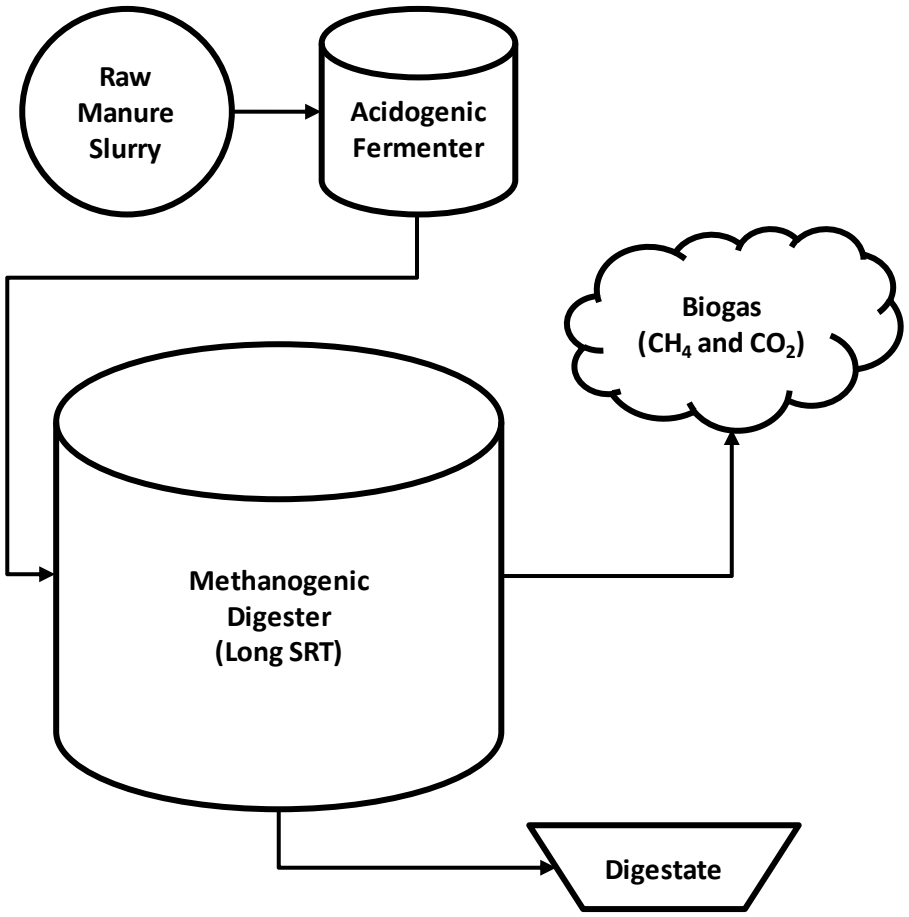

Figure 4. Two-phase anaerobic digestion configuration.

Although acetate and hydrogen both serve as direct precursors for methane formation, methanogens lack the ability to directly metabolize propionate. Instead, it is first metabolized to acetate, bicarbonate, and hydrogen or formate by members of the genus *Syntrophobacter*, several of which have been isolated and identified in pure culture. The Gibbs free energy for the oxidation of propionate is positive at standard conditions, and the reaction therefore will not proceed unless the products acetate, hydrogen and formate are removed. This is accomplished via a syntrophic relationship between the propionate-oxidizing bacteria and hydrogenotrophic methanogens, which causes the reaction equilibrium to shift and the free energy change to become negative. This relationship operates most efficiently at short inter-bacterial distances, which maximizes the flux of the electron carriers hydrogen and formate between the producing and consuming species (de

Bok et al. 2004). Disruption of this relationship, for example by intense mixing, has been shown to cause propionate to accumulate in a digester (Stroot et al. 2001).

Rates of fermentation and methanogenesis both increase with temperature, but due to their higher growth rates fermentative bacteria are impacted less by lower temperatures than methanogens (Grady et al. 2011, Speece 2008). Fermentation-only reactors used to produce VFAs for other processes (e.g., biological phosphorus removal or PHA production) are typically operated at ambient temperatures in order to minimize energy consumption and the growth of acetoclastic methanogens (Grady et al. 2011), while anaerobic digesters are usually operated in the mesophilic (30-38 °C) or thermophilic (50-57 °C) temperature ranges (Tchobanoglous et al. 2003). Thermophilic digestion has several advantages over mesophilic digestion, including faster biochemical reactions and microbial growth rates and better pathogen reduction, but is subject to problems including reduced stability, VFA accumulation, and greater energy requirements for heating (Speece 2008). Free ammonia (NH_3), which has a toxic or inhibitory effect on methanogens, will be present at higher concentrations in thermophilic digesters due to the increased temperature, and can cause inhibition or process failure due to washout (Grady et al. 2011, Weiland 2010). Despite the larger reactor volumes required for mesophilic systems, they have been more widely adopted due to reduced energy consumption and greater process stability (Speece 2008).

1.6 Research Background and Overview of Laboratory Fermenter and Digester Operations

In order to demonstrate the feasibility of the proposed manure treatment method (Chapter 1.1) and to collect performance data, the University of Idaho Environmental Engineering Lab operated two anaerobic digestion systems since 2010. The specific operating parameters (SRT, organic loading rate (OLR), active volume, etc.) and reactor configuration have been changed periodically, but in general the systems were operated using our modified two-phase approach. Each reactor is operated as a fed batch continuously stirred tank reactor (CSTR), with feeding and wasting occurring once per day. In a fed batch reactor, the influent is introduced and the effluent withdrawn (also referred to as cycling) in discrete steps over a

given time period. The fed batch approach reduces short circuiting (removal of a fraction of the reactor contents at a time much less than the theoretical reactor retention time) because the feed is retained for at least one full feed/waste cycle, while in a continuous flow reactor some feed would exit as soon as it entered. The behavior of a fed batch CSTR approaches that of a continuous flow CSTR as the period of time between cycling intervals approaches zero.

Raw manure is diluted with water and fed to the fermenter, and the fermenter effluent undergoes liquid/solid separation by either centrifugation or screening followed by centrifugation in order to divert a portion of the VFA-rich liquid fraction to PHA production experiments. The remaining liquid fraction and the solids, which consist primarily of slowly biodegradable cellulosic plant material, are fed to the anaerobic digester to produce methane. The digestate is either discarded or used in other experiments.

Prior to the beginning of the research described herein, liquid/solid separation of the fermenter effluent had been performed using centrifugation, which is effective but time-consuming. In an effort to make the process of cycling the reactors less onerous, the decision was made to switch the separation method to the faster method of screening. Although this method was more efficient, material fine enough to pass through the screen (~1 mm mesh size) was excluded from the digester feed. Centrifugation of the screened liquid effectively separated this material, which was desirable as the presence of particulate material interferes with the PHA process. After the switch was made to screening, a decrease in digester biogas content and production volume due to the diversion of the fine material was observed. In order to obtain greater understanding of the effect of this material on the anaerobic digestion process, a 500 mL digester fed the fine solids was operated for a period of several months. Although the feed to the digester was low in volatile solids (VS) (~75% of total solids), the biogas produced was higher in methane content (~69% vs. ~55% for the main digesters). Microbial population analyses showed the consortium present in the fine solids digester differed from those present in the two main digesters (Briones et al. 2014).

It was unclear if the greater methane content was simply a reflection of the characteristics of the substrate, or if separate digestion of the fine material from the coarse cellulosic material was somehow allowing the the methanation process to proceed more efficiently. If the second possibility was correct, it was conceivable that the methane yield of a two-phase digestion system could be increased using digesters operated in parallel, one receiving fine and one receiving coarse solids. Other research using cow manure as the substrate has been carried out using anaerobic digesters operated in series without phase separation (Boe and Angelidaki 2009, Kaparaju et al. 2009), as well as the two-phase approach (Coats et al. 2012, Demirer and Chen 2005, Yilmaz and Demirer 2011), but to our knowledge a parallel digestion system utilizing separate digesters to treat different components of the feed has not been studied. Consequently, design parameter ranges (SRT and organic loading rate (OLR) range, potential for foaming, etc.) for a fine-fraction digester were unavailable, and a batch reactor experiment was conducted to develop rough estimates of OLRs and reactor solids concentrations that would be likely to result in stable performance if applied to a fed batch reactor. In a batch reactor the theoretical and actual SRTs are identical, but this is not the case in a fed batch system, and consequently performance estimates developed using each approach will differ. In order to further refine the design parameter estimates, the performance data from the batch reactors was used as the basis for the design of three fed batch reactors. Performance data from these reactors was used to establish design organic loading rates and volume requirements for a single digester capable of efficiently treating the entire mass of fine solids produced daily by the fermenter of one of the existing laboratory AD systems. The fine solids digester was operated in parallel with a larger digester fed the coarse solids fraction, and the performance of this system was compared to that of a simultaneously operated conventional two-phase AD system fed the entire solids fraction in order to evaluate the potential of the parallel digester configuration to improve the process methane yield. The batch reactor, fed batch reactor, and system comparison experiments are described in detail in Chapters 2, 3, and 4.

Chapter 2 Batch Reactor Experiment

2.1 Overview

Given the lack of operational data from which to design this unique two-stage AD system, a batch reactor experiment was conducted as an initial step toward developing an estimate of the range of OLRs that would result in stable operating conditions for a fine solids fed batch digester. The experiment was carried out using a method similar to the biomethane potential (BMP) test (Angelidaki et al. 2009). The performance of a fed batch anaerobic digester was approximated using batch reactors having an equivalent OLR calculated as

$$OLR_{eq} = \frac{VS_{initial}}{V*SRT}$$

where $VS_{initial}$ refers to the mass of volatile solids added to the batch reactor, V is the active volume of the batch reactor, and SRT refers the solids retention time of the fed batch reactor being modeled, which is equal to the length of the batch reactor operation period. This approximation ignores the effect of wasting, which removes inhibitory degradation products such as ammonia, as well as a fraction of previously introduced feed prior to the theoretical retention time. The maximum loading rate tolerated by a fed batch reactor may therefore be higher than indicated by this test. The digesters were not operated long enough to determine the actual BMP, as that procedure is used to assess and compare the digestibility of different substrates, while in this case the parameter of interest was instead the methane yield of the fine solids at different OLRs within a typical anaerobic digester SRT of 15-25 days. This is a sufficiently long retention time to extract the bulk of the methane potential from the substrate; the marginal increase in methane production achieved through the use of longer retention times is not cost-effective due to the larger reactor volume required (Speece 2008).

2.2 Materials and Methods

2.2.1 Experimental Design

A fed batch raw manure fermenter was operated to provide substrate for the batch reactor AD experiment. Fine solids were collected by sequential screening and centrifugation of the effluent of fermenter F3-b (details concerning this reactor and its operation are found in Chapter 3.2.1.1) over a period of 16 days and stored at 4 ^{o}C until startup. Centrifugation of the screened effluent was performed at 8,000 rpm for 5 minutes at room temperature (20 °C to 25 °C). At the end of the fine solids collection period, the solids were homogenized by manual mixing and tested for total solids (TS) and VS. Five 0.5 L batch reactors were operated (referred to as digesters 1 through 5), each at a different equivalent OLR. Dilutions were made using tap water as required to make up the active volume. One liter capacity screw top glass bottles were used as reactor vessels, and were sealed with plastic caps containing rubber septa. Biogas was collected in Tedlar gas sampling bags (Smith Air Sample Supply Co., Hillsborough, NC, USA) equipped with a valve and septa for sampling. The bags were connected with Tygon tubing to a syringe and needle, which was inserted through the septa in the cap to the reactor headspace. The needle was secured in the septa by an adapter which fit over the cap and served to immobilize the needle during mixing to prevent leakage. The bottles were placed in a New Brunswick environmental incubator shaker (Eppendorf Inc., Enfield, CT, USA) maintained at 35 °C. The shaker table mixing speed was set at a level sufficient to prevent solids from settling. The digesters were not inoculated on startup, as methanogens are present in both raw dairy manure and fermenter effluent. Gas production and content data were collected for 22 days to approximate the effect of a 22-day SRT.

2.2.2 Analytical Techniques

Samples were collected to i) quantify total and volatile solids in the fine solids used to initiate the reactors, and ii) to assess biogas volume produced, and biogas methane and carbon dioxide content. It should be noted that the oven-drying procedure used for solids determination will result in losses of volatile components (e.g. VFAs, carbon dioxide, and

ammonia), and the true dry matter content will be somewhat higher than reported using Standard Methods 2540G (Porter and Murray 2001). Although research conducted in the agricultural sciences frequently corrects for this effect, anaerobic digestion research has traditionally followed the non-corrected approach described in Standard Methods (Kreuger et al. 2011). The degree of volatilization loss is determined by a range of factors including the pK_a of the volatile compound, sample pH, the change in pH occurring during drying, pH buffering capacity, sorption of volatile compounds to inert solids, and the presence of other ions with which volatile components can form salts (e.g. sodium, calcium, magnesium, etc.). Consequently, the mass of volatile compounds lost during drying can vary widely depending on substrate or digestate composition, as described in a technical report published by the Swedish biogas firm SGC (Vahlberg et al. 2013). Water may be retained by mechanical occlusion or by the formation of hydrates, counteracting the effect of volatilization. The complexity of the interactions governing the degree of volatilization, and the ease of the oven-drying approach to TS and VS quantification, are most likely why the effort is typically not made to quantify volatilization losses in digestion research or in the operation of full-scale plants. Solids concentrations are typically used in the context of anaerobic digestion as general indicators of the efficiency of the process or the nature of a feedstock, and the greater degree of accuracy obtained through adjustment for volatilization may not be necessary. In dealing with digestion of substrates having a high potential for volatilization losses (e.g., an industrial waste stream high in acetate with low pH lacking buffering capacity), it may be desirable to correct solids measurements for losses, or to use a more accurate technique (e.g., total organic carbon). All TS and VS measurements made during the research described herein were conducted following Standard Methods 2540G (Clesceri 1998) due to the focus of the research on dairy manure, which is high in volatilization-limiting alkalinity (Huchzermeier and Tao 2012), the typical reactor pH of 6.6-7.6, the distance of the reactor pH from the pK_a of VFAs of ~4.8, the ease with which the procedure can be conducted on large numbers of samples, as well as to maintain consistency with the method of reporting of the majority of studies conducted in the anaerobic digestion field.

The volume of biogas collected in the sampling bags at room temperature was measured on a daily basis using the principle of liquid displacement. The biogas was evacuated from the bags after the volume was measured, and the empty bags were reattached to the reactors. Measured volumes were corrected for the presence of water vapor under the assumptions the gas was saturated with water vapor and was in a state of dynamic equilibrium with the liquid phase. The Clausius-Clapeyron equation (Brown et al. 1994) was used to calculate the vapor pressure of water in the biogas at room temperature, which under saturated conditions is equal to the partial pressure. The gas collection bags were not allowed to become pressurized so that the biogas contained was at atmospheric pressure, which was estimated at the elevation of Moscow, ID (786 m) using the formula found in Metcalf & Eddy (Tchobanoglous et al. 2003). The resulting estimate of water vapor content was 3.5%, and all biogas volume measurements were reduced by this percentage.

Methane and carbon dioxide were quantified using a Gow-Mac (Bethlehem, PA, USA) Series 550P Gas Chromatograph equipped with a thermal conductivity detector (TCD). The temperature of the column (Grace Davison Discovery Sciences, Deerfield, IL, USA, Alltech® Hayeseop ® DB 100/120 column, 30 ft x 1/8 in. x 0.085 in., stainless steel) and injector was held constant at 36 $^{\circ}$C. The detector was operated at 175 $^{\circ}$C using a current of 220 mA. Helium was used as the carrier gas at a flow rate of 22 mL/min. One milliliter of sample was injected using a gas-tight syringe (SGE Analytical Science, Austin, TX, USA). The presence of methane and carbon dioxide were confirmed by matching with known standards and converted to volumes using a standard curve (r^2 = 0.999). Samples for methane and carbon dioxide analysis were taken from the gas collection bags prior to measurement of gas production volumes, and the methane fraction was used to convert biogas volumes to methane volumes.

2.3 Results and Discussion

The solids content of the fine material used to inoculate the reactors was 12.1% +/- 0.1% TS, of which 74.8% +/- 0.1% VS (n = 4). Each digester was loaded based on a dilution factor, with 1 part fine solids and 4 parts water added to digester 1, 2 parts solids to 3 parts water in

digester 2 and so forth, with digester 5 consisting of undiluted solids. This approach resulted in the loading rates shown in Table 1.

Table 1. Batch digester organic loading rates.

Digester Number	Mass of Wet Solids Added (g)	Mass of Volatile Solids (g)	Equivalent OLR (g VS/L*22 d)
1	90	8.16	0.74
2	190	17.22	1.57
3	280	25.38	2.31
4	390	35.35	3.21
5	480	43.51	3.96

Results from this batch AD experiment are presented in Figure 5. Digester 2 is not included because it never produced a measureable quantity of biogas, possibly due to a leak in the gas collection apparatus or to failure of a viable microbial population to become established. In retrospect, it would have been desirable to use effluent from one of the established digesters as an inoculant and operate an additional reactor to control for the methane production due to the addition of the inoculant. Reactor 5 was terminated after 14 days because it began to produce foam, which filled the headspace and repeatedly clogged the needle attached to the gas collection bag, making measurement of methane production impossible.

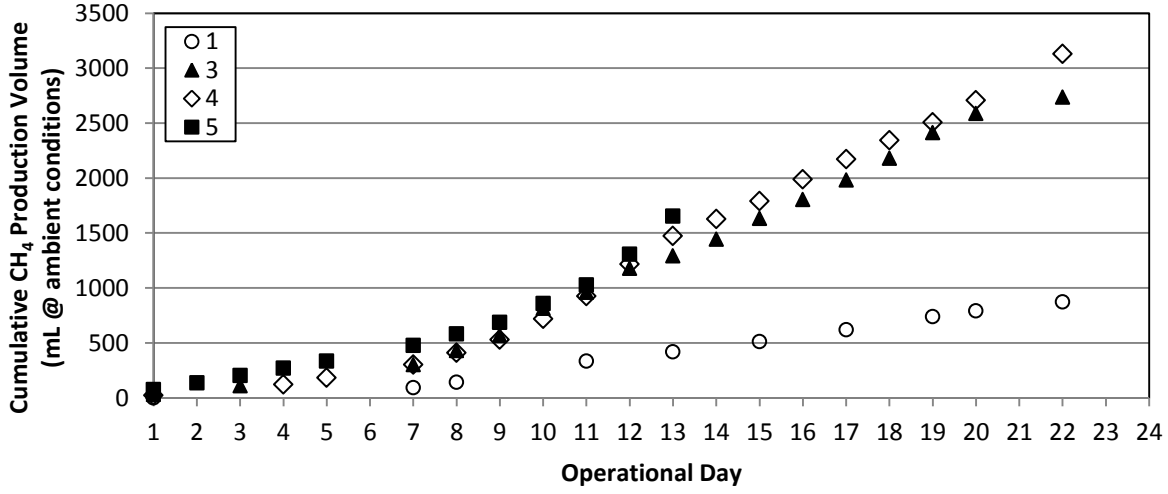

Figure 5. Cumulative methane production by batch digesters 1, 3, 4, and 5.

Reactors 3, 4, and 5 displayed similar rates of methane production (Figure 5), despite the differences in organic loading, once methanogenic cultures became established at about Day 7, as indicated by the increase in biogas methane content shown in Figure 6. In contrast, Reactor 1, which received the smallest amount of fine solids, produced biogas at a much lower rate. This indicates substrate availability was likely limited, as might be expected given the low organic loading. The methane content in Reactor 1 demonstrated little variability over the course of the experiment, indicating equilibrium between the methanogenic and fermentative populations was achieved quickly due to the dilute composition of the reactor.

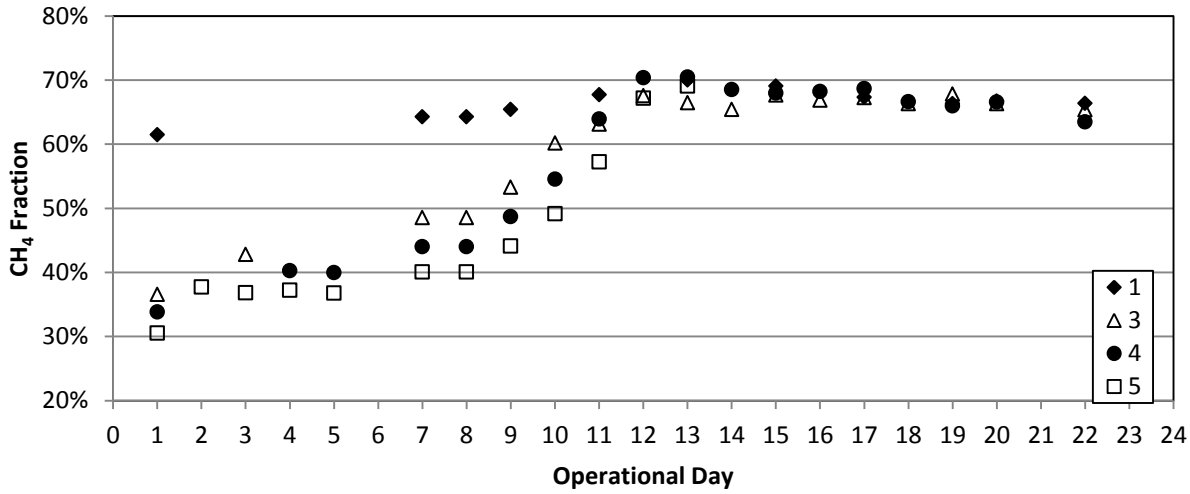

Figure 6. Methane fraction (of CH_4 + CO_2) of batch digesters 1, 3, 4, and 5.

In the remaining digesters methane content increased rapidly between days 7 and 12, corresponding to establishment of a population of acetoclastic methanogens and balanced conditions between fermentation and methanogenesis. The presence of methane between days 1 and 6 indicates methanogens were initially present in the fine solids. Methane production associated with faster-growing hydrogenotrophic methanogens would not be expected to occur until about day 3 based on the Anaerobic Digestion Model (ADM) No. 1 (Batstone et al. 2002, Grady et al. 2011), although the time required for establishment of acetoclastic methanogens generally agrees with predicted values. Average methane content from day 12 to day 22 was 67.6% in Reactor 1, 66.6% in Reactor 3, and 67.4% in Reactor 4. Methane yields for digesters 1, 3, and 4 were 0.107, 0.159, and 0.123 L CH_4/g VS applied,

respectively. The lower yield in digester 4 could be due to a buildup of inhibitory compounds such as VFAs, hydrogen, or ammonia, but is more likely a reflection of the rate-limiting effect of hydrolysis, as the total quantity of methane produced was similar to that of digester 3. If more VS was present in digester 4 than the microbial population was capable of hydrolyzing within the data collection period, less complete conversion of biodegradable VS to methane would result, thereby reducing the yield in comparison to digester 3, which was operated at a lower OLR. This suggests the OLR applied to a fed batch digester with an SRT of ~22 days should be similar to that of digester 3 (2.31 g VS/L*d) and that OLRs lower than this will be inefficient in terms of digester volume utilization due to underloading. Digestion at OLRs near 4 g VS/L*d should be avoided based on the development of foaming in digester 5 and the decrease in methane yield observed in digester 4.

The solids concentrations of digesters 1, 3, and 4, assuming a concentration of 1% equals ~10,000 mg/L, were 2.2%, 6.8%, and 9.5%, respectively, at the start of the experiment. Solids content would have declined somewhat by the end of the experiment due to the production of gas, although solids analysis was not performed to quantify the degree of reduction. An alternative interpretation of the methane yield data for design of a fed batch reactor is that the concentration of solids should be maintained at approximately 7% or less to avoid overloading at SRTs in the 20 day range. In addition, the energy input required for mixing and pumping increases with solids content (Tchobanoglous et al. 2003), providing an additional reason for avoiding high solids concentrations in reactor design.

With approximate estimates of OLR range and total solids concentration developed in the batch reactor experiment, a more rigorous and comprehensive investigation into the digestion of the fine solids was conducted using fed batch reactors. These would be operated to obtain more accurate performance data for the design of a larger fed batch digester, which would be used to evaluate the potential of the solids separation approach to increase digester methane yield.

Chapter 3 Fed Batch Reactor Investigation

3.1 Overview

Building upon the batch AD investigations (Chapter 2), these studies were conducted to quantify the performance of digesters fed the fermented fine solids operated over a range of SRT/OLR combinations with the primary goal of identifying the SRT/OLR range which would result in effective treatment based on VS reduction, effluent VFA concentrations, and methane production. The results could then be used as the basis of design for a larger fine solids digester capable of treating all the fine material produced daily by the fermenter of one of the two larger-scale lab AD systems (Chapter 1.6) in order to facilitate a direct performance comparison between the parallel and single-stream digestion approaches.

Three fed batch digesters having a volume of 1.25 L were operated at SRTs of 15, 22.5, and 30 days, referred to as AD7-15, AD7-22.5, and AD7-30. The digesters were operated from June 2013 (AD7-30) or July 2013 (AD7-22.5 and AD7-15) to January 2014. The digesters were fed fine solids isolated from the effluent of the fermenter of one of the two-phase digestion systems operated in the lab (Chapter 1.6). Samples were collected for quantification of TS/VS, biogas production and methane content, VFAs, total carbon (TC), and total nitrogen (TN).

3.2 Materials and Methods

3.2.1 Experimental Design

3.2.1.1 Fermenter F3-b

Fine solids were collected from a fermenter (referred to as F3-b) constructed from a 22.7 L (6 gallon) HDPE bucket. A valve was installed in the base for wasting, and it was sealed with an HDPE lid containing a rubber gasket. The fermenter was operated at an active volume of 20 L, a 4-day SRT, and a target OLR of 8.75 g VS/L*d, with feeding/wasting conducted once per day. Fermentation took place at room temperature, typically between 22 °C and 25 °C. Mixing was accomplished using a 3.75” diameter helical impeller driven by an Oriental Motor (San Jose, CA, USA) USM315-401W 15 W AC speed control motor connected to 3GN35SA

reduction gearbox operated at a speed sufficient to provide uniform mixing of the reactor contents. In order to minimize the daily exposure of the contents of the reactor to oxygen, gasketed bulkhead fittings were used to install two draft tubes constructed of Schedule 40 ¾" PVC pipe through the lid and extending below the liquid surface. One tube housed the impeller shaft and the other was used for feeding. Biogas produced was vented through an airlock via Tygon tubing inserted through a rubber grommet in the lid. The airlock was disconnected during feeding and wasting, which limited atmospheric exposure of the reactor contents to about 5 L of air per day.

3.2.1.2 Fed Batch Digesters

Each digester was operated in a rectangular 2 L Nalgene polyethylene terephthalate glycol-modified (PETG) bottle (Thermo Fisher Scientific, Waltham, MA, USA) placed in a New Brunswick environmental incubator shaker (Eppendorf Inc., Enfield, CT, USA) maintained at 35 °C and shaken at 74 rpm, which provided sufficient intensity to maintain completely mixed conditions. Tubing extending beneath the liquid surface in the bottle was attached to fittings in the caps, which allowed a 150 mL capacity syringe to be attached and used for feeding and wasting. Biogas was vented through a third fitting in the caps, and conveyed through Tygon tubing to meters operated on the principle of water displacement. A 0.5 L capacity Tedlar gas sampling bag was installed at a tee in the tubing connecting the reactors to the meters which allowed the headspace volume to vary so that constant pressure was maintained in the reactor headspace, and the contents were not exposed to oxygen during feeding and wasting. Biogas samples analyzed for methane and carbon dioxide content were collected from a septum installed in an additional tee in the tubing near the top of the reactor. Each digester was seeded with a mixture of digestate from one of the two-phase systems (Chapter 1.6) and fine solids from the fermenter. The experimental setup for the digesters and gas meters is shown in Figure 7.

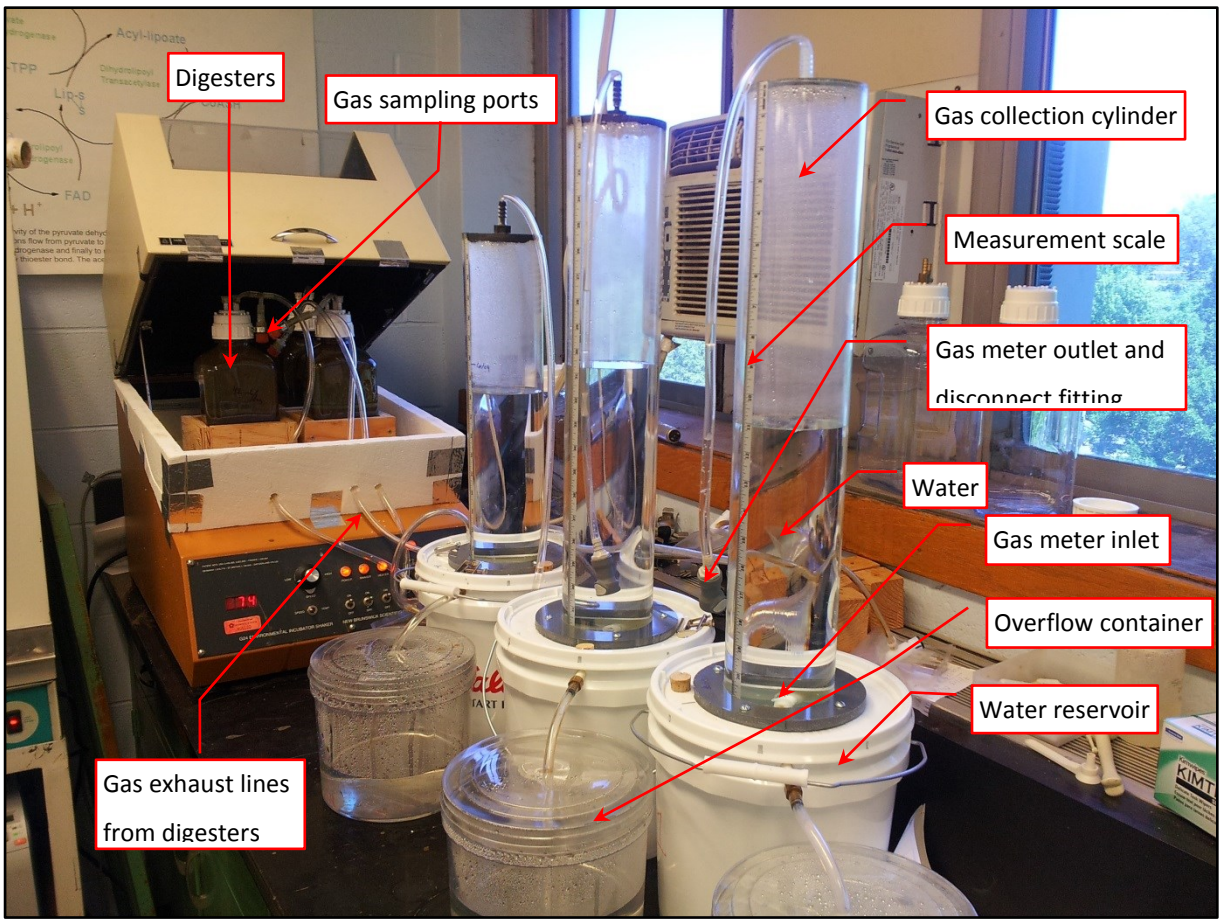

Figure 7. Experimental setup for fed batch digester system.

3.2.1.3 Operational Approach

The feed to the fermenter consisted of a mixture of raw dairy manure and enough tap water to bring the total feed volume to 5 L. Manure was collected bi-weekly from the floor of the University of Idaho dairy and stored at 4 °C prior to use. The manure was sampled for total and volatile solids content at the time of collection, and these measurements were used to calculate the mass of manure required daily to maintain the 8.75 g VS/L*d (175 g VS/d total) target OLR. Collection of manure from areas of the dairy where it would be likely to be mixed with refractory lignocellulosic bedding material was avoided.

Fine solids were separated by screening the effluent from the fermenter through a kitchen strainer with a mesh size of approximately one millimeter (Norpro Inc., Everett, WA). The liquid passing through the strainer was then centrifuged at 8000 rpm for 5 minutes at room

temperature to isolate the fine solids. Solids were typically collected on a daily basis, although solids production fluctuated and made daily collection unnecessary at times. The collected solids were stored at 4 °C, and were homogenized and diluted to 9.5% TS using tap water to make up feed for the three digesters.

Both the fermenter and the digesters were cycled at approximately the same time each day throughout the testing and analysis period. Prior to cycling, biogas samples were collected from each digester and the time and volume of water displaced were recorded for each gas meter. The meters were refilled and reconnected to the digesters after cycling.

3.2.2 Analytical Techniques

Biogas was characterized using a Gow-Mac (Bethlehem, PA, USA) Series 550P Gas Chromatograph equipped with a thermal conductivity detector (TCD). The temperature of the column (Grace Davison Discovery Sciences, Deerfield, IL, USA, Alltech® Hayeseop ® DB 100/120, 30 ft x 1/8 in. x 0.085 in., stainless steel) was held constant at 70 °C, and the injector temperature constant at 39 °C. The detector was operated at 160 °C with 180 mA current. Helium was used as the carrier gas at a flow rate of 22 mL/min, and 0.6 mL samples were injected using a gas-tight syringe (SGE Analytical Science, Austin, TX, USA). The presence of methane and carbon dioxide were confirmed by matching with known standards and converted to volumes using a standard curve ($r^2 = 0.999$).

Samples taken for total and volatile solids content were analyzed as described in Chapter 2.2.2.

Samples collected for quantification of soluble VFAs were centrifuged at 16,000 rpm for 20 minutes and the supernatant was passed through a 0.22 micron PVDF syringe filter (Millipore Corp., Billerica, MA, USA). Diluted solutions of the supernatant for analysis were prepared using 0.5 mL of sample and 0.5 mL of double deionized water (DDI) and then acidified using 0.35 to 0.50 µL of 2 N HNO_3 depending on sample alkalinity in order to shift the pH to approximately 2 and VFA speciation to the volatile, protonated form. VFA concentrations were measured using a Hewlett-Packard (Palo Alto, CA, USA) 6890 series gas chromatograph and flame ionization detector. The column was a Grace (Grace Davison

Discovery Sciences, Deerfield, IL, USA) AT™-AquaWax-DA (30m x 0.25mm) capillary column (PN 14537). Samples were injected using the Hewlett-Packard model 7679 auto-injector equipped with a 5 µL syringe using an injected volume of 0.5 µL in a 1:20 split ratio using helium as the carrier gas at a flow rate of 1.2 mL/min and nitrogen as the makeup gas. The temperature program used consisted of heating the oven to 50 ^{o}C for 2 min, ramp at 25 ^{o}C/min to 95 ^{o}C, then ramping at 10 ^{o}C/min to 150 ^{o}C, hold 3 min, ramping at 25 ^{o}C/min to 200 ^{o}C and hold for 12 minutes for a total method length including cooldown of approximately 33 minutes.

As shown in Figure 7, biogas produced in the 1.25 L anaerobic digesters was vented through tubing attached to fittings in the caps and captured and quantified volumetrically in a liquid displacement meter. The setup of the meters and reactors was similar to that used by Eastman and Ferguson (Eastman and Ferguson 1981). The meters consisted of a section of 4" diameter acrylic tubing inverted in an HDPE container and sealed at the upper end. The gas line from the reactor was extended through the wall of the tubing near the top of the container so that gas generated in the reactor would be captured inside. Holes were drilled in the lower end of the acrylic tubing to allow water displaced by the biogas to flow out of the tubing and into the container. The water level in the container was fixed at the level of the gas line installed in the acrylic tubing by a drain fitting in the wall of the container, which allowed displaced water to flow out of the container and into a separate receptacle. In this arrangement the pressure at the outlet of the gas line is equal to atmospheric pressure, and the liquid level in the meter will not influence the headspace pressure in the reactor. The meters were reset on a daily basis by clamping off the line leading to the reactor, attaching a vacuum pump to a fitting installed in the upper end of the acrylic tubing and evacuating the biogas to refill the meter. Water displaced into the overflow container was then transferred back to the meter and the clamp removed from the gas line. Biogas volume was measured using an adhesive rule graduated in 32^{nds} of an inch affixed to the outside of the acrylic tubing, and calibrated to relate vertical liquid displacement and gas volume. Measured gas volumes were reduced to account for the sub-atmospheric pressure within the meter and by

an additional 3.5% to allow for the presence of water vapor as described in Chapter 2.2.2, and then adjusted to standard ambient temperature and pressure (SATP).

Due to the high partial pressure of carbon dioxide in the biogas, some of the carbon dioxide entered solution with the water in the meter and was subsequently lost to the atmosphere. The water in the meters was not changed in an attempt to allow the system to approach equilibrium and minimize this effect, but the loss of carbon dioxide was not completely eliminated. The volume of biogas lost per day was estimated at between 3.6% and 5.7% of the measured average volume produced. As the underestimation of biogas production was similar for each meter, and the experimental results were being used to develop a design OLR/SRT combination for a large fine solids digester to be used in a future experimental comparison, measures to reduce the volume of carbon dioxide lost (e.g., acidification of the water in the meters) were deemed unnecessary. Methane production by each digester was calculated based on measured biogas volume, meaning loss of carbon dioxide from solution in the meter resulted in underestimation of the actual methane volume generated by approximately 2.5% to 4.0%. Data presented herein does not take this effect into account.

Samples were collected on two occasions for total carbon and total nitrogen content on a dry solids basis. Samples were collected from the influent and effluent of fermenter F3-b and each of the fed batch digesters. The analysis was performed by the University of Idaho Analytical Laboratory (Nelson and Sommers 1982).

3.2.3 Statistical Methods

Calculations were performed using the R statistics program (Team 2012) and the Agricolae (de Mendiburu) package. Single factor ANOVA was used to establish differences in means, with significance declared at $p < 0.05$. If the ANOVA procedure indicated a significant difference in means existed between reactors, the Tukey test was used to identify which reactors were different, with significance declared at $p < 0.05$.

3.3 Results

3.3.1 Fed Batch Digester Performance

In order to compare performance between reactors, a steady-state analysis period based on consistent daily biogas production rates and low effluent VFA concentrations lasting from August 4th to September 28th 2013 (56 days) was isolated from the larger dataset. The substrate fed to all digesters was identical (Table 2), but each received different quantities depending on the SRT. The resulting OLRs were between 2.4 and 4.8 g VS/L*d, similar to the range evaluated in the initial batch reactor test, with the highest OLR associated with the shortest SRT.

Table 2. Average fed batch digester influent characteristics.

TS (% dry matter)	9.3 +/- 0.3 (n = 15)
VS (% of TS)	77.4 +/- 2.0 (n = 13)
Influent VFAs (mg/L as COD)	5351 +/- 777 (n = 6)

As shown, daily methane production increased as the organic load increased (Figure 8), but methane yield on a gram VS applied basis decreased as the SRT was reduced (Table 3), which is in agreement with other observations in the literature (Lawrence 1971, Speece 2008).

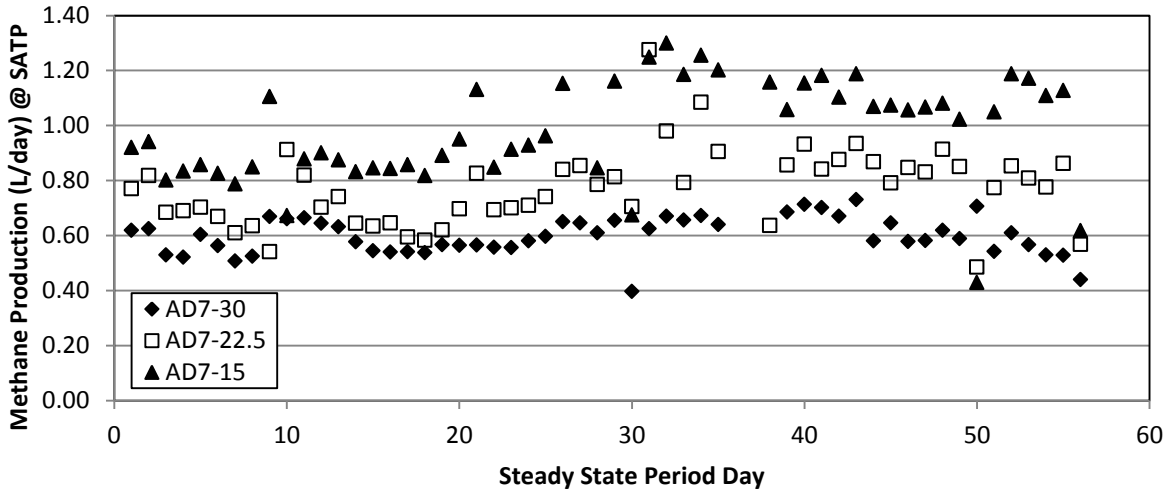

Figure 8. Methane production rates of each digester during steady-state period.

Referring to the effluent sampling and performance data summarized in Table 3, yield for the 22.5 day SRT digester was 17% higher than that of the 15 day SRT digester, while there was essentially no difference between the yield of the 22.5 and 30 day SRT digesters. Methane content was similar for all digesters, although it did decrease somewhat at the 30 day SRT (AD7-30 < AD7-15 and AD7-22.5, p = 0.004 and 0.02, respectively, Tukey test). The inverse relationship between methane content and SRT is possibly due to greater CO_2 production resulting from additional hydrolysis and fermentation of particulate material at longer SRTs. Shorter retention times correlated with lower VS destruction; AD7-15 and AD7-22.5 achieved about 8.5% and 4% less VS destruction than AD7-30, respectively.

Table 3. Summary of effluent sampling and steady-state performance data for fed batch digesters. Confidence intervals represent one standard deviation. Standard deviations for OLR, VS destruction, and methane yield were calculated using the error propagation approach described in Chapter 4.2.3.

	AD7-30	AD7-22.5	AD7-15
TS (% dry matter)	6.9 +/- 0.3 (n = 15)	7.1 +/- 0.4 (n = 15)	7.5 +/- 0.2 (n = 15)
VS (% of TS)	67.9 +/- 1.3 (n = 14)	69.3 +/- 1.8 (n = 13)	70.6 +/- 2.0 (n = 13)
OLR (g VS/L*d)	2.4 +/- 0.1	3.2 +/- 0.1	4.8 +/- 0.2
VS Destruction (%)	35.0 +/- 2.0	31.2 +/- 2.3	26.6 +/- 1.7
CH_4 production (L/d)	0.60 +/- 0.07 (n = 53)	0.77 +/- 0.14 (n = 54)	0.98 +/- 0.18 (n = 53)
Biogas CH_4 Content (%)	66.5 +/- 1.2 (n = 9)	67.8 +/- 0.8 (n = 8)	68.1 +/- 0.8 (n = 9)
Yield, L CH_4/g VS applied	0.20 +/- 0.02	0.19 +/- 0.04	0.16 +/- 0.03
VFAs (mg/L as COD)	110 +/- 57 (n = 6)	170 +/- 126 (n = 6)	498 +/- 448 (n = 6)

Digesters AD7-30 and AD7-22.5 both produced good quality effluent, with average total VFA concentrations less than 200 mg/L as COD, representing about 97% removal of influent VFAs (Table 2). Differences in effluent quality between these reactors were not significant (p = 0.88, Tukey test), as was also the case when comparing AD7-22.5 and AD7-15 (p = 0.11, Tukey test). However, the effluent quality of AD7-15 was significantly lower than that of AD7-30, (p = 0.04, Tukey test) (Figure 9), with only 91% removal of influent VFAs achieved on average. This digester suffered from VFA accumulation; by day 34 the acetate concentration had built to 835 mg/L and propionate to 124 mg/L. This is an indicator of imbalance and/or organic overloading, as properly designed and operated anaerobic digesters are capable of producing total effluent VFA concentrations of 100 mg/L or less (Speece 2008).

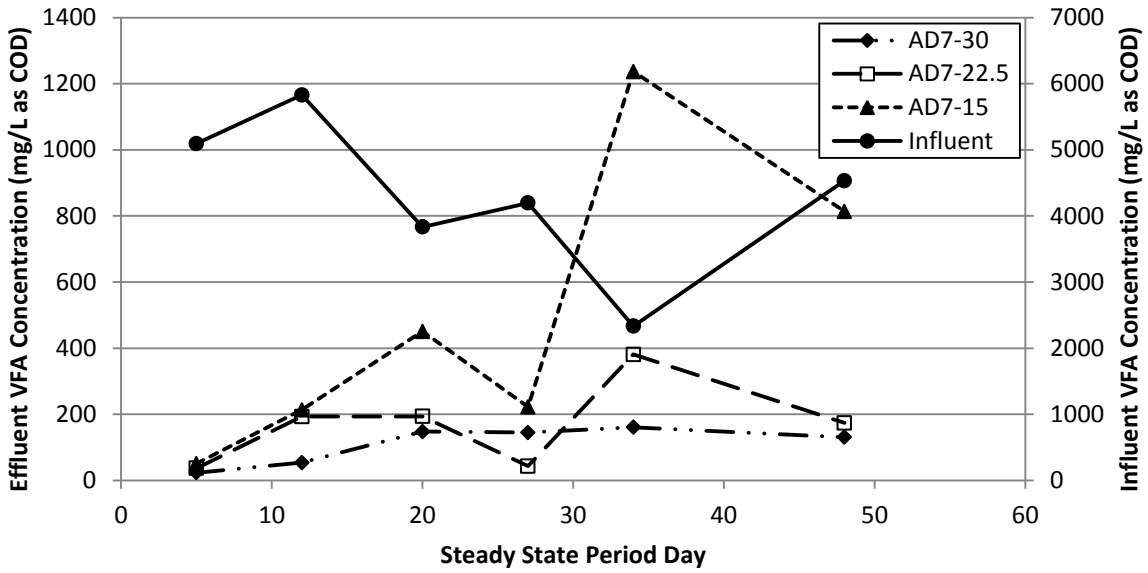

Figure 9. Total influent and effluent VFA concentrations in fed batch digesters.

The digesters were sampled for total carbon and nitrogen content, as measured on a dry mass basis. The second set of samples was collected after the end of the steady-state period (due to an upset caused by problems with the thermostat in the shaker incubator), but the results were similar to those of the first sampling run.

Table 4. Results of TC and TN sampling.

	Raw Manure	F3-b Effluent	AD7-15 Effluent	AD7-22.5 Effluent	AD7-30 Effluent
TC (n = 2)	42.7 +/- 0.5	41.3 +/- 0.9	38.5 +/- 0.5	38.5 +/- 0.5	38.5 +/- 0.5
TN (n = 2)	2.7 +/- 0.5	2.9 +/- 0.2	3.8 +/- 0.1	3.8 +/- 0.0	3.8 +/- 0.1

Several observations can be made based on the data shown in Table 4. Most importantly, the fine solids digester feed is enriched in nitrogen in comparison to the fermenter effluent or the raw manure. Organic material contains nitrogen, primarily in the form of protein and amino acids (Grady et al. 2011), indicating that biomass is likely concentrated in the fine solids fraction, as might be expected given the method used to recover this substrate. Total nitrogen can be used as an estimator of protein content; crude protein can be calculated as as TKN*6.25 (Latimer 2012), indicating the fine solids are composed of approximately 26%

protein. Nitrogen loss was observed in the digesters, probably due to a combination of generation of gas phase ammonia within the reactors, which operated at an average pH of ~7.6, and volatilization of ammonia during the sample drying process. Carbon appeared to be slightly more concentrated in the fine solids feed than in the bulk fermenter effluent, and was present at a lower concentration in the digester effluent due to biogas production, while little reduction occurred in the fermenter. There was no apparent difference in the carbon content of the three digesters.

3.4 Organic Loading Rate Determination

In order to avoid oversizing the fine solids digester, it was desirable to determine the highest OLR that could be used without compromising effluent quality or AD stability. Accumulation of VFAs and/or reduced methane yield is indicative of organic overloading, and the determination of a design OLR was consequently based on the observed effluent VFA concentration and methane yield in each of the fine solids digesters.

AD7-15 performed poorly in comparison to the other two digesters, with reduced VS destruction and methane yield. VFA accumulation was observed in this digester as well, although it did not experienced the type of severe imbalance characterized by VFA buildup and declining pH that is sometimes observed in municipal sludge digesters and described as “sour” or “stuck” digestion (Grady et al. 2011). This is due to the high buffering capacity of the manure substrate; anaerobically digested dairy manure may contain concentrations of alkalinity as high as several thousand mg/L as $CaCO_3$ (Huchzermeier and Tao 2012). The excess alkalinity prevents the reduction in pH and methanogenic activity typically associated with VFA imbalance, and allows dairy digesters to operate (albeit inefficiently) at higher VFA concentrations. Nevertheless, given the inferior performance of digester AD7-15, the 15 day SRT/4.8 g VS/L*d OLR combination should be avoided. AD7-22.5 and AD7-30 exhibited similar levels of performance, with AD7-30 possibly having a slight advantage over AD7-22.5 in terms of methane yield and VS reduction. Effluent VFA concentrations from both digesters were similar, and neither experienced the VFA accumulation observed in AD7-15. Therefore, it appeared OLRs in the range of 2.4 to 3.2 g VS/L*d would result in acceptable performance

at SRTs in the 20-30 day range, and that while a higher OLR would result in reduced treatment efficiency, it would not cause digestion to stall. The negligible difference in performance between AD7-22.5 and AD7-30 indicated an extended SRT/reduced OLR would not justify the increased tank size required to treat a given mass of solids.

With the range of OLRs resulting in acceptable digester performance known, it was possible to calculate the digester volume required to treat the average mass of fine solids produced daily in our 20 L laboratory manure fermenter, while avoiding organic overloading and digester oversizing. The next step was to construct such a digester in order to directly compare the performance of a two-digester system treating fine and coarse solids separately with that of a conventional combined solids digester.

Chapter 4 Direct Comparison of Parallel and Single-Stream Digestion Approaches

4.1 Overview

Building upon the preliminary AD investigations, the purpose of this investigation was to conclusively determine if separate digestion of the fine and coarse material present in fermented dairy manure using two parallel CSTRs would deliver a greater methane yield than the conventional approach in which all solids are digested in a single CSTR. The two AD systems described in Chapter 1.6, one of which was modified to utilize the parallel digestion approach, were used to conduct the experiment. Each of these systems consisted of a 20 L fermenter operated at a 4 day SRT and 40 L of digester volume operated at a 20 day SRT. In both systems, the 5 L of fermenter effluent was subjected to solids separation, with 3 L of liquid directed to PHA production reactors and the remaining 2 L of solids and residual liquid going to digestion. Solids separation for one of these systems was performed using centrifugation, which retained both coarse and fine solids in the digester feed. In the second system, solids separation was performed using sequential screening and centrifugation, which separated the coarse and fine fractions into separate streams. Sampling for total nitrogen indicated the fine material was enriched in nitrogen in comparison with the fermenter effluent and raw manure (Chapter 3.3.1). Nitrogen is associated with the presence of proteins and biomass, suggesting the possibility the fine material could also be enriched in lipids in comparison with the coarse fraction, which consisted mainly of seeds and plant fibers based on visual inspection. While hydrolysis of simple carbohydrates occurs in the fermenter, the bulk of molecules such as LCFAs, proteins, and complex carbohydrates such as cellulose are hydrolyzed and fermented in the digester (Mahmoud, N., Ph.D thesis, as cited in (Speece 2008)). Given the apparent differences in composition in the fine and coarse material, it was hypothesized that treatment of these two fractions in separate ADs would select for distinct microbial consortia optimized to hydrolyze, ferment, and convert to methane the separated and more specific forms of substrate. An optimized microbial consortium would potentially be capable of carrying out hydrolysis as well as

methanogenesis at an increased rate, as hydrolysis of complex organic material is usually rate limiting in anaerobic digestion (Eastman and Ferguson 1981, Vavilin et al. 2008).

The two phase-separated AD systems used to conduct the comparison are referred to as System 1 and System 2. System 1 (Figure 10) was operated as the experimental control, and consisted of a fermenter (F6-c) and a digester (AD6-c) receiving the centrifuge-separated solids fraction of the fermenter effluent. System 2 (Figure 11) consisted of a fermenter (F3-c) and the parallel digesters AD3-c and AD8. AD3-c received the coarse solids fraction of the fermenter effluent separated by screening, while AD8 received the fine solids fraction separated by sequential screening and centrifugation. Data was collected during an 85 day steady state period lasting from April 1st, 2014 to June 24th, 2014 defined based on stable AD biogas production rates and low AD effluent VFA concentrations. Secondary goals included in-depth solids characterization sampling and comparison of microbial populations between reactors using the quantitative polymerase chain reaction (qPCR) technique in order to better understand the conversion processes occurring in each reactor, and provide possible explanations for differences in performance between the two systems. At the conclusion of the methane production comparison period, the OLR on both systems was increased in steps of 44 g VS steps at two week intervals, and performance was monitored to determine if the organic loading capacity of one system was greater than that of the other.

4.2 Material and Methods

4.2.1 Experimental Design

4.2.1.1 Anaerobic Digesters AD3-c and AD6-c

Digesters AD3-c and AD6-c were operated at a 20-day SRT with active volumes of 30 L and 40 L, respectively, and were fed effluent from fermenters F3-c and F6-c. These fermenters were identical in construction and operation to fermenter F3-b described in Chapter 3.2.1.1. The reactors were constructed from Chem-Tainer 56.8 L (15 gallon) cone bottom HDPE tanks (West Babylon, NY, USA) fitted with a polypropylene lid and sealed using RTV silicone gasket sealant. Sections of 1.9 cm diameter Schedule 40 PVC pipe were extended beneath the liquid surface to allow feeding and insertion of the impeller shaft while maintaining anaerobic

conditions. The digesters were mixed using 6” diameter two-blade impact-resistant plastic propellers driven by Oriental Motor (San Jose, CA, USA) BHF62AT-50 40-watt AC speed control motors connected to 5GN3.6SA reduction gearboxes. The mixing intensity generated was sufficient to homogenize the digester contents, although a floating scum layer approximately 2”-3” thick developed on the surface of AD6-c which resisted breakup and dissipation. The digesters were heated by circulation of hot water from a 15.1 L point-of-use water heater through 15.24 m sections of 0.95 cm diameter copper tubing wrapped around the exterior of the tanks. The cycling frequency of the hot water was dictated by a programmable logic controller (PLC) connected to resistance temperature detector (RTD) probes. The PLC opened a set of solenoid valves in response to a drop in digester temperature below the 35 °C setpoint, initiating circulation of hot water through the tubing for 20 seconds. If the temperature did not return to the setpoint after five minutes, another 20 second flush of hot water through the coils would begin, and this loop would continue until the setpoint was reached. This control scheme maintained the temperature in both digesters at 35 +/- 0.1 °C on average. In order to avoid the need to re-pressurize the headspace after cycling to quantify gas production (the wet tip gas meters employed require pressure equal to about 4” of water to operate; see Chapter 4.2.1.3), as well as prevent exposure of the anaerobic microbial population to oxygen, Tedlar gas sampling bags (Smith Air Sample Supply, Mebane, NC, USA) were spliced into the digester gas exhaust lines to allow the headspace volume to vary during feeding and wasting. Biogas produced was vented through 3/8” Tygon tubing attached to a fitting installed in the top of the tank.

4.2.1.2 Anaerobic Digester AD8

Digester AD8 was designed to treat the fine solids separated from the fermenter effluent via sequential screening and centrifugation, and was operated at a 20-day SRT to match the other two digesters. The required active volume was calculated based on fine solids production by F3-c and the performance data collected using the digesters described in Chapter 3. The average mass of fine solids produced during Aug.-Nov. 2013 was 272 +/- 94 g, with a maximum of 515 g and a minimum of 165 g. The recovered fine material averaged 10.2% TS, 76.0% of which was VS, and the digester was designed to treat 320 g of wet fine

solids per day at average TS/VS concentrations. The upper OLR limit based on the fed batch reactor study was between 3.2 and 4.8 g VS/L*d, with the ideal loading range between 2.4 and 3.2 g VS/L*d (Chapter 3.4). Based on this information, a design OLR of 2.5 g VS/L*d was selected for AD8. Using the anticipated VS production (25 g/d), the OLR, and the SRT, the required digester volume was calculated to be 10 L. At 410 g/d of wet solids production, the resulting OLR would be 3.2 g VS/L*d, at the upper end of the ideal treatment range. If wet solids production was to increase to 500 g/d (i.e., the fine solids would not be diluted with fermenter supernatant) the resulting OLR would be 3.9 g VS/L*d, which is above the upper end of the ideal OLR range, but would probably not result in overloading provided solids production was not sustained at that level. The lower end of the wet solids production range would result in an OLR of 1.7 g VS/L*d, which is less than was applied to any of the fed batch digesters but still within the range of 1.6-4.8 g VS/L*d recommended for AD in Metcalf & Eddy (Tchobanoglous et al. 2003).

A square 18.9 L (5 gallon) medium density polyethylene (MDPE) tank with a sloped bottom and 8” diameter lid (Den Hartog Industries, Hospers, IA, USA) was selected for the reactor body so the impeller draft tube height would be sufficient to operate a wet tip gas meter, and the large headspace volume would prevent foaming from interfering with the gas exhaust line if it were to occur. Feeding was accomplished manually through a 3/4” Schedule 40 PVC pipe inserted through a bulkhead fitting on the top of the tank and extending below the liquid surface. Mixing was provided by a 4” diameter three-blade stainless steel propeller fixed to a 3/8” diameter, 20” long stainless steel shaft. The shaft extended through a 3/4” Schedule 40 PVC draft tube submerged below the liquid surface in the digester, and was attached to an Oriental Motor USM315-401 15-watt AC speed control motor using a 36N5SA reduction gearbox (Oriental Motor, San Jose, CA, USA). The mixing speed was constant and the intensity was sufficient to cause complete turnover of the reactor contents. The digester was heated using two 5” x 10” silicon rubber heaters rated at 120V/63W (Watlow Controls, St. Louis, MO, USA). Heater cycling was controlled with a proportional/integral/derivative (PID) controller (Red Lion Controls, York, PA, USA) connected to a R385 RTD temperature probe installed in the side of the digester. The controller maintained digester temperature at

35.0 °C except during feeding/wasting. Temperature dropped to ~34 °C immediately after feeding, and could rise as high as 36 °C after feeding due to controller overshoot, but typically stabilized at 35.0 °C within one hour. Wasting was accomplished using a manually operated ball valve installed in the bottom of the tank. Digester gas was vented through 3/8" diameter Tygon tubing attached to an adapter installed on the top of the tank. A Tedlar gas sampling bag (Smith Air Sample Supply, Mebane, NC) was installed in the gas exhaust line to allow the headspace volume to fluctuate during feeding and wasting. All fittings installed in the tank were sealed using RTV silicone to ensure the reactor remained gastight. The reactor was mounted in a support stand and covered with 1.5" foam insulating panels to minimize heat loss, ensure a homogenous temperature distribution within the reactor, and minimize the amount of time the heaters were switched on. AD8 operations commenced January 2014 using the contents of the three fed batch reactors used in the fed batch experiment described in Chapter 3 and a mixture of fine solids and liquid fermenter effluent.

4.2.1.3 Operational Approach

Raw dairy manure collected from the University of Idaho dairy was fed to fermenters F3-c and F6-c in the same manner as used with fermenter F3-b described in Chapter 3.2.1.3. The five liters of effluent produced daily from F6-c was centrifuged at 8000 rpm for five minutes at room temperature (22-25 °C) to separate the liquid and solid fractions. Feed for AD6-c was batched using the separated solids and sufficient liquid to make two liters of feed. Fine and coarse solids were separated in the F3-c effluent by sequential screening and centrifugation as described in Chapter 3.2.1.3. The feed for digester AD3-c was batched using the solids retained on the strainer along with sufficient centrifuged liquid to make 1.5 L of feed, while the feed for AD8 was batched using the fine solids and sufficient centrifuged liquid to make 0.5 L of feed. The remaining liquid effluent from both F3-c and F6-c was diverted to PHA production experiments or discarded. The OLR of AD8 was not fixed, and fluctuated in response to the mass of fine solids produced by F3-c.

Diversion of the entire VFA-rich liquid fraction to PHA production, while digester feed is batched using water or a VFA-depleted recycle from the PHA process, could be desirable

from the standpoint of maximizing PHA production, but was found to be impractical for AD. Specifically, the tap water dilution approach to preparing digester feed was attempted for a two week period prior to the start of data collection, and caused effluent VFA concentrations from AD3-c and AD6-c to increase from trace levels to several hundred milligrams per liter. This response was likely due to washout of the acetoclastic methanogenic population caused by lack of readily available substrate, as VFA concentrations decreased once feed dilution using liquid fermenter effluent resumed. The presence of VFAs in the digester feed appears to enhance process stability by providing a food source that can be utilized immediately, while the slowly biodegradable residual material from the fermenter is hydrolyzed and fermented to produce VFAs that can be utilized later in the operational cycle. Data collection did not begin until VFA production by the fermenters was stable, biogas production by all digesters was stable, and total effluent VFA concentrations from all digesters were consistently less than 200 mg/L as COD.

In summary, System 1 (F6-c coupled to AD6-c, Figure 10) acted as the experimental control against System 2 (F3-c coupled to AD3-c and AD8, Figure 11) to establish if separate digestion of fine and course material present in the fermenter effluent would produce more methane than the conventional approach of digestion of all solids in a single reactor by selecting for a microbial consortium optimized to degrade each solids fraction. The same loading rate was applied to both systems (175 g VS/d), and the volumes of both systems were identical (20 L fermenter, 40 L digester, 60 L total). The SRT applied to both systems was the same also (4 days fermenter, 20 days digester, 24 days total). Thus, the only factor that varied between the systems was the approach to digestion.

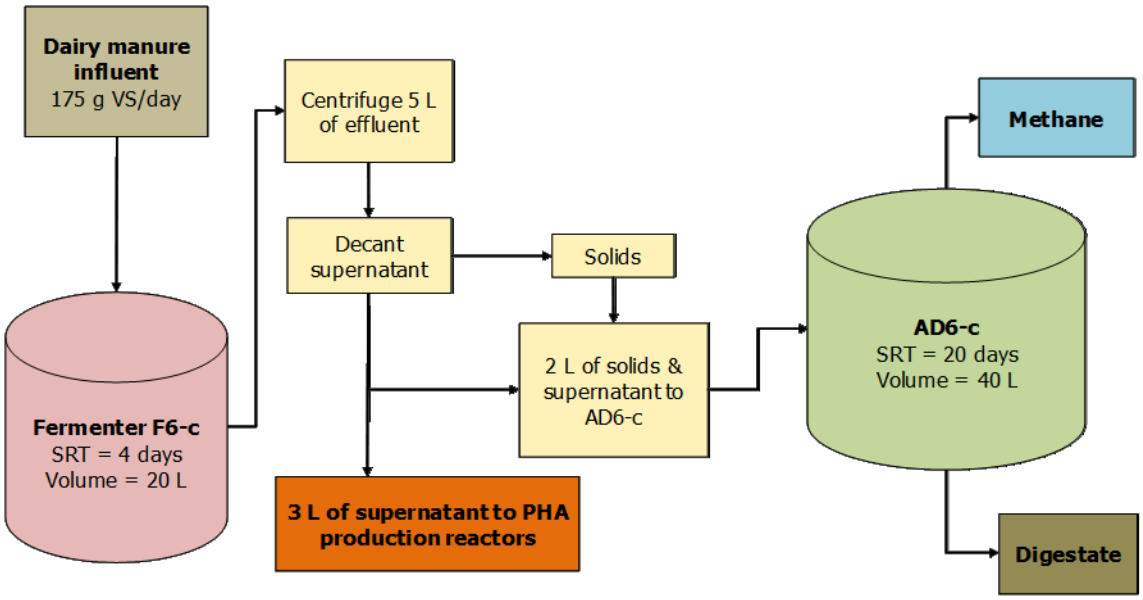

Figure 10. AD System 1 process flow chart (conventional two-phase AD).

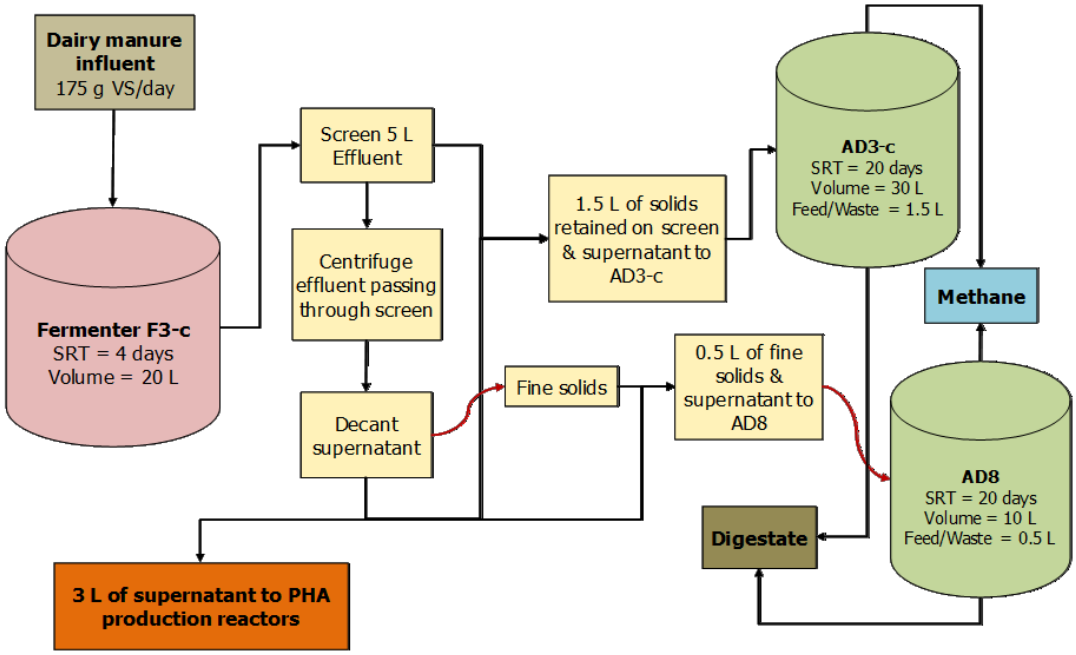

Figure 11. AD System 2 process flow chart (parallel two-phase AD).

4.2.2 Analytical Techniques

Digester gas production was quantified using wet tip gas meters (Wet Tip Gas Meter Co., Nashville, TN, USA). One meter was attached to each digester, although during the latter 48 days of the 85 day experiment the gas exhaust lines of AD3-c and AD8 were spliced together and the combined production was measured by a single meter. The meters were calibrated by connection to an Aera PI-98 mass flow controller (Hitachi Metals America Ltd., Purchase, NY, USA) dispensing air at 20 mL/min. The meters were connected to data loggers which recorded the time at which the meter released accumulated gas from one cycle (between 80 and 110 mL). Calibration data was recorded for 2-4 days for each meter depending on the degree of variability in cycle volume. In this manner both the average volume of gas measured per cycle by each meter could be established, as well as the uncertainty in the average cycle volume. The uncertainty varied somewhat between meters, and ranged from 1% to 3% of the mean cycle volume.

Methane, carbon dioxide, and nitrogen content of the digester gas were quantified daily by gas chromatography using the method described in Chapter 3.2.2. Methane, carbon dioxide, and nitrogen were quantified by matching with known standards. The solubility of carbon dioxide in water is sensitive to temperature, and sampling was avoided during periods when the digester temperature was not at the setpoint (e.g., soon after feeding) to ensure the sample was representative of the average biogas composition. The volume of carbon dioxide and methane produced daily was estimated by reducing the volume of digester gas recorded daily by the gas meter by the percentage of compounds other than methane and carbon dioxide present in the daily chromatography sample (primarily water vapor and nitrogen, along with trace quantities of hydrogen sulfide, ammonia, hydrogen, and volatile organic compounds). This volume was adjusted to standard temperature and pressure (STP) using the average temperature and barometric pressure in the lab and the ideal gas law. Temperature and pressure data was collected using an Extech® RHT50 humidity/temperature/pressure data logger (Extech Instruments, Nashua, NH, USA). Methane and carbon dioxide volumes at STP were calculated using the following equations:

$$V_{CH_4} = \left(V_{gas}\right)\left(\frac{V_{CH_{4,GC}}}{V_{CH_{4,GC}} + V_{CO_{2,GC}}}\right)$$

$$V_{CO_2} = V_{gas} - V_{CH_4}$$

V_{CH_4} = volume of methane produced

V_{CO_2} = volume of carbon dioxide produced

V_{gas} = volume of methane and carbon dioxide produced daily

$V_{CH_{4,GC}}$ = volume of methane in 0.6 mL daily gas chromatography sample

$V_{CO_{2,GC}}$ = volume of carbon dioxide in 0.6 mL daily gas chromatography sample

On days when no chromatography sample was available, the average methane content of the four previous daily samples was used to calculate methane production.

Samples collected for quantification of soluble constituents were centrifuged at 15,000 rpm for 20 minutes and the supernatant was passed through a 0.22 micron PVDF syringe filter (Millipore Corp., Billerica, MA, USA). VFAs were quantified as described in 3.2.2, with the exception that samples were undiluted and consisted of 1 mL of sample and 80 to 100 µL of 2.0 N HNO_3 for acidification, depending on sample alkalinity. Total and volatile solids were quantified in accordance with Standard Methods 2540G as described in Chapter 2.2.2, and care was taken to ensure the material to be sampled was completely mixed prior to sample collection. A Thermo Fisher Scientific Accumet AP85 Waterproof pH/Conductivity Meter (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure pH, and was calibrated on a monthly basis.

Soluble reactive phosphorus (SRP) was determined in accordance with Hach (Loveland, CO, USA) method 8048 (equivalent to Standard Methods 4500-PE (Clesceri 1998)), while ammonia testing followed Hach method 10031. A Spectronic® 20 Genesys™ spectrophotometer (Thermo-Fisher Scientific Corp, Waltham, MA, USA) was used to measure the absorbance of the reacted sample at a wavelength of 890 nm for SRP and 655

nm for NH_3. Phosphate and NH_3-N concentrations were determined using a standard curve ($R^2 > 0.99$).

4.2.2.1 Manure, Fermenter, and Digester Solids Characterization Analysis

Samples were collected once during the analysis period for quantification of total carbon (TC) and total nitrogen (TN) content by the University of Idaho Analytical Sciences Laboratory (Nelson and Sommers 1982). Samples were collected on two occasions during the analysis period and shipped to the Dairy One Forage Laboratory (Ithaca, New York, USA) for quantification of acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignins (ADL), starch, crude fat, total phosphorus (TP), and crude protein. The methods and equipment used by the Dairy One laboratory are available at the following web address:

http://dairyone.com/wp-content/uploads/2014/02/Forage-Lab-Analytical-Procedures.pdf

or may be obtained by contacting the lab directly. The methods of analysis used are briefly summarized below:

ADF: Solutions are as in AOAC 973.18 – Fiber (Acid Detergent) and Lignin (H_2SO_4) in Animal Feed (Latimer 2012) using ANKOM Technology Method 5 and an ANKOM A200 digestion unit. A solution of cationic detergent and 0.5 M H_2SO_4 is used to remove most carbohydrates, proteins, and fats, with the residue consisting of cellulose, lignin, and insoluble proteins.

NDF: Solutions are as in Van Soest (Van Soest et al. 1991) using ANKOM Technology Method 6 and an ANKOM A200 digestion unit. The sample is treated with a neutral detergent solution and α-amylase enzyme, leaving behind cellulose, hemicellulose, and lignin.

ADL: Solution as in AOAC 973.18 – Fiber (Acid Detergent) and Lignin (H_2SO_4) in Animal Feed (Latimer 2012). Analysis is performed on residue of the ADF method using ANKOM Method 9 after digestion in 72% H_2SO_4 for three hours in a Daisy Incubator. ADL is defined as the residue remaining after removal of the acid-soluble material.

Starch: Analysis is performed using an YSI 2700 SELECT Biochemistry Analyzer. Starches present in samples are hydrolyzed to dextrose, which is quantified by the analyzer. Starch is calculated as 90% of the measured dextrose content.

Crude Fat: Determined in accordance with AOAC 2003.05 – Crude Fat in Feeds, Cereal Grains, and Forages (Latimer 2012). The procedure uses anhydrous diethyl ether as the solvent in a Soxtec HT6 System, with crude fat residue determined gravimetrically after evaporation of the solvent.

TP: Determined using a Thermo ICAP 6300 Inductively Coupled Plasma (ICP) Radial Spectrometer after microwave accelerated digestion in HCl and H_2O_2.

Crude Protein: Determined in accordance with AOAC 990.03—Protein (Crude) in Animal Feed (Latimer 2012), using a Leco FP-528 Nitrogen/Protein Analyzer. The sample is combusted in pure oxygen, and total nitrogen is quantified in the gas produced using a thermal conductivity detector. Crude protein is calculated as % total nitrogen (w/w) * 6.25.

The results of the analysis were used to estimate cellulose (ADF - ADL), hemicellulose (NDF - ADF), and total carbohydrate (NDF + starch) content.

4.2.2.2 Comparative Analysis of Microbial Populations

Microbial population analyses were conducted by the University of Idaho Civil Engineering Environmental Laboratory using the quantitative real-time polymerase chain reaction (qPCR) method and 16S rDNA-based oligonucleotide primers following an approach similar to that described in detail in Coats (Coats et al. 2012). Biomass samples were collected on three occasions from all digesters and once from the fermenters and raw manure. Genomic DNA was extracted from each sample using the MO BIO PowerSoil® DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA USA). 16S rDNA-based oligonucleotide primers were used to target the three principle orders of hydrogenotrophic methanogens (*Methanococcales (MCC), Methanobacteriales (MBT),* and *Methanomicrobiales (MMB)*), the two families of acetoclastic methanogens (*Methanosarcinaceae (MSC)* and *Methanosaetaceae (MST))*, and all members of the domains Archaea and Bacteria (Table 5).

Table 5. Primers and genetic sequences used in qPCR analysis.

Group	Primer	Sequence	Reference
Methanococcales	MCC 495F	TAA GGG CTG GGC AAG T	(Lee et al. 2009, Yu et al. 2005)
	MCC 832R	CAC CTA GTY CGC ARA GTT TA	
Methanobacteriales	MBT 857F	CGW AGG GAA GCT GTT AAG T	
	MBT 1196R	TAC CGT CGT CCA CTC CTT	
Methanomicrobiales	MMB 282F	ATC GRT ACG GGT TGT GGG	
	MMB 832R	CAC CTA ACG CRC ATH GTT TAC	
Methanosarcinaceae	MSC 492F	GAA ACC GYG ATA AGG GGA	
	MSC 828R	TAG CGA RCA TCG TTT ACG	
Methanosaetaceae	MST 702F	TAA TCC TYG ARG GAC CAC CA	
	MST 862R	CCT ACG GCA CCR ACM AC	
All members of *Archae*	ARC 349F	GYG CAS CAG KCG MGA AW	(Takai and Horikoshi 2000)
	ARC 806R	GGA CTA CVS GGG TAT CTA AT	
All members of *Bacteria*	BAC 338F	ACT CCT ACG GGA GGC AGC AG	(Huse et al. 2008)
	BAC 515R	TTA CCG CGG CTG CTG GCA C	

qPCR was performed on a StepOne Plus™ Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) using iTaq™ SYBR® Green Supermix with ROX (Bio-RAD Laboratories Inc., Hercules, CA, USA) with a total reaction volume of 25 µL. The qPCR process was performed under the following conditions: 3 min at 95 °C, 45 cycles of 30 s at 95 °C, 45 s annealing (annealing temperature varied from 55 to 59 °C depending on the primer set), and 30 s at 72 °C. Samples were processed in quadruplicate (digester samples) or in duplicate (fermenter and manure samples) using 5 ng of total genomic DNA quantified using a BioTek H1 hybrid multi-mode plate reader (BioTek, Winooski, VT, USA) and a 500 nM final concentration of each primer per reaction. Annealing temperatures and primer concentrations were determined through an optimization process. All qPCR melting curves were evaluated to confirm a single melting peak, and agarose gel analysis confirmed one signal for each primer set. Amplification efficiency was calculated for each primer set using baseline-corrected fluorescence data (StepOne software v2.0) and the LinRegPCR program (Ramakers et al. 2003). The cycle threshold was set at a constant value within the log-linear region across all samples for determination of quantification cycle (Cq) values, i.e., the cycle number at which the fluorescence value exceeded the threshold value. The average amplification efficiency

across all samples and primers was 1.79, which is comparable to that observed by Yu et al. (Yu et al. 2006). Comparisons of target gene quantities between samples were made by calculation of a relative expression ratio (RER) following an approach similar to that described by Cikos and Koppel (Cikos and Koppel 2009). The calculation is derived from the general qPCR equation

$$X_n = X_0 * (E + 1)^n$$

where X_n is the amount of PCR product at the end of cycle n, X_0 is the amount of PCR template, and E is the PCR amplification efficiency ranging from 0 (no product is produced) to 2 (doubling of product in every amplification cycle). The intensity of the fluorescence produced is assumed to be directly proportional to the amount of PCR product present, and provided the cycle threshold is set to a constant value for all samples, the same quantity of product will be present at the end of the amplification process for both samples being compared. This allows a RER to be calculated between samples from two reactors by writing

$$\frac{X_{n(A)}}{X_{n(B)}} = \frac{X_{0(A)} * (E(A)+1)^{Cq_{(A)}}}{X_{0(B)} * (E(B)+1)^{Cq_{(B)}}}$$

where *(A)* and *(B)* refer to each of the samples. As the amount of PCR product at the end of the process is the same for both samples, the term $\frac{X_{n(A)}}{X_{n(B)}} = 1$, and the equation can be rearranged to solve for $\frac{X_{0(A)}}{X_{0(B)}}$, the ratio of the quantity of target genetic material present in sample A and sample B:

$$\frac{X_{0(A)}}{X_{0(B)}} = \frac{(E(B) + 1)^{Cq_{(B)}}}{(E(A) + 1)^{Cq_{(A)}}} = RER$$

This approach assumes the 16S rDNA gene copy numbers for the groups being compared are identical in sample A and sample B, and that multiple copies are not present.

4.2.3 Statistical Methods

Single factor or two-factor ANOVA was used to establish differences in means using Microsoft Excel, with significance declared at $p < 0.05$. When calculating the value of

parameters involving average values of data sets (e.g., methane yield, VS reduction, mass balances, etc.), the National Institute of Standards Engineering Statistics Handbook recommends the use of one of two approaches for estimation of the standard deviation in the resulting parameter: the "top-down method" and the formal propagation of error approach (Croarkin and Tobias 2013). The top-down method is the preferred approach and was used when the sizes of the data sets of interest were similar. The value of the parameter is calculated for every set of measurements, and the standard deviation in the parameter is found using the results. Propagation of error formulas were used when the data sets being combined were not of similar sizes (Ku 1966). When adding or subtracting the means of two data sets, the appropriate equation for the standard deviation of the result is

$$\sigma_x = \sqrt{{\sigma_a}^2 + {\sigma_b}^2}$$

where σ_x is the standard deviation of the sum or difference of means, and σ_a and σ_b are the standard deviations of the two data sets a and b being combined. This approach assumes the covariance between the two sets is zero. Similarly, when finding the standard deviation σ_x of the mean product or quotient x of sets a and b, the equation is

$$\frac{\sigma_x}{x} = \sqrt{\left(\frac{\sigma_a}{\bar{x}_a}\right)^2 + \left(\frac{\sigma_b}{\bar{x}_b}\right)^2}$$

where $\bar{x}_a$ and $\bar{x}_b$ refer to the means of sets a and b. Expressions for the standard deviation of parameters involving both addition/subtraction and multiplication/division (e.g. methane yield) can be derived through combination of these equations.

4.3 Results

4.3.1 Methane Production, Yield, and Volatile Solids Reduction

Methane content in the biogas over the analysis period was stable for all three digesters individually and for the combined system measurements (Table 6). From day 38 to day 85 the biogas exhaust lines of reactors AD3-c and AD8 were spliced together and biogas production from both digesters was measured using a single meter, which also allowed

direct measurement of the composition of the System 2 biogas. During this period the average methane content of AD6-c was 61.3%, while that of System 2 was 59.9%, a statistically significant difference ($p = 6.46*10^{-22}$).

Table 6. Mean biogas production, composition and methane yield. Confidence intervals represent one standard deviation. Values shown for AD3-c and AD8 are based on the first 37 days of data collection.

Reactor	Biogas (L/d @ STP)	Methane Content (%)	Methane (L/d @ STP)
AD3-c	28.5 +/- 1.9 (n = 36)	57.1% +/- 0.7% (n= 30)	16.3 +/- 1.0 (n = 36)
AD8	9.7 +/- 1.2 (n = 35)	64.4% +/- 1.0% (n = 30)	6.3 +/- 0.8 (n = 35)
AD6-c (System 1)	37.2 +/- 2.4 (n = 76)	60.9% +/- 1.2% (n = 71)	22.7 +/- 1.6 (n = 76)
AD3-c + AD8 (System 2)	35.0 +/- 3.2 (n = 83)	59.9% +/- 0.6% (n= 41)	20.8 +/- 1.8 (n = 82)

Midway through the experiment (specifically on Day 39), statistical analysis of fermenter effluent VFA concentrations indicated there was a significant difference between F3-c and F6-c. As the fermenters were fed equal masses of manure from the same batch, it is possible the difference in VFA concentrations was due to unequal hydrolysis rates of simple carbohydrates associated with differences in the makeup of the microbial populations of the two fermenters. The ultimate degree of hydrolysis occurring in the AD process is limited in the digestion phase by the presence of recalcitrant compounds such as lipids and proteins (Speece 2008). Consequently, the degree of hydrolysis of readily biodegradable compounds (e.g. sugars or starch) occurring in the fermenter of a two-phase AD system will not impact the quantity of methane produced because any residual readily biodegradable compounds present in the fermenter effluent will be degraded in the digester. Alternatively the difference could have been caused by greater removal of VFAs by a larger population of acetoclastic methanogens or sulfate-reducing bacteria in one of the fermenters, which would have reduced the amount of substrate entering the digester and the methane yield. In order to ensure the difference in fermenter VFA yield did not impact digester performance, the effluent from the fermenters was switched to the opposite digester, i.e. F3-c effluent was used to make AD6-c feed and vice versa.

Two-factor ANOVA was performed using those sections of the dataset where methane production data was available for both reactors (days for which data was missing or available

for only one reactor were excluded) to compare both methane production and the effect of switching the fermenters. In addition, the length of the dataset was truncated so that an equal number of points from prior to and after the switch were included due to the requirements of the ANOVA procedure. The fermenter used to batch the feed had no effect on methane production (p=0.95), and average methane production was significantly higher from System 1 (p=6.82*10^{-6}), indicating the separated digestion approach is detrimental to methane production. This result was confirmed using single factor ANOVA analysis of the entire methane production data set (p = 5.33*10^{-11}).

Table 7. Data used in ANOVA analysis of CH_4 production.

Two-factor ANOVA			
	Count	**Average**	**Variance**
System 2	60	20.85	3.61
System 1	60	22.64	2.88
Single factor ANOVA			
System 2	82	20.82	3.07
System 1	76	22.69	2.49

Daily average methane production from System 1 was higher than that of System 2 (Table 6, Figure 12). Methane production volumes from both systems were stable considering raw manure, an inherently heterogeneous substance, was used as the substrate. Similar variability has been observed in AD research involving dairy manure at both pilot scale (Rico et al. 2011) and lab scale (Demirer and Chen 2005), and would be observed at full scale as well. Methane yields are a function of a range of factors, including the composition of the substrate, reactor configuration, and mixing effectiveness, and a range of values have been reported in the literature. Yields of 0.075-0.223 L CH_4/g VS applied were observed by Ogejo and Li (Ogejo and Li 2010) from dairy manure, and Hawkes (Hawkes et al. 1984) reported yields of 0.166-0.204 L CH_4/g VS applied at SRTs from 5-15 days from screened dairy manure. Using the L CH_4/L active reactor volume basis, Dugba and Zhang (Dugba and Zhang 1999) reported a yield of 0.82 from screened dairy manure for a two-stage temperature-phased thermophilic-mesophilic AD system, while Wen et al. (Wen et al. 2007) reported yields of

0.12-0.57 for a mesophilic system consisting of four CSTRs in series using liquid dairy manure of three different strengths. The International Panel on Climate Change (IPCC) reports an average value for the ultimate methane production potential of dairy manure (i.e., the value that would be generated by a BMP test) of 0.240 L CH_4/g VS applied (IPCC 1997). The digestion system examined in this research is intended to treat residual organic material remaining after the diversion of much of the soluble carbon to PHA production. Consequently, the yields of both systems normalized to the VS mass applied to the fermenters (i.e., the mass of VS in the raw manure entering the system and available for combined PHA and CH_4 production) fall into the lower end of the range of yields per gram VS applied reported in the literature due to diversion of VFAs (Table 8), at about 50% of the IPCC ultimate yield value.

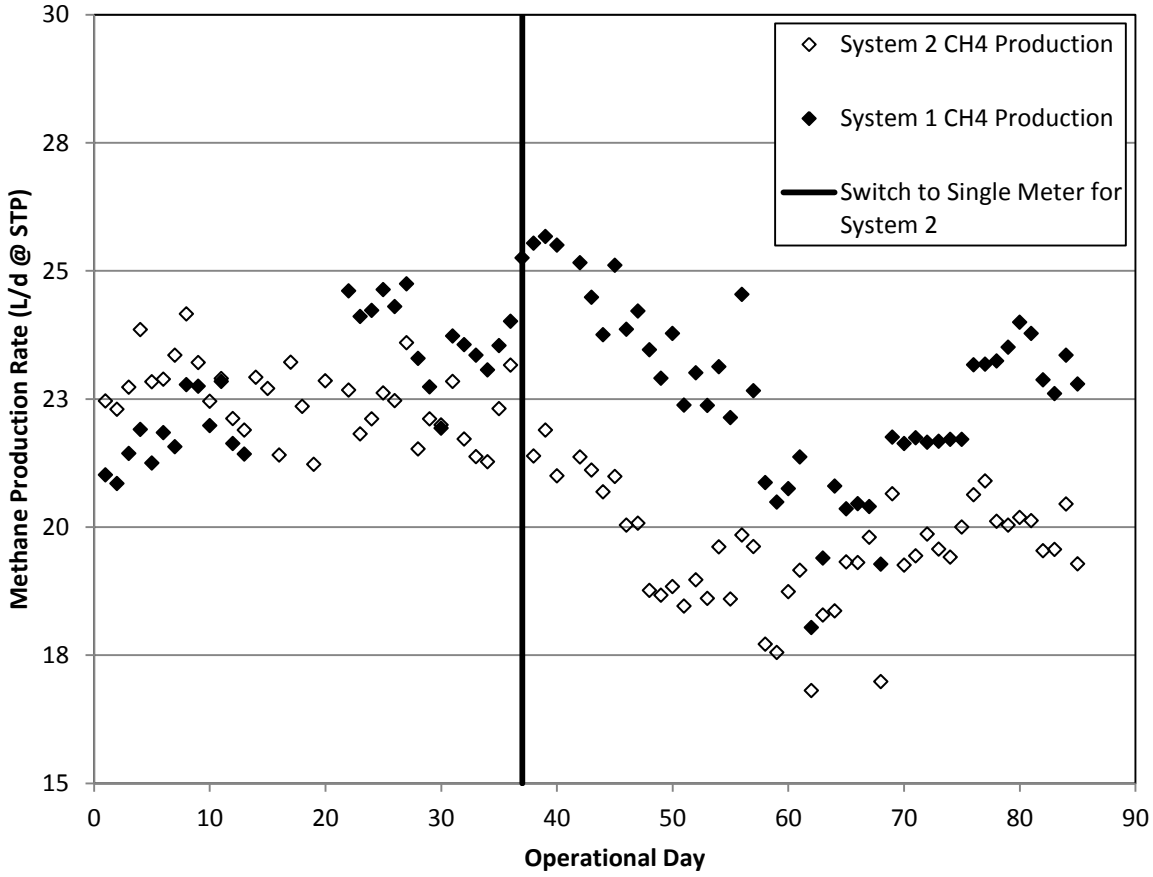

Figure 12. Daily methane production over the analysis period for System 1 (AD6-c) and System 2 (AD3-c + AD8 combined).

Table 8. Methane production normalized to grams VS applied, g VS destroyed, and liters of active digester volume. Values shown for AD3-c and AD8 are based on the first 37 days of data collection. Confidence intervals represent one standard deviation.

Reactor	L CH_4/g VS applied to digester	L CH_4/g VS applied to fermenter	L CH_4/g VS destroyed	L CH_4/L active volume
AD3-c	0.17 +/- 0.01	N/A	0.55 +/- 0.08	0.69 +/- 0.06
AD8	0.22 +/- 0.05	N/A	0.55 +/- 0.24	0.63 +/- 0.08
AD6-c (System 1)	0.18 +/- 0.02	0.12 +/- 0.01	0.52 +/- 0.08	0.57 +/- 0.16
AD3-c + AD8 (System 2)	0.17 +/- 0.02	0.12 +/- 0.01	0.54 +/- 0.11	0.52 +/- 0.18

If production is normalized to the VS mass actually applied to each digester (Table 8), the values are comparatively in the higher end of those typically reported, indicating effective digester performance. Digester yields on a unit active volume basis were comparable or higher than those reported in the literature, although this parameter is of limited utility because it does not account for differences in solids loading between systems. Ultimately, comparison of methane yields between data sets generated by other investigators is complicated by the fact biogas measurements in AD research are frequently not reported in units of standard temperature and pressure (STP), and it is usually unclear if the reported production volumes were adjusted to reflect the presence of water vapor (both corrections were made in this study).

VS reduction averaged 14% to 17% in the fermenters (Table 9), and was subject to substantial uncertainty associated with variability in the composition of the raw manure. Some of the reduction was due to methane production; the methane content of biogas produced by F3-c averaged 45.8% +/- 2.4% (n = 10), while that of F6-c averaged 42.9% +/- 1.46% (n = 5). The volume of gas produced by fermenters F3-c and F6-c was not measured during this experiment. However, the volume of biogas produced by a similar 15 L fermenter used to generate VFAs for PHA production experiments in the lab during the OLR comparison (Chapter 4.3.6) and operated at the same OLR and SRT as used for F3-c and F6-c was measured for a 15 day period. Assuming a 20 L fermenter would produce a directly proportional quantity of biogas, F3-c and F6-c would have produced about 7.4 L/d of biogas, or 3.3 L/d of methane at the average methane concentration at STP. These volumes may be

underestimated as the fermenter was not originally designed to facilitate capture and measurement of biogas, and was impossible to seal reliably. The estimated methane production is about 15% of the methane volume produced by the digesters, indicating at full scale it would be desirable to collect the gas produced by the fermenters in addition to that produced by the digesters.

Table 9. Average organic loading rate and VS destruction in each reactor. Confidence intervals represent one standard deviation.

Reactor	OLR (g VS/L*d)	VS Destruction (%)
F3-c	9.0 +/- 0.7 (n = 16)	14.0% +/- 7.1% (n = 16)
F6-c	9.1 +/- 0.7 (n = 16)	16.8% +/- 5.6% (n = 16)
AD3-c	3.1 +/- 0.2 (n = 16)	30.4% +/- 4.2% (n = 15)
AD8	2.8 +/- 0.4 (n = 16)	34.2% +/- 9.0% (n = 16)
AD6-c (System 1)	3.1 +/- 0.2 (n = 16)	34.9% +/- 3.4% (n = 14)
AD3-c + AD8 (System 2)	3.1 +/- 0.2 (n = 16)	31.6% +/- 4.2% (n = 15)

Care was taken to ensure the OLRs applied to each system were similar so that differences in loading did not influence the results. The OLRs of the digesters were not regulated directly, and were instead controlled by the loading applied to, and the reactions occurring within, the fermenters. Moreover, the production of fine solids by F3-c was variable, causing the loading rate of AD8 to fluctuate more than that of AD3-c and AD6-c (Figure 13), but there was no statistical difference between the mass of VS applied to the two digestion systems or to their respective fermenters (Table 10). The observed VS destruction in System 1 was statistically higher than that of System 2 (p = 0.02), which is in agreement with the higher average daily methane production volume observed in AD6-c (Table 6). Given that the organic loading applied to both systems was the same, these results indicate separation of the fine solids did not improve VS reduction, and in fact appears to inhibit it. If VS reduction is compared between individual digesters, AD8 and AD6-c exhibited similar reduction (approximately 34%, Table 9), while that of AD3-c was only 30.4%, indicating the biodegradable component of the coarse solids fraction was smaller than that of the fine solids fraction.

Table 10. Average solids characteristics and pH in feed and effluent of each reactor. Confidence intervals represent one standard deviation.

Reactor	TS (%)	VS (% of TS)	Total VS mass into or out of reactor per day (g)	pH
F3-c Influent	15.7% +/- 1.5% (n = 16)	83.0% +/- 1.4% (n = 16)	179.3 +/- 13.0 (n = 16)	7.4 +/- 0.6 (n = 16)
F6-c Influent	15.9% +/- 1.6% (n = 16)	83.1% +/- 1.4% (n = 16)	182.1 +/- 13.1 (n = 16)	7.4 +/- 0.4 (n = 16)
F3-c Effluent	3.7% +/- 0.3% (n = 16)	81.4% +/- 1.3% (n = 16)	153.5 +/- 8.8 (n = 16)	6.7 +/- 0.1 (n = 16)
F6-c Effluent	3.6% +/- 0.2% (n = 16)	81.2% +/- 1.1% (n = 16)	151.1 +/- 6.8 (n = 16)	6.6 +/- 0.1 (n = 16)
AD3-c Influent	6.6% +/- 0.3% (n = 16)	88.6% +/- 1.3% (n=16)	94.3 +/- 4.8 (n = 16)	6.8 +/- 0.2 (n = 16)
AD6-c Influent	6.8 % +/- 0.4% (n = 16)	85.7 +/- 1.3% (n = 16)	123.2 +/- 6.4 (n = 16)	6.8 +/- 0.1 (n = 16)
AD8 Influent	7.2% +/- 1.1% (n = 16)	77.3% +/- 1.6% (n =16)	28.1 +/- 4.0 (m = 16)	6.9 +/- 0.1 (n = 15)
AD3-c Effluent	5.0% +/- 0.2% (n= 16)	85.5% +/- 1.0% (n = 16)	65.5 +/- 2.1 (n = 15)	7.3 +/- 0.1 (n = 16)
AD6-c Effluent	4.9% +/- 0.2% (n = 17)	81.2% +/- 1.6% (n = 17)	80.0 +/- 2.3 (n = 14)	7.4 +/- 0.1 (n = 17)
AD8 Effluent	5.2% +/- 0.4% (n = 16)	70.5% +/- 1.4% (n = 16)	18.2 +/- 1.1 (n = 16)	7.6 +/- 0.1 (n = 16)

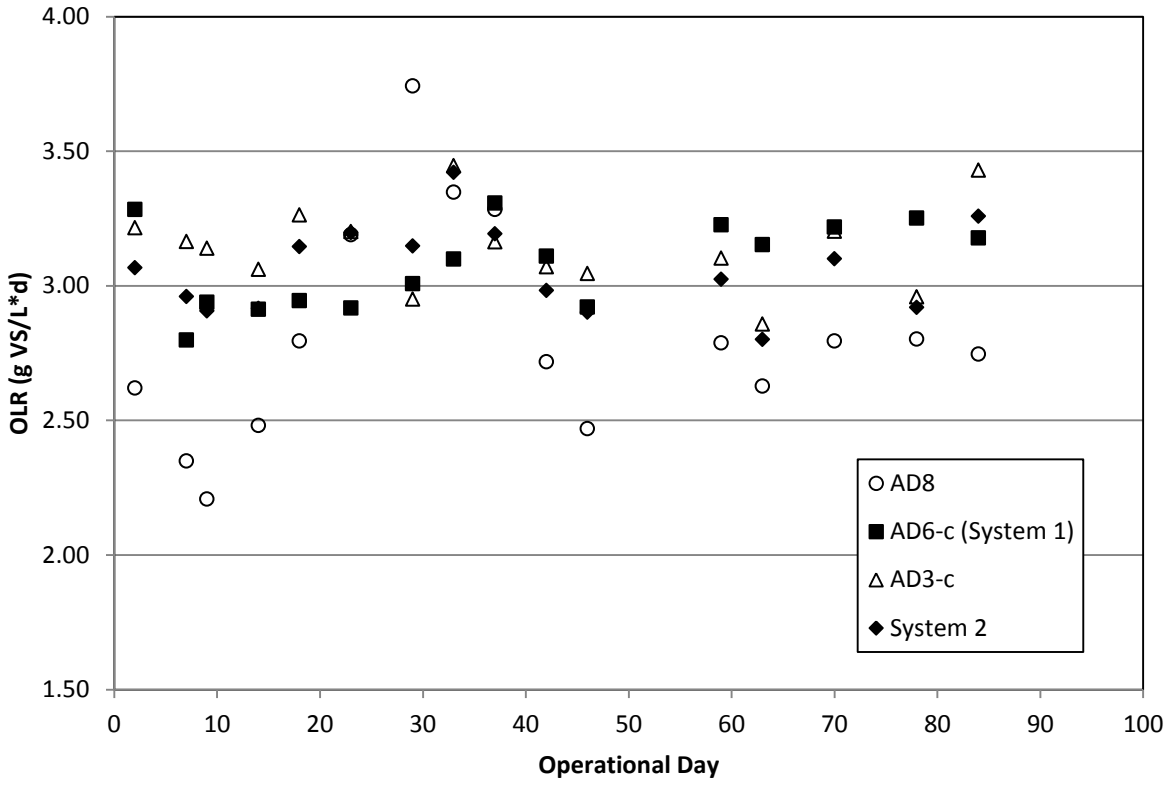

Figure 13. Individual digester and System 2 (AD3-c + AD8) organic loading rates.

4.3.2 VFA Analysis

Average total effluent VFA concentrations were similar (p = 0.075) between the two fermenters. The ratio of average total effluent VFAs to average total influent VFAs was slightly higher in F6-c than in F3-c (2.44 vs 2.26), indicating a slightly higher VFA yield, but this difference did not impact digester performance, as described in Chapter 4.3.1.

Each digester effectively removed essentially all VFAs present in the influent, along with those generated in the digester (Figure 14). AD8 performed the best out of the three digesters, with effluent VFA concentrations verging on non-detectable, while the average effluent VFA concentration of AD3-c was somewhat higher than that of AD6-c, although the difference was not statistically significant (p = 0.25). There was no statistical difference in the average mass of VFAs present in the effluent of the two AD systems (p = 0.86). Considering the similarity in influent and effluent VFA measurements for the two AD systems, it is unlikely the difference in methane production was associated with methanogenesis, and was instead probably due to a limitation in the fermentative processes of hydrolysis and/or acidogenesis in one or both of the System 2 digesters.

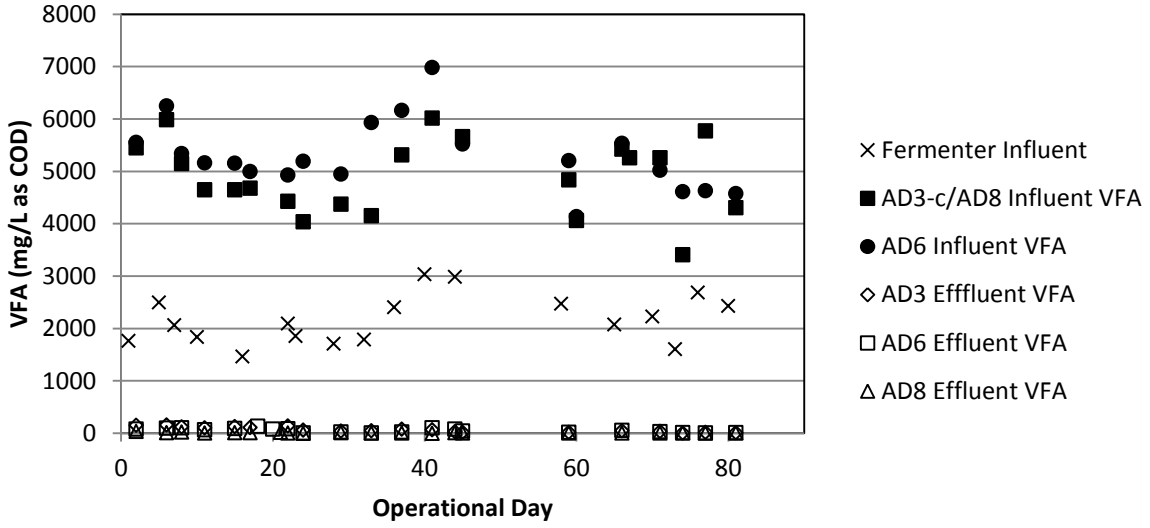

Figure 14. Influent and effluent VFA concentrations of each reactor.

4.3.3 Carbon Mass Balance Analysis

In order to increase the level of confidence in the solids sampling and gas production data, a carbon mass balance was performed on the fermenters and digesters of each system. The mass balance was based on reactor influent/effluent total solids (Table 10) and the carbon dioxide/methane produced by each reactor (Table 6). Total solids measurements were converted to carbon units based on total carbon (TC) analysis of one sample taken during this experiment and two samples taken during the batch reactor experiment of Chapter 3.

Methane and carbon dioxide volumes at STP were converted to carbon units using gas density and molecular weight. As shown in Table 11, the balances on the two digestion systems closed within 5%, indicating good reliability of both the solids sampling and gas production measurements, while the balance on the fermenters closed to ~10%. As the fermenters were not the primary focus of this research, their gas production was not measured directly. The average biogas production and composition produced by the 15 L fermenter, as described in Chapter 4.3.1, was instead used as an estimate of fermenter biogas production in the carbon balance calculations. The raw manure fed to the fermenters was the primary source of variability in VS and TC measurements (Table 10), and this variability in combination with the uncertainty associated with the quantity of biogas produced, as well as losses of VFAs and other volatile compounds during the sample drying process (Chapter 2.2.2), appear to be the primary contributors to the fermenter carbon imbalance.

Table 11. Results of reactor carbon mass balance analysis based on average reactor performance. Confidence intervals represent one standard deviation about the mean. The standard deviation was not calculated for fermenter biogas due to lack of reliable data.

	Reactor			
	F3-c (g C)	**F6-c (g C)**	**AD3-c + AD8 (g C)**	**AD6-c (g C)**
Influent solids	92.2 +/- 12.7	94.2 +/- 13.3	63.2 +/- 3.6	63.3 +/- 4.1
Effluent solids	83.2 +/- 6.5	84 +/- 5.1	42.6 +/- 1.6	40.2 +/- 2.1
CH_4 + CO_2	5.2	5.2	18.8 +/- 2.3	20.0 +/- 1.0
Balance	9.1 +/- 14.3 (10% of influent carbon mass)	10.2 +/- 14.3 (11% of influent carbon mass)	1.7 +/- 4.5 (3% of influent carbon mass)	3.0 +/- 4.7 (5% of influent carbon mass)

4.3.4 Solids Characterization Analysis

Sampling for NDF, ADF, lignin, starch, crude fat, crude protein, and total phosphorus was conducted on two occasions in order to better understand the feedstock and reactor composition. The analysis does not account for the entire mass of dried material in the samples, and it was not possible to demonstrate statistically significant differences in means between reactors for most of the components summarized in Table 12. This was due to the combination of variability between samples and small sample size, both of which increase the sample variance. If the variance associated with two means is high, the difference in means must be very large in order to declare statistical significance at the commonly used 95% confidence level. However, the data provide some insight into the nature of the feedstock and how its composition changes as it moves through each digestion system. Comparisons should be made only between the influent and effluent of the fermenters/digesters due to the diversion of a portion of the fermenter effluent to PHA production.

Table 12. Results of solids characterization analysis. Values represent the average mass contained in the influent/effluent of each reactor plus/minus one standard deviation. Component masses with the exception of those in the fermenter influent are based on the average mass fed/wasted from each reactor during the analysis period. Fermenter influent masses are based on the mass of raw manure used to batch the feed when the samples were taken.

Sample Location (n = 2)	Crude Protein (g)	ADF (g)	NDF (g)	Crude Fat (g)	TP (g)	Lignin (g)	Starch (g)	Cellulose (g)	Hemicellulose (g)	Total Carbohydrates (g)
Fermenter Influent	37.1 +/- 3.2	65.2 +/- 9.1	88.1 +/- 0.8	4.4 +/- 0.3	1.9 +/- 0.1	22.9 +/- 5.7	10.2 +/- 4.3	42.3 +/- 3.5	22.9 +/- 8.3	98.3 +/- 3.5
F3-c Effluent	37.1 +/- 4.9	65.9 +/- 1.1	94.4 +/- 0.1	7.1 +/- 1.4	1.9 +/- 0.2	24.8 +/- 2.9	1.1 +/- 0.8	41.1 +/- 4.0	28.5 +/- 1.0	95.5 +/- 0.9
F6-c Effluent	36.7 +/- 5.9	61.5 +/- 4.3	83.5 +/- 7.1	7.7 +/- 0.7	1.9 +/- 0.2	23.0 +/- 0.1	1.0 +/- 0.6	38.4 +/- 4.2	22.0 +/- 2.8	84.5 +/- 6.5
AD3-c Influent	11.3 +/- 0.4	48.6 +/- 0.7	67.7 +/- 4.2	1.8 +/- 0.1	0.5 +/- 0.0	16.9 +/- 0.9	2.5 +/- 0.2	31.7 +/- 1.6	19.1 +/- 4.9	70.2 +/- 4.0
AD3-c Effluent	9.0 +/- 0.3	39.2 +/- 0.3	46.6 +/- 4.1	0.9 +/- 0.1	0.5 +/- 0.0	16.9 +/- 2.2	0.3 +/- 0.0	22.4 +/- 2.0	7.4 +/- 3.9	47.0 +/- 4.1
AD8 Influent	13.2 +/- 2.4	9.1 +/- 1.6	16.5 +/- 2.5	2.3 +/- 0.9	0.7 +/- 0.2	4.0 +/- 0.5	0.1 +/- 0.1	5.1 +/- 1.1	7.3 +/- 0.9	16.6 +/- 2.4
AD8 Effluent	6.9 +/- 0.5	7.7 +/- 0.4	9.8 +/- 0.2	0.4 +/- 0.4	0.5 +/- 0.0	4.0 +/- 0.3	0.1 +/- 0.0	3.7 +/- 0.1	2.1 +/- 0.2	9.9 +/- 0.2
System 1 Influent	25.3 +/- 2.2	62.5 +/- 7.5	83.9 +/- 5.3	4.1 +/- 1.2	1.2 +/- 0.0	24.7 +/- 5.3	2.1 +/- 1.7	37.8 +/- 2.2	21.4 +/- 12.7	86.0 +/- 3.5
System 2 Effluent	14.7 +/- 0.1	43.0 +/- 1.6	54.1 +/- 7.5	1.2 +/- 0.1	0.9 +/- 0.1	20.2 +/- 0.1	0.3 +/- 0.1	22.8 +/- 1.7	11.1 +/- 5.8	54.4 +/- 7.4
System 2 Influent	24.5 +/- 2.0	57.7 +/- 0.9	84.1 +/- 6.7	4.2 +/- 1.0	1.2 +/- 0.2	20.8 +/- 1.5	2.7 +/- 0.3	36.8 +/- 0.5	26.5 +/- 5.8	86.8 +/- 6.4
System 2 Effluent	16.0 +/- 0.8	46.9 +/- 0.6	56.4 +/- 4.3	1.3 +/- 0.3	1.0 +/- 0.0	20.8 +/- 2.6	0.5 +/- 0.0	26.1 +/- 1.9	9.5 +/- 3.7	56.9 +/- 4.3

Table 13. Average reactor influent/effluent composition on a percent of dry matter basis. Confidence intervals represent one standard deviation.

Sample Location (n = 2)	Crude Protein	ADF	NDF	Crude Fat	TP	Lignin	Starch
Fermenter Influent	17.7% +/- 1.5%	31.1% +/- 4.3%	42.0% +/- 0.4%	2.1% +/- 0.1%	0.9% +/- 0.1%	10.9% +/- 2.7%	4.9% +/- 2.1%
F3-c Effluent	18.7% +/- 3.8%	33.1% +/- 1.9%	47.4% +/- 3.5%	3.6% +/- 1.0%	1.0% +/- 0.1%	12.5% +/- 2.4%	0.6% +/- 0.4%
F6-c Effluent	19.4% +/- 2.3%	32.5% +/- 1.0%	44.1% +/- 2.0%	4.1% +/- 0.2%	1.0% +/- 0.1%	12.2% +/- 0.4%	0.6 +/- 0.4%
AD3-c Influent	10.7% +/- 0.4%	46.0% +/- 0.5%	64.1% +/- 4.2%	1.8% +/- 0.1%	0.5% +/- 0.0%	16.0% +/- 0.9%	2.4% +/- 0.1%
AD3-c Effluent	12.5% +/- 0.9%	54.1% +/- 2.1%	64.1% +/- 2.8%	1.2% +/- 0.1%	0.7% +/- 0.0%	23.2% +/- 2.1%	0.5% +/- 0.1%
AD8 Influent	30.4% +/- 0.1%	20.9% +/- 0.1%	37.9% +/- 1.2%	5.3% +/- 1.1%	1.6% +/- 0.1%	9.2% +/- 0.4%	0.3% +/- 0.3%
AD8 Effluent	26.6% +/- 1.3%	29.4% +/- 2.0%	37.3% +/- 1.4%	1.5% +/-1.6%	2.0% +/- 0.0%	15.3% +/- 1.5%	0.6% +/- 0.1%
AD6-c Influent	17.1% +/- 1.8%	42.1% +/- 4.1%	56.6% +/- 4.8%	2.8% +/- 0.8%	0.8% +/- 0.0%	16.7% +/- 3.2%	1.4% +/- 1.1%
AD6-c Effluent	15.8% +/- 0.8%	46.1% +/- 0.3%	57.9% +/- 5.4%	1.3% +/- 0.2%	1.0% +/- 0.0%	21.7% +/- 1.1%	0.4% +/- 0.1%

The primary component of all samples was NDF (total fiber including cellulose, hemicellulose, and lignin), which would be expected given that the diet of a dairy cow consists of a mixture of plant materials and grains. Lignin is highly resistant to biodegradation, and its presence will limit the ultimate degree of substrate utilization that can be achieved (Lynd et al. 2002). This is reflected in the similar masses of lignin observed in the influent and effluent of each AD system, and between each fermenter and the raw manure (Table 12). The mass of lignin represents about 13% of the 175 g of VS applied to the fermenter, and provides an estimate of the non-biodegradable VS contained in the raw manure. The remaining cellulose and hemicellulose is a source of slowly biodegradable VS, and is degraded primarily in the digesters, as indicated by similar quantities of NDF in the raw manure and fermenter effluent (Table 12). In contrast, effluent NDF masses from both AD systems were lower than the influent masses (Table 12; $p = 0.03$ and $p = 0.04$ for Systems 1 and 2, respectively), and the mass of NDF lost accounts for about 70% of the VS reduction occurring in each system. These results indicate VS reduction in the digesters is primarily due to degradation of cellulose and hemicellulose, with some of the remaining reduction likely associated with the breakdown of proteins and lipids.

Starch is present primarily in the fermenter influent (~6% of the organic load), along with a small amount of crude fat (3% of the organic load). The majority of the starch is likely hydrolyzed in the fermenter, although the apparent difference in effluent and influent masses is not significant at a 95% confidence level ($p = 0.10$ for both fermenters). The mass of crude fat increases ($p = 0.03$) in the F6-c effluent, probably due to cell synthesis, although the difference was not significant ($p = 0.12$) in the F3-c effluent. Starch is reduced to trace levels in the effluent of both digestion systems, while some crude fat, likely associated with cellular material, remains. The influent to AD8 contained elevated percentages of both crude protein and crude fat, while the opposite is observed in the AD3-c influent, which contained elevated levels of NDF relative to AD8. As expected, the crude protein, crude fat, and NDF content of the influent to AD6-c, which includes the material comprising the AD8 feed, fell between that of AD3-c and AD8 (Table 13).

Sampling for ammonium and soluble reactive phosphorus (SRP) was conducted on eight occasions during the analysis period. Ammonium comprised about half the total nitrogen present in the raw manure (Table 14). Some production of ammonium from protein hydrolysis would be expected to occur in the fermenters (Speece 2008), although the apparent difference in average soluble nitrogen content between the fermenter influent and effluent is not statistically significant (p = 0.055 (F3-c) and p = 0.675 F6-c) due to variability in effluent and influent ammonia measurements. Anaerobic digestion releases ammonium during hydrolysis of proteins, and approximately 85% of the total nitrogen in the digestate from the two systems is present as ammonia (Table 14). TN content estimates for the fermenters and AD6-c are based on the average of one sample taken during the analysis period and two samples taken during collection of performance data for digester AD6-b (Appendix A). TN calculations for AD3-c and AD8 are based on one sample taken during the analysis period. TP measurements are based on the values shown in Table 12.

Table 14. Average soluble and total nitrogen and phosphorus summary data. Confidence intervals represent one standard deviation.

	Soluble NH_4-N Concentration (mg/L) (n = 8)	Soluble PO_4-P Concentration (mg/L) (n = 8)	TN Mass (g)	Soluble PO_4-P Mass (g)	NH_4-N/TN Ratio	PO_4-P/TP Ratio
Fermenter Influent	646.9 +/- 143.3	30.9 +/- 16.1	5.7 +/- 0.3	0.15 +/- 0.08	56% +/- 11%	8% +/- 4%
F3-c Effluent	827.9 +/- 174.4	77.0 +/- 44.6	5.5 +/- 0.2	0.38 +/- 0.22	75% +/- 17%	20% +/- 11%
F6-c Effluent	688.9 +/- 214.4	75.1 +/- 43.5	5.0 +/- 0.1	0.38 +/- 0.22	69% +/- 21%	21% +/- 12%
AD3-c Influent	Equal to F3-c effluent		1.5 +/- 0.0	0.12 +/- 0.07	85% +/- 17%	24% +/- 14%
AD3-c Effluent	913.5 +/- 174.2	16.8 +/- 8.8	1.5 +/- 0.0	0.03 +/- 0.01	90% +/- 18%	5% +/- 3%
AD8 Influent	Equal to F3-c effluent		1.6 +/- 0.2	0.04 +/- 0.02	25% +/- 3%	6% +/- 4%
AD8 Effluent	1588.6 +/- 164.8	15.7 +/- 8.0	1.0 +/- 0.1	0.01 +/- 0.00	76% +/- 10%	1% +/- 0%
AD6-c Influent	Equal to F6-c effluent		4.1 +/- 0.2	0.15 +/- 0.09	34% +/- 12%	12% +/- 7%
AD6-c Effluent	1092.9 +/- 257.6	11.3 +/- 3.6	2.5 +/- 0.1	0.02 +/- 0.01	86% +/- 19%	2% +/- 1%
System 2 Influent	Equal to F6-c effluent		3.1 +/- 0.3	0.15 +/- 0.09	53% +/- 8%	14% +/- 9%
System 2 Effluent	Not sampled on a combined basis		2.6 +/- 0.1	0.03 +/- 0.02	84% +/- 14%	3% +/- 2%

In traditional single-stage AD systems, the concentration of SRP is in the range of 100-350 mg/L (Huchzermeier and Tao 2012, Zeng and Li 2006). The presence of SRP in combination with large amounts of available nitrogen makes anaerobic digestate an excellent plant fertilizer. In contrast to typical AD effluent P speciation, the phosphorus present in the effluent from both the System 1 and System 2 digesters of this study was almost entirely in a non-SRP form. SRP was instead observed in the effluent from the fermenters, with subsequent transformation to insoluble phosphorus in the digester effluent (Table 14). The difference in average mass of SRP in the influent vs. the effluent of System 1 and System 2 was statistically significant (p = 0.004 and p = 0.002, respectively). The mildly basic pH and concentration of ammonia in the digesters (Table 10, Table 14) suggest SRP removal through precipitation of struvite ($NH_4MgPO_4 \cdot 6H_2O$), a common nuisance in AD systems, or possibly as calcium phosphates (Huchzermeier and Tao 2012, Speece 2008, Zeng and Li 2006). The solubility of these compounds in water may be relatively high (monocalcium phosphate, which is used as fertilizer) or quite low (tricalcium phosphate). Land application of digestate at rates high enough to satisfy plant nitrogen requirements results in over-application of phosphorus (Harris et al. 2008), which can cause eutrophication if SRP-containing runoff reaches water bodies. A two-phase AD system could potentially help to reduce phosphorus-related water pollution problems associated with land application of digestate by providing more phosphorus in a slowly released form instead of as SRP, as would be the case in a conventional AD system.

4.3.5 Microbial Population Comparisons

Samples for qPCR analysis of the microbial consortia were collected on days 14, 29, and 38 from the three digesters and once on day 14 from the fermenters and the raw manure feed. The relative expression ratio (RER) was calculated for each targeted gene in each reactor comparison combination. The average results of the three sets of samples are shown in (Table 15), and although the standard deviation of some of the results is high, requiring caution in interpretation, some general trends emerge from the data that provide additional insight into the behavior of the two digestion systems.

Table 15. Summary of RER comparisons between reactors. Confidence intervals represent one standard deviation. Sample number = 3 for AD and 1 for fermenters and manure.

Comparison	MCC	MBT	MMB	MSC	MST	ARC	BAC
AD3-c /AD6-c	0.4 +/- 0.2	1.6 +/- 2.0	0.3 +/- 0.2	2.8 +/- 4.4	0.2 +/- 0.1	0.8 +/- 0.5	0.8 +/- 0.9
AD8/AD6-c	2.7 +/- 2.8	2.0 +/- 1.1	2.9 +/- 0.8	0.2 +/- 0.2	5.9 +/- 4.9	2.3 +/- 1.0	1.8 +/- 0.9
AD8/AD3-c	8.2 +/- 5.0	5.3 +/- 5.5	85.5 +/- 106.7	2.1 +/- 3.1	76.0 +/- 72.9	9.7 +/- 9.0	6.6 +/- 4.9
F3-c/F6-c	0.1 +/- 0.0	0.5 +/- 0.2	235.7 +/- 289.6	476.3 +/- 250.7	0.2 +/- 0.1	2.7 +/- 1.2	1.0 +/- 0.4
Manure/F6-c	0.1 +/- 0.0	0.4 +/- 0.5	0.1 +/- 0.0	no data	0.0 +/- 0.1	0.2 +/- 0.2	0.2 +/- 0.2
Manure/F3-c	0.7 +/- 0.7	0.7 +/- 0.7	0.0 +/- 0.0	no data	0.1 +/- 0.1	0.1 +/- 0.1	0.2 +/- 0.2
Summary (Digesters)	8 > 6 > 3	8 > 3 > 6	8 > 6 > 3	inconclusive	8 > 6 > 3	8 > 6 > 3	8 > 6 > 3
Summary (Fermenters and Manure)	6 > 3 > M	6 > 3 > M	3 > 6 > M	3 > 6	6 > 3 > M	3 > 6 > M	3 = 6 > M

The fermenters contained similar quantities of bacteria and differed primarily in the presence of elevated quantities of *Methanosarcina*, *Methanomicrobiales*, and overall archaea in F3-c, while the raw manure contained lower populations of both archaea and bacteria than either of the fermenters. However, the presence of methanogenic organisms in the fermenters and raw manure was not surprising, considering that dairy cows are ruminants.

In comparison with AD6-c and AD3-c, AD8 was enriched in all orders of hydrogenotrophic methanogens targeted, while AD6-c had larger populations of *Methanococcales* and *Methanomicrobiales*. Greater abundance of hydrogenotrophs therefore appears to be correlated with the inclusion of the fine solids material in the digester feed, possibly due to the production of hydrogen through β-oxidation of fats, which are present in the fine solids at elevated levels (Table 13). Larger populations of hydrogenotrophs were correlated with increasing biogas methane content; the methane content of AD8 was greater than that of AD6-c, which was greater than that of AD3. A larger hydrogenotrophic population would be expected to produce a biogas with a higher methane fraction, as these organisms remove carbon dioxide during methane synthesis.

Acetate concentrations in the AD3-c effluent were on average the highest of the three digesters, followed by AD6-c and AD8 (44, 31, and 5 mg/L, respectively). The maximum

specific growth rate μ_{max}, as well as the half-saturation coefficient K_s, are higher in *Methanosarcina* than in *Methanosaeta* (Figure 3). Consequently, the presence of *Methanosarcina* tends to correlate with higher digester effluent acetate concentrations, while *Methanosaeta* is more likely to be found in digesters operated at lower acetate concentrations (De Vrieze et al. 2012). This relationship was generally reflected by the qPCR data, with AD8 and AD6-c dominated by *Methanosaeta* compared to AD3-c. AD8 had the largest archaeal population overall, as might be expected based on the low VFA concentrations in that reactor.

Comparison of relative quantities of the domain Bacteria is of interest as its members are responsible for the hydrolysis and subsequent fermentation of cellulosic material (Lynd et al. 2002, Thomas et al. 2011), and a digester having a larger bacterial population would be more likely to be capable of an increased degree of cellulose degradation relative to one with a smaller population. Bacteria were most prevalent in AD8 and least prevalent in AD3-c, indicating a possible correlation between bacterial abundance and the quantity of fine material present in the feed. This effect may have been caused by diversion of biomass to AD8 from AD3-c by the sequential screening and centrifugation approach used to batch the feed to these two digesters. Biomass present in the liquid phase, or bound to particles smaller than the mesh size, would have passed through screen and been separated into the AD8 feed by centrifugation. The greater fraction of crude protein and fat, components associated with cellular material, present in the fine fraction in comparison with the coarse fraction (Table 13) provides support for this interpretation. Alternatively, it is possible the fine material could simply be more bioavailable due to greater specific surface area. Higher surface area enhances the hydrolysis rate (Vavilin et al. 2008), increases the quantity of available substrate, and would allow a larger bacterial population to develop. However, if the differences in bacterial populations between the digesters were due to biomass diversion, it would mean the digester containing the largest bacterial population that would be most suited to degrade plant fiber (AD8) was given feed containing the smallest fraction of this substrate. The digester containing the smallest bacterial population that would be

least suited to degrade fiber (AD3-c) was given the feed containing the largest fiber fraction (Table 13).

4.3.6 Organic Loading Rate Comparison

Provided good methane production efficiency and effluent quality can be maintained, operation of a digester at a high organic loading rate for a given SRT (i.e., by increasing the feedstock solids concentration) increases process efficiency by allowing treatment of a larger volume of waste per unit active digester volume. The required tank size for a higher rate system is less than for a low rate system, resulting in savings in construction and operations costs. If the System 2 digesters were capable of operating at a higher OLR than the System 1 digester, it could potentially be economically desirable to construct and operate such a system despite the ~10% lower daily methane production rate in System 2. In order to test this possibility, the OLR applied to the fermenters of each system was increased from 175 g VS/d to 219 g VS/d, and then to 263 g VS/d following a stabilization period.

The time required for steady-state conditions to develop after a change in operating conditions at a 20-day SRT is about 60 days. It was anticipated that two or more OLR increases would be required to induce failure in one or both of the AD systems. At the beginning of the OLR comparison about four months were available for collection of the data required for completion of this thesis, and in order to ensure the deadline was met the stabilization period between OLR increases was reduced to two weeks. Consequently, the observed response of the AD systems provides an indication of their resilience to shock loading, but should not be used as the basis for steady-state digester design.

Methane production and TS/VS in all reactors were the primary variables monitored. VFA concentrations into and out of the fermenters and digesters were monitored as well, but the concentrations were significantly underestimated due to systematic error caused by mechanical problems with the GC-FID system. These sampling results have not been included as the accuracy is insufficient to compare the performance of the two digestion systems, although the data does indicate VFA production in the fermenters and VFA destruction in the digesters. It was not possible to re-analyze samples other than those

taken from each reactor on day 102 and from AD6-c on day 108, as they had been disposed of by the time the inaccuracy was discovered.

4.3.6.1 Results of OLR Comparison

System 1 performed well at the 219 g VS/d OLR, with methane production and yield increasing (Figure 15), and no indication of excessive foaming. VFA concentrations after two weeks of operation at the higher OLR had not changed appreciably from levels observed at the 175 g VS/d loading rate (Table 16). The rate of methane production from System 2 increased at the 219 g VS/d OLR, and was comparable to that of System 1 (Figure 15), while VFA concentrations remained low. However, digester AD8 was less stable at this OLR and began producing foam after daily feeding, although the volume produced was not sufficient to foul the gas exhaust tubing and cause operational difficulties. The mass of fine solids produced daily by F3-c increased at the higher OLR to 500-550 g/d, which met or exceeded the maximum feed volume associated with the operating conditions of AD8 (SRT = 20 d, V = 10 L, Q = 0.5 L), and the feed solids concentration increased to 9-10% as no dilution with liquid fermenter effluent was possible. Solids produced by F3-c in excess of 0.5 L were directed away from AD8 and into AD3-c in order to avoid changing the ratio of feed volumes between the two digesters or the SRT, which are two alternative approaches to handling the excess fine solids. As noted previously, foaming was observed in the batch reactor experiment in the reactor containing undiluted (i.e., high concentration of) fine solids (Chapter 2.3), so it was not surprising that foam began to develop in AD8 as feed dilution decreased. Once the OLR was increased to 263 g VS/d, the volume of foam produced was sufficient to fill the headspace of the digester (~10 L) and contaminate the gas exhaust line. Foaming may be caused by a range of factors, and is common under high organic loading conditions. It has been hypothesized to be due to an imbalance in the production/consumption of intermediate compounds having surfactant properties, protein denaturation, high VFA concentrations, or bacterial production of extra-cellular polymers (Speece 2008). Considering that the effluent VFA concentration in AD8 was low, the foaming was probably due to a combination of the other factors.

Table 16. VFA concentrations and speciation in each reactor on day 102.

		VFA speciation as percentage of total VFA COD						
Reactor	**Total VFA as COD (mg/L)**	**Acetate**	**Propionate**	**Butyrate**	**Isobutyrate**	**Valerate**	**Isovalerate**	**Caproate**
Fermenter Influent	6059.7	58.8%	27.9%	9.1%	1.2%	1.4%	1.5%	0.0%
F3-c	8386.8	53.3%	20.2%	9.9%	1.7%	8.9%	2.4%	3.7%
F6-c	10258.1	46.0%	22.5%	19.3%	2.8%	4.5%	3.7%	1.2%
AD3-c	93.6	83.3%	12.9%	1.7%	1.1%	0.0%	1.0%	0.0%
AD6-c	152.8	78.5%	14.9%	4.1%	1.2%	0.5%	0.8%	0.0%
AD8	19.3	94.0%	6.0%	0.0%	0.0%	0.0%	0.0%	0.0%

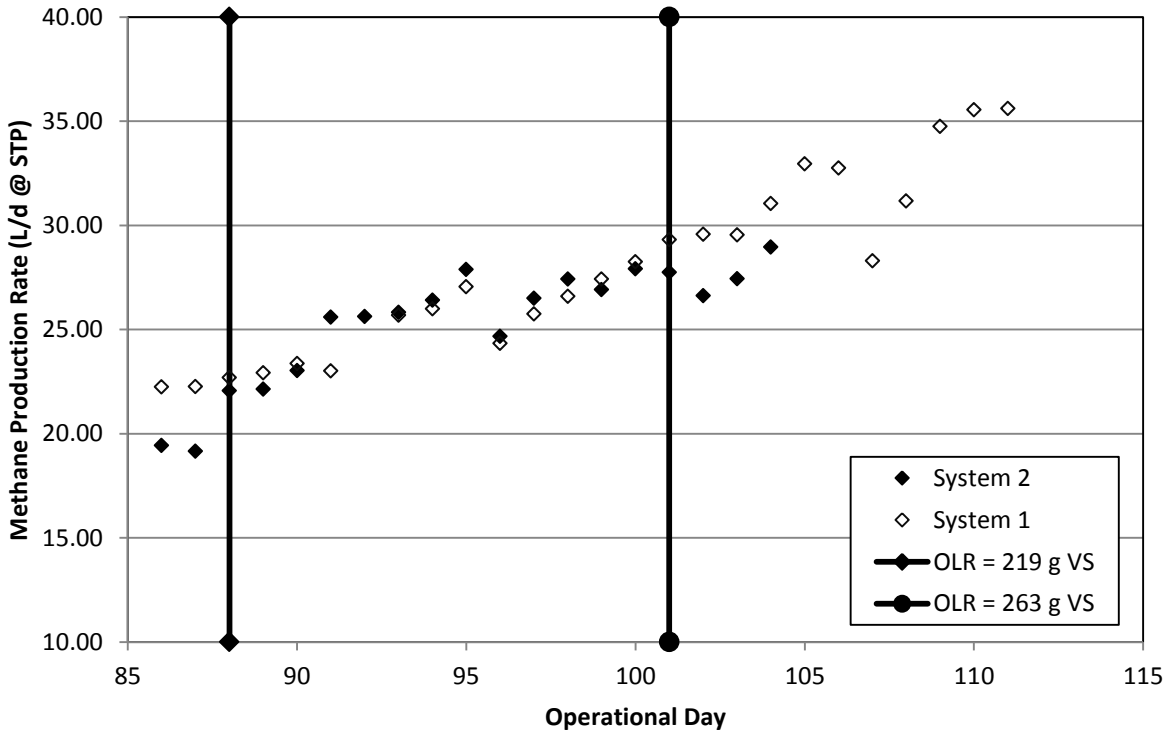

Figure 15. System 1 and 2 methane production rates at increased OLRs.

System 2 was shut down on day 105 due to foaming in AD8, and System 1 was operated for another seven days. Foaming did not develop in AD6-c, although the effluent VFA concentration increased and reached 567 mg/L as COD on day 108. Biogas methane content decreased from ~62% to ~60% as well, indicating that although the digester was capable of operation at the higher OLR, it was not operating efficiently. Foaming began to be

problematic in F6-c as well, and resulted in blockage of the gas exhaust line and overflows. The feed concentration in AD6-c increased to ~10% TS, causing blockage of the digester feed tube during cycling. Due to the problems with solids handling and foam production in the fermenter, the 263 g VS/d OLR was deemed too high to be practical. The experiment was terminated after 111 days of operation.

4.4 Summary, Discussion, and Recommendations

4.4.1 Methane Production—combined vs. separated digestion of fine solids

There are a number of reasons for dairy operators to consider installation of an anaerobic digester for manure treatment, including greenhouse gas reduction (assuming the methane produced is combusted to produce CO_2), odor control, energy production, recycling of bedding material, and the potential for revenue generation through co-digestion tipping fees. However, digester construction is often not an economically viable option for all but the largest dairy operators due to the economies of scale required to overcome high facility construction and operating costs coupled with low electrical power prices. Innovative treatment approaches, such as coupling anaerobic digestion with PHA production, can maximize substrate resource recovery and offset costs, thereby making the treatment process more competitive with less intensive, lower-cost options such as lagoons.

Maximization of methane yield is of primary concern with any digestion system, as this will maximize electrical power output. This research focused on the potential for enhancing the methane yield of a two-phase, PHA production-coupled digestion system through separation of fermented solids into two fractions based on particle size, and digestion of each fraction in a separate reactor. It was hypothesized that due to the differing characteristics of each solids fraction, the separate reactors would act as selectors for unique bacterial and methanogenic cultures optimized for the hydrolysis and methanation of each substrate, thereby increasing the methane yield in comparison to that of a single digester.

In order to test the solids separation hypothesis, it was necessary to conduct a comparison of the performance of a two-digester system with that of a single-digester system. Due to the fact no similar research had been conducted, it was unclear what size the digester

treating the fine fraction should be, or what organic loading rate should be applied. In order to obtain an approximate sense of how the fine fraction would react in a lab-scale digester, a batch test was conducted which showed the solids content of the digestate should be kept at 6.5-7.0% or less in order to avoid foaming and a possible decrease in methane yield. More accurate design guidance was developed through operation of three 1.5 L digesters at different SRTs and OLRs. The results of that experiment indicated the target OLR range should be between 2.4 and 3.2 g VS/L*d, and less than 4 g VS/L*d. This information, in combination with measurements of fine solids production by fermenter F3, was used to establish the 10 L active volume of AD8. This digester was operated in conjunction with the 30 L digester AD3-c (System 2), and the performance of this system was compared to that of the 40 L digester AD6-c (System 1). The fermenters providing digester feedstock were loading equally, and equal amounts of liquid fermenter effluent were separated from both systems and directed to PHA production. Both digestion systems were operated for 85 days at the same SRT, VS, and VFA loading to isolate the effect of separated digestion.

In contrast to the hypothesized outcome, average daily methane production from System 2 was statistically lower than that of System 1. Both systems achieved essentially complete removal of all VFAs produced, with average effluent concentrations equal to about 1% of the influent concentrations for each system. This result indicates methanogenesis was not rate-limiting in either case, and differences in performance are therefore most likely primarily due to dissimilarities in cellulose and hemicellulose hydrolysis rates, as these compounds account for the majority of the volatile solids in the digester feed. Although proteins and fats made up a much smaller fraction of the feed than fiber, differences in protein and LCFA hydrolysis rates could have contributed to the performance difference as well.

Cellulose is a complex polysaccharide comprising the majority of the mass of plant cell walls, and is typically embedded in a matrix of the decay-resistant biopolymers hemicellulose and lignin (Lynd et al. 2002). Depending on the species, bacterial hydrolysis of cellulose under anaerobic conditions is accomplished by either secretion of various extracellular cellulase enzymes into bulk solution, or adhesion of the bacterial cell to the cellulose particle and

secretion the cellulolytic enzymes directly onto the surface. The soluble end products of cellulose hydrolysis are cellobiose and cellodextrins, which are subsequently hydrolyzed further to glucose and glucose-1-phosphate. These compounds are then catabolized by the bacteria as a carbon and energy source. The end products of fermentation are primarily acetate and carbon dioxide, but may also include ethanol, succinate, lactate, or hydrogen, depending on the species of bacteria (Lynd et al. 2002). Acetate, carbon dioxide, and hydrogen are converted to methane gas by methanogens.

In both digestion systems, NDF (a lumped parameter comprising cellulose, hemicellulose, and lignin) accounted for about 68% of the influent VS mass and 70% of VS destruction, meaning fermentation of cellulosic material provided a major source of substrate for the methanogenic population. Solids composition analyses suggested the AD8 feedstock, and to a lesser extent the AD6-c feedstock, were enriched in crude fat and protein in comparison to the AD3-c feedstock, which was enriched in NDF (Table 13). Both crude fat and protein are indicators of the presence of cellular material, meaning the fine material likely contains more bacteria than the coarse material treated by AD3-c. Comparisons of bacterial abundance made using qPCR indicated the two digesters receiving the fine solids (AD8 and AD6-c) contained greater amounts of biomass than AD3-c. Although the frequency of sampling for both the qPCR technique and the solids characterization was too low to allow statistically supported conclusions to be drawn regarding bacterial abundance and the nature of the digester feed and contents, the data circumstantially suggests the observed difference in methane production between the two systems could have been due to greater bacterial abundance in AD6-c. This difference in abundance could have resulted from the solids separation approach, in which the microbial biomass contained in the F3-c effluent was directed disproportionately to AD8, while the cellulosic substrate needed by these bacteria was directed to AD3-c. In contrast, AD6-c received both cellulosic substrate and the biomass required to hydrolyze and ferment it, resulting in a higher rate of biomass retention, a larger population of fermenting bacteria, and greater VFA and methane production.

4.4.2 Organic Loading Rate

Although separated digestion did not increase methane production in comparison to a conventional digester, it was possible the approach would have a greater tolerance of high organic loads, resulting in less required reactor volume and lower construction costs. This was not the case at the two higher OLRs examined (219 g VS/d and 263 g VS/d to the fermenter). In fact, System 2 was less stable than System 1 because of increased production of fine solids by F3-c at the higher OLRs, which resulted in overloading and failure due to foaming in AD8. It is possible the failure at the 263 g VS/d level and the production of foam at the 219 g VS/d level could have been avoided by increasing the volume of AD8 and decreasing the volume of AD3-c while maintaining the same SRT. This would have diluted the feed going to AD8 while concentrating the feed going to AD3-c, which had a lower proclivity toward foam generation. However, this increase in operational complexity to avoid failure is unjustified because System 1 showed no indications of foaming while producing an equal or greater quantity of methane.

4.4.3 Recommendations

This research was focused on investigating two configurations of a phase-separated anaerobic digestion system in which the majority of VFAs produced in the acid fermentation phase are directed to PHA production, and the remaining material treated in an anaerobic digester, although the conclusions regarding the effectiveness of the solids separation approach are likely applicable to conventional two-phase AD systems treating dairy manure as well. Recommendations based on this research are as follows:

1) The conventional single CSTR configuration or CSTRs in series (Appendix A) should be employed in a full scale two-phase PHA/methane production facility utilizing dairy manure vs. the parallel digester configuration examined.

    a) Separation and separate digestion of fine material and coarse material present in the fermenter effluent decreases methane production in comparison with treatment of fine and coarse material simultaneously in a conventional single CSTR at the 175 g

VS/d loading rate, while approximately equal levels of production were observed at the 219 g VS/d loading rate.

b) Separate digestion of fine and coarse material does not allow treatment at a higher OLR than would be possible in a conventional single CSTR. The separate digestion system failed before the conventional system at the 263 g VS/d loading rate due to foam production in the digester receiving the fine solids fraction.

c) Separate digestion is mechanically more complex than a single CSTR, requiring duplication of control hardware, mixers, and tanks and additional solids separation equipment, piping, and valving. The increase in cost and complexity associated with designing and operating such a system is unjustified given the reduced methane yield and tolerance of short-term increases in organic loads in comparison with a conventional CSTR.

2) Diversion of the entire liquid fraction of fermenter effluent to PHA production and dilution of the remaining solids entering the digester with non-VFA containing liquid is not a viable operational approach for combined PHA and methane production. A portion of the VFAs generated in the fermenter must be directed to the digester in order to provide adequate substrate for the methanogenic population to maintain its growth rate at a level sufficient to avoid washout.

3) The maximum OLR applied to the fermenter should be less than 263 g VS/d to avoid excessive foam production.

4) The effluent of both AD systems was low in soluble phosphate, in contrast to what is typically observed in a conventional single phase digester. This suggests land application of digestate from a two phase AD system could generate less runoff of SRP compared to a conventional digester. Further research should be conducted to evaluate this possibility.

Chapter 5 Mixing Comparison Study

5.1 Mixing Theory and Impact on AD Operations

The primary functions of mixing in an anaerobic digester are (EPA 1979, Grady et al. 2011, Speece 2008, Ward et al. 2008):

- Maintenance of close contact between the incoming substrate and the biomass
- Formation and maintenance of uniform physical, chemical and biological conditions
- Dispersion of inhibitory toxic substances that may be present in the feed, as well as metabolic end products, to maximize biological activity
- Reduction of the potential for dead zone formation, short circuiting of influent, and scum formation
- Stripping gas bubbles from the liquid phase of the digester

A tracer study of 10 conventional pancake design digesters found inadequate mixing resulted in dead zone formation and HRT/SRT values that were on average only 65%, and as low as 18%, of the theoretical value (Smart 1978). Short-circuiting of feed was a major problem in the digesters examined, with as much as 72% of the feed received undergoing negligible digestion (Smart 1978). Additional problems associated with poor mixing include reduced pathogen reduction, and more importantly, reduced methane production, as the sludge is allowed to leave the digester before an adequate amount of time has passed for volatile solids conversion (Speece 2008).

Despite the general consensus on the benefits of mixing, the literature is unclear as to the degree of mixing constituting adequacy (EPA 1979, Smith et al. 1996, Ward et al. 2008). Although inadequate mixing is undesirable, excessive mixing has been correlated with impaired digester performance as well, particularly during digester startup (Ghanimeh et al. 2012, Hoffmann et al. 2008, McMahon et al. 2001, Stroot et al. 2001). Vavilin (Vavilin and Angelidaki 2005) suggests that higher mixing levels may be tolerated once a large

methanogenic biomass inventory has been developed, and that vigorous mixing may improve the hydrolysis rate by physically breaking down particulate material, thereby increasing the surface area available for reaction with hydrolysis enzymes. The hydrolysis step is usually rate-limiting in AD, meaning that a greater hydrolysis rate will result in an increased biogas production rate. During digester startup, when methanogenic populations are not well established, vigorous mixing may be inhibitory due to disruption and exposure of methanogenic growth initiation centers to high levels of VFAs produced by the faster growing fermentative bacteria. Vavilin's theory is in agreement in some respects with observations of digester performance and recommendations made by other researchers (Ghanimeh et al. 2012), (Hoffmann et al. 2008), (Stroot et al. 2001), although it does not explain observations of mixing-induced propionate accumulation (McMahon et al. 2001, Stroot et al. 2001).

Intense mixing may also impair digester performance and lead to propionate accumulation (McMahon et al. 2001, Stroot et al. 2001) through spatial disruption of the syntrophic relationship between hydrogen-oxidizing methanogens and propionate-oxidizing bacteria, allowing the partial pressure of hydrogen to increase and causing product inhibition. As approximately 30% of the biodegradable COD in the feed passes through propionate (McCarty and Smith 1986), disruption of this pathway will result in reduced methane production and VFA accumulation.

Propionate can be difficult to remove anaerobically because oxidation of propionate to acetate and hydrogen or formate is thermodynamically unfeasible under standard conditions, with a positive Gibbs free energy change of 71.67 kJ (de Bok et al. 2004, McCarty and Smith 1986). In order for energy to be obtained from the reaction, hydrogen must be removed at a rate sufficient to keep its partial pressure at 10^{-4} atmospheres or less. This is accomplished through maintenance of close physical proximity of hydrogen/formate consuming methanogens and propionate-oxidizing bacteria, which results in a steep hydrogen concentration gradient between the organisms and maximizes the rate at which hydrogen and formate can be removed (McCarty and Smith 1986). The total amount of

energy released from conversion of 1 mole of propionate to carbon dioxide and methane is about 1 ATP, which must be split between both acetoclastic and hydrogenotrophic methanogens and propionate-oxidizing bacteria (de Bok et al. 2004). Several species of syntrophic propionate oxidizing bacteria have been isolated to date, including *Syntrophobacter wolinii, S. fumaroxidans, S. Pfennigii,* and *Smithella propionica.* The population size of propionate oxidizing bacteria may be a limiting factor in AD; for example, in a study of anaerobic digestion of glucose, the population of bacteria capable of performing propionate degradation was smaller than that of either glucose or acetate-consuming microorganisms, and the degradation rate of propionate was shown to be slow (Ito et al. 2012). In a digester subjected to intense mixing, the close association between propionate oxidizing bacteria and methanogens can be disrupted, and the contents are instead homogenized. The even distribution of these organisms results in a greater average separation distance, the rate of hydrogen/formate removal is reduced, and the potential for imbalance and propionate accumulation increases (de Bok et al. 2004, McCarty and Smith 1986).

The combination of low bacterial populations, obligate syntrophy, and minimal energy yield due to unfavorable thermodynamics makes propionate more likely to accumulate in anaerobic digesters than longer chain VFAs or LCFAs. This is partially due to more favorable thermodynamics for oxidation of these compounds; the free energy changes associated with anaerobic propionate, butyrate, and palmitate oxidation (a common LCFA) are 0.68, 0.30, and 0.55 kJ/g COD at standard conditions, respectively (Batstone et al. 2002). Propionate is typically considered the intermediate compound most difficult to effectively treat (Speece 2008).

Given the sensitivity of propionate concentrations to imbalance in the AD process, its use as a performance indicator has been recommended. While research has shown digesters can in fact operate at elevated propionate/acetate ratios and/or high acetate concentrations, provided sufficient alkalinity is present to prevent digester acidification (Ahring et al. 1995, Nielsen et al. 2007, Pullammanappallil et al. 2001), the treatment efficiency and methane

yield of such a digester would obviously be compromised. Hill proposed a value of the ratio of the concentrations of propionate to acetate of 1.4 or greater, in combination with acetic acid concentrations of 800 mg/L or greater, as being indicative of a 90% probability of digester failure (Hill et al. 1987). This combination of indicator values was derived from statistical analysis of data from 70 full-scale mesophilic and thermophilic digesters for which methane yields (L CH_4/g VS applied) and acetate and propionate concentrations were available in the literature. Digester failure was defined based on the assumption that a healthy digester would achieve VS destruction of at least 50% and a methane yield of at least 0.5 L CH_4/g VS destroyed. Digesters with yields less than 0.25 L CH_4/g VS applied were defined as having failed. However, given the probabilistic and somewhat arbitrary nature of the failure criteria applied, these should not be taken as hard and fast rules. For example, a digester operating with a propionate to acetate ratio of 1.3 combined with acetate concentrations of 750 mg/L would not be performing optimally, although it would meet Hill et al.'s criteria for successful operation. Additionally, no distinction was made between thermophilic and mesophilic operation, and correction factors taking into account differences in non-biodegradable VS content and alkalinity requirements between substrates would be required in order to extrapolate the recommendations beyond the swine and beef cattle wastes on which the analysis was based. A better approach entails observation of the concentration, and rate of change in concentration, of propionate as a primary control parameter. High, increasing, or persistent propionate levels, or sudden spikes in concentration, would be indicative of current or impending digester imbalance (Nielsen et al. 2007).

5.1.1 Background on Research Conducted

This research was performed using a two-phase AD system consisting of a 40 L digester operated at a 20 day SRT (referred to as AD3) coupled to a 20 L fermenter operated at a 4 day SRT. The OLR applied to the fermenter was initially 219 g VS/d. Solid/liquid separation of the fermenter effluent was accomplished by screening (as was used with AD3-c described in Chapter 4) meaning AD3 was fed the coarse solids fraction. Digester temperature was maintained in the mesophilic range by periodic cycling of hot water through the heating coils

(passive control vs. the active control used with AD6-c of Chapter 4). Mixing was initially provided by a 4.25” pitched-blade turbine and four interior baffles installed to prevent vortexing. The design and operation of the fermenter and digester was otherwise the same as that of System 1 described in Chapter 4.

Observation of digester performance during the spring of 2013 indicated the original impeller was undersized and did not provided sufficient mixing to keep the contents homogenized and prevent dead zone formation. To remedy this, a 6” diameter, two-bladed high impact plastic propeller was installed in the digester at the end of May 2013. Mixing speed guidelines in the literature are somewhat vague due to the dependence of mixing effectiveness on the impeller number, viscosity, impeller rotational speed, tank geometry, and the presence or lack of baffles. Consequently, an “intermediate” level of mixing (Smith et al. 1996) is frequently recommended. In keeping with this empirical approach, the mixing speed for the new impeller was selected based on visual observation of mixing intensity at the liquid surface in the digester, and was set sufficiently high to generate turbulent conditions with frequent turnover of the contents. No other changes were made to the method of digester operation. Samples taken during the period February-March 2013 prior to replacement of the impeller indicated the average effluent VFA concentration was low (171 mg/L total as COD). No samples were taken until two weeks after replacement, by which time significant buildup up of propionate and acetate had occurred (Figure 16), indicating severe inhibition of propionate oxidation.

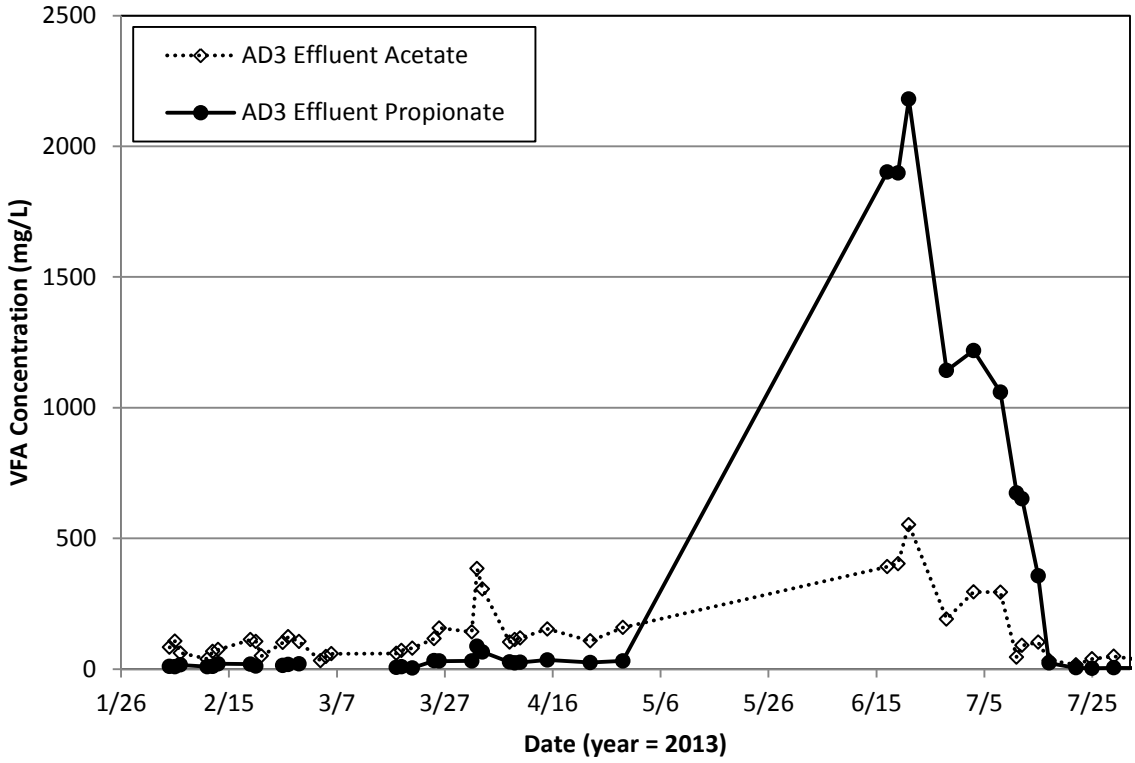

Figure 16. VFA imbalance in digester AD3 prior to and after increase in mixing intensity at the end of May 2013.

As the digester had been performing acceptably prior to the impeller replacement, a search of the literature was conducted to determine if any research had been published regarding the effect of mixing on digester VFA concentrations. In addition, the OLR applied to the system fermenter was reduced from 216 g VS/L*d to 175 g VS/L*d on July 3rd in an attempt to reduce the imbalance. Although this appeared to help, it did not solve the problem, and feeding was suspended on July 9th. On July 11th, a paper by Stroot et al. (Stroot et al. 2001) was located, which described similar patterns of VFA accumulation in anaerobic digesters subjected to intense mixing on a shaker table. The digesters described recovered after the mixing intensity was reduced, and so the impeller speed in AD3 was adjusted to a level that produced visible currents on the liquid surface, but did not cause significant turbulence. The VFA concentration in AD3 continued to decrease, feeding was resumed on July 12th, and the effluent VFA concentration subsequently returned to the level seen prior to the impeller replacement.

Due to the fact several variables affecting VFAs were changed simultaneously, it is not possible to conclude with certainty whether the upset was brought under control by the reduction in mixing intensity, the reduction in OLR, the temporary suspension of feeding, or some combination thereof. Thus, the following experiment was designed with the goal of replicating the observations of Ghanimeh et al. (Ghanimeh et al. 2012), Hoffmann et al. (Hoffmann et al. 2008), and particularly Stroot et al. (Stroot et al. 2001) regarding the detrimental effect of high intensity mixing on digester performance.

5.2 Materials and Methods

5.2.1 Experimental Design

This research was conducted using a two-phase AD system consisting of a 20 L fermenter (F3-b, described in Chapter 3.2.1.1) operated at a 4 day SRT and a 40 L digester (AD3-b) operated at a 20 day SRT. The system had been at steady state for several months at the beginning of the data collection period. The mixing intensity was increased on the first day of data collection to a level sufficient to generate intense, turbulent mixing and frequent turnover of the digester contents.

5.2.1.1 Digester AD3-b

Digester construction was the same as that of AD3-c described in Chapter 4.2.1.1, with the exception that PLC temperature control had not yet been implemented, and the Tedlar headspace equalization bag was not yet installed. The digester was instead heated by cycling hot water through the heating coils for a fixed amount of time at set intervals. The temperature was maintained within about 2 °C of the 35 °C setpoint by manual adjustment of the cycling period length and frequency, the water heater thermostat setting, and the hot water flow rate through the heating coils. Due to the fact there was no provision for headspace volume equalization, it was necessary to vent the digester headspace to the atmosphere during feeding/wasting using a valve. Feed to AD3-b consisted of fermenter supernatant and the coarse solids fraction separated by screening as described in Chapter 4.2.1.3.

5.2.2 Analytical Techniques

Samples were collected on a semi-daily basis for digester influent and effluent VFA concentrations, total and volatile solids content, biogas volume, and methane content of biogas. Reactor pH was monitored as well, but was unresponsive to changes in VFA concentration due to the high alkalinity and buffering capacity of the manure. Quantification of VFA concentrations and methane content was carried out as described in Chapter 3.2.2, and testing for total and volatile solids was conducted as described in Chapter 2.2.2. Biogas production volumes were quantified using wet tip gas meters (Wet Tip Gas Meter Co., Nashville, TN, USA) calibrated manually to record production in 100 mL increments using a 150 mL gas tight syringe.

5.3 Results

5.3.1 Fermenter F3-b

The effluent VFA concentration of F3-b was monitored over the course of the experiment. VFA production was stable (Figure 17 and Figure 18), indicating fermenter performance was unlikely to have influenced the behavior of AD3-b. Total average VFA as COD concentrations plus/minus one standard deviation were 4266.2 +/- 561.2 mg/L.

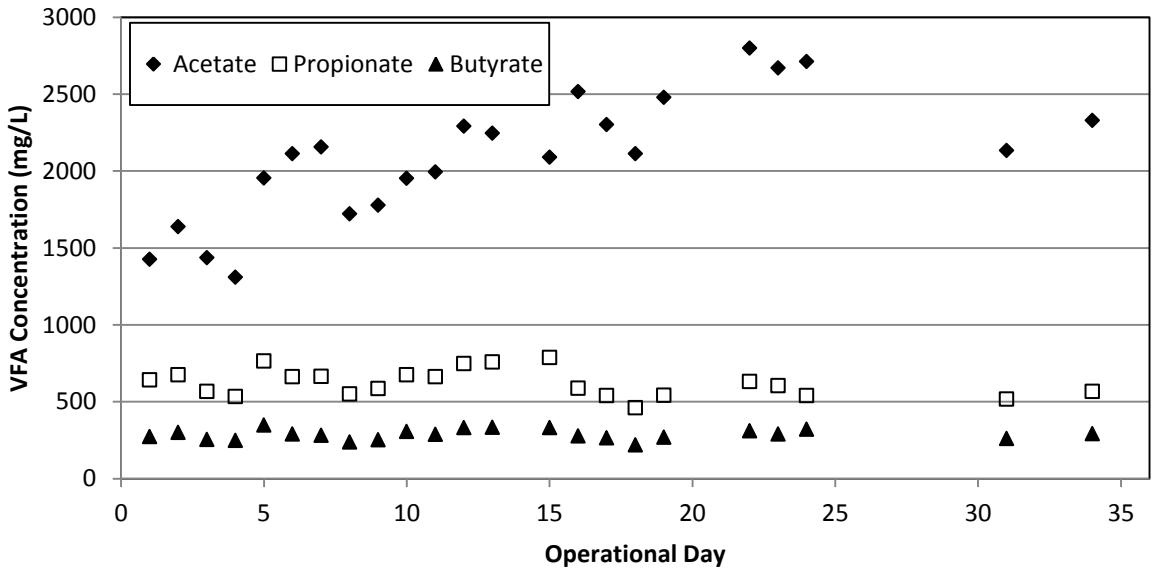

Figure 17. F3-b effluent acetate, propionate, and n-butyrate concentrations.

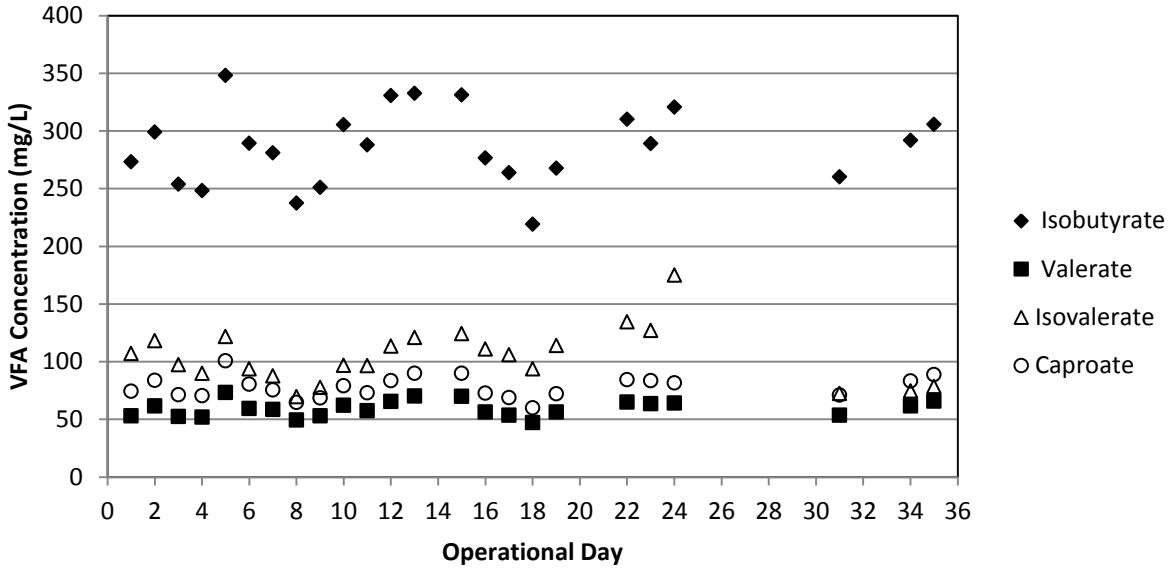

Figure 18. F3-b effluent isobutyrate, iso- and n-valerate, and caproate concentrations.

5.3.2 Digester AD3-b

The concentration of VFAs from acetate through caproate was the primary response variable in this study. Propionate was of particular interest, and was expected to be the first VFA to be affected after mixing intensity was increased. This was observed to be the case, and propionate began to accumulate within days of changing the mixing speed (Figure 19). The propionate concentration exceeded that of acetate within one week, and the difference between the two continued to increase until the mixing rate was decreased to the original level after two weeks of operation at the higher speed. At this point, it was anticipated that VFA levels in the digester would begin to drop as the syntrophic relationship between hydrogenotrophic methanogens and LCFA and propionate-degrading bacteria was reestablished under more quiescent mixing conditions. However, volatile acid concentrations, with the exception of propionate, continued to rapidly increase (Figure 19 and Figure 20). In order to rectify the VFA imbalance, feeding/wasting was suspended for a total of six days. This had the desired effect of stabilizing the digester, and VFA levels remained low after daily feeding was resumed.

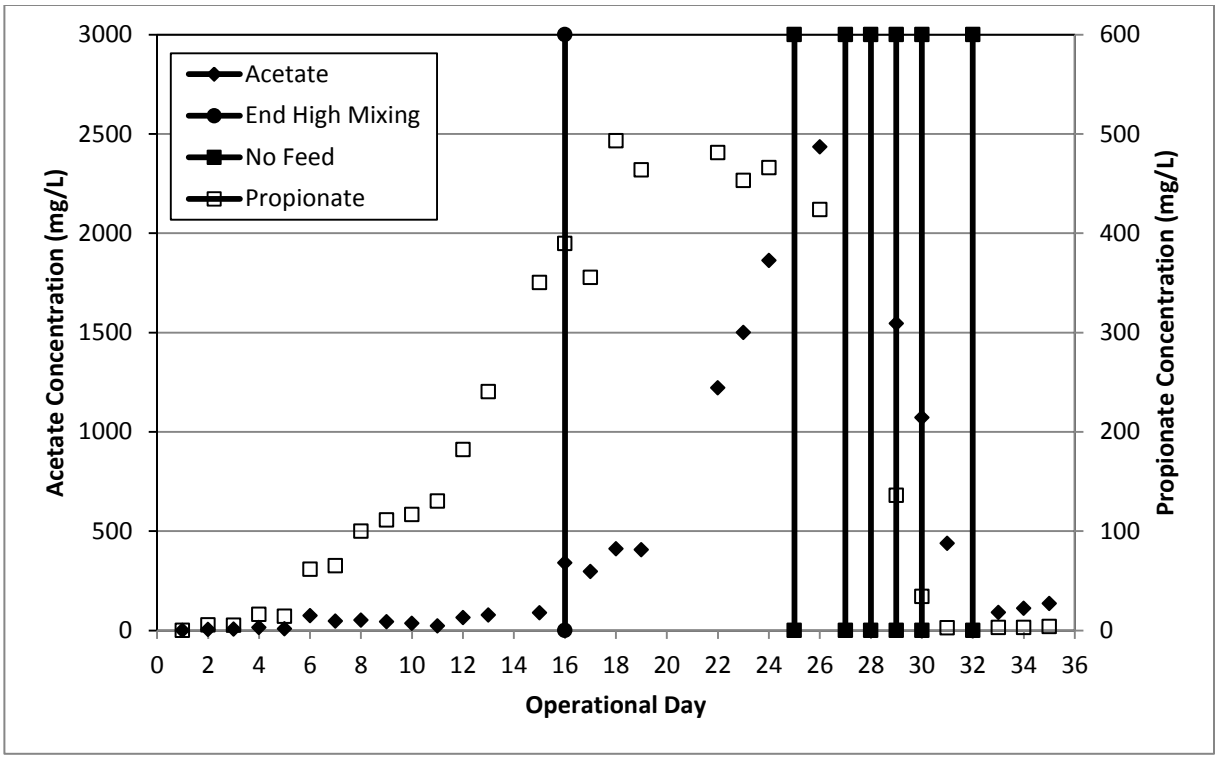

Figure 19. AD3-b effluent acetate and propionate concentrations. Lines indicate days on which mixing intensity was reduced, or feeding was suspended to allow VFA concentrations to decrease.

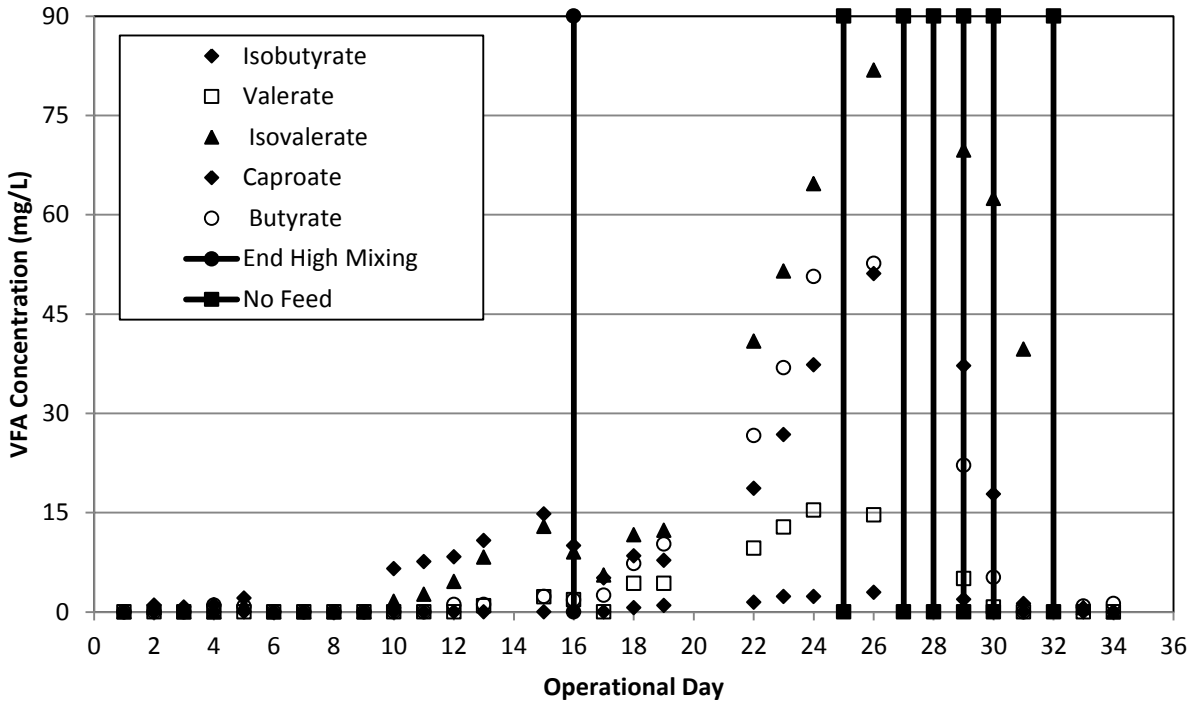

Figure 20. AD3-b effluent 4-6 carbon VFA concentrations.

Total and volatile solids concentrations in the digester effluent remained essentially constant over the course of the experiment. Although the degree of volatile solids destruction might be expected to decrease (i.e. an increase in effluent total and/or volatile solids concentrations) based on the observed VFA accumulation and the reduced biogas production shown in Figure 22, no discernible trends are present in the solids sampling data (Figure 21). It is possible the upset was not allowed to continue for an adequate period of time, or that it was not of sufficient severity, for the effects to be reflected by TS/VS, a parameter which is insensitive to short-term changes in performance due to the long SRT at which the digester operated.

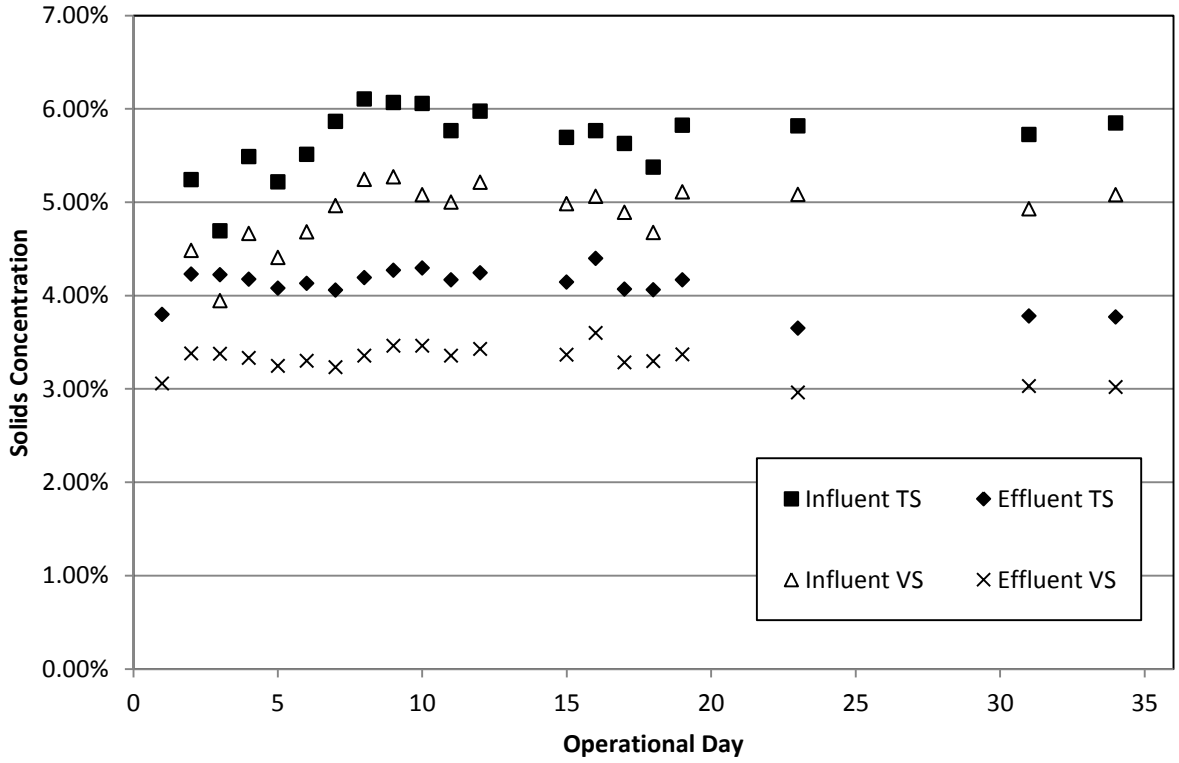

Figure 21. AD3-b influent and effluent total and volatile solids concentrations.

While there was no observed effect on VS destruction, the impact of the process upset was quite apparent in its impact on biogas production and methane content. Biogas production averaged 33.4 L/d over the month prior to the increase in mixing. Biogas production spiked to 45 L on the day after mixing was increased (Figure 22), probably due to stripping of

dissolved CO_2 and CH_4 from solution, and then declined sharply to about 30 L/d until the mixing speed was reduced. Production then increased to about 35 L/d, but after several days decreased to 26 L/d, which correlates with the rapid accumulation of acetate and longer-chain VFAs beginning on day 19. After feeding was suspended on day 25, daily gas production initially increased, and then began to decline as the accumulated VFAs were consumed. Once feeding resumed, gas production recovered to the 30 L/d level within a week. The digester was operated for another two months using the original mixing intensity, with about three weeks required for gas production to recover to a level comparable to the daily average prior to the upset (Figure 22).

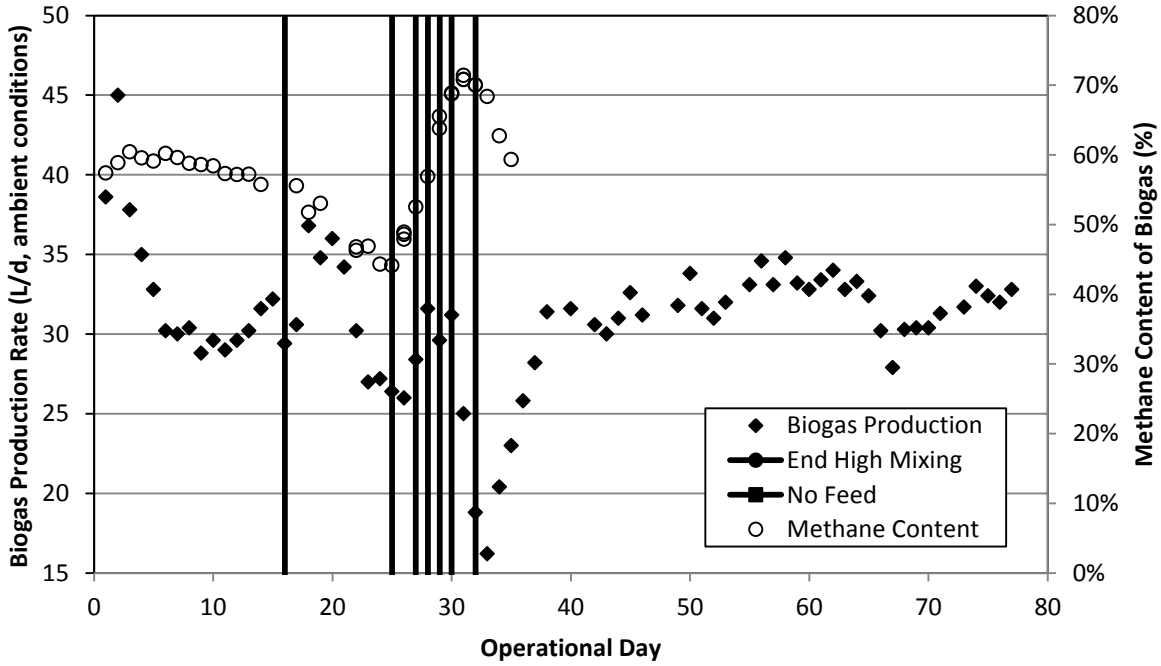

Figure 22. AD3-b biogas production (primary y-axis) and methane content of biogas (secondary y-axis) at ambient conditions in the lab during and after the mixing experiment.

The methane content of the biogas initially began to rise after mixing was increased, but then began to decrease as VFAs built up in the digester (Figure 22). Methane content did not recover when the mixing level was reduced, and continued to decrease until feeding was discontinued. At this point the methane fraction began to rapidly increase as balanced conditions were reestablished. Once the digester stabilized and VFA concentrations returned

to pre-upset levels (200 mg/L as COD or less), the methane content returned to the pre-experiment level of ~58%-59%.

5.4 Discussion

Stroot et al. (Stroot et al. 2001) found that a stable digester subjected to intense mixing would destabilize and begin accumulating propionate and acetate, while an unbalanced digester containing high levels of propionate and acetate and subjected to intense mixing would recover to low VFA concentrations if the mixing level was reduced. The pattern of VFA accumulation observed in AD3-b after the mixing speed was increased was essentially the same as that observed by Stroot et al., despite differences in feedstock (dairy manure vs. mixed primary sludge and municipal solid waste), mode of mixing (impeller vs. shaker table), and reactor construction and size (externally heated 15 gallon HDPE tank with baffles vs. 2 L Pyrex bottles in an incubator). Similar to the results observed by Stroot et al., reduction in mixing speed by itself was not sufficient to normalize operation of AD3-b, and it was necessary to suspend feeding for several days as well. The primary differences between AD3-b and the results of Stroot et al. were the lower rate of VFA accumulation in AD3-b, and the additional time required for acetate to build to a high level. AD3-b stabilized more quickly, but was subjected to a shorter period of intense mixing (16 days vs. 65 days), so the level of disruption of the microbial consortium was likely not as great.

It is highly unlikely the cause of the observed upset in AD3-b was due to a factor other than mixing intensity. The digester had been operating in a stable manner for several months prior to the increase in mixing, with propionate concentrations of 15 mg/L or less. Propionate began to accumulate immediately after mixing intensity increased, and accumulated to 490 mg/L from non-detectable levels within 15 days of the change. During the period of increased mixing, the influent propionate concentration averaged 657 mg/L, and the rate of increase of propionate concentration in the digester effluent implies inhibition of propionate oxidation. Once the mixing level was reduced and the accumulated VFAs were allowed to degrade through reduction in feeding, the digester stabilized, and no further upsets were experienced for the duration of the research. In fact, the only two

upsets experienced by this digester over two years of operation occurred shortly after substantial increases in mixing intensity.

5.5 Conclusions

This study provides additional support for evidence presented in the literature linking poor digester performance with high mixing intensity. Effluent concentrations of all VFAs increased during the mixing-induced process upset, although propionate was the first to accumulate. Resumption of propionate degradation correlated with a return to stable operation. Once the upset was underway, reduction of mixing intensity to the original level was necessary, but not sufficient, to quickly stabilize the digester. Temporary suspension of feeding was required as well to halt and reverse VFA accumulation. In contrast to the other VFAs, particularly acetate, propionate concentrations did not continue to increase after mixing was reduced.

The cause of the increase in acetate concentration observed in AD3 after the reduction in mixing speed is less clear, as anaerobic acetate oxidation is more thermodynamically favorable than propionate oxidation. High-rate anaerobic treatment processes, such as the upflow anaerobic sludge blanket configuration, achieve high substrate conversion rates by encouraging the development of biomass granules that minimize the distance between fermentative and methanogenic organisms, thereby maximizing concentration gradients and consumption rates. It may be that the intensified mixing increased the average distance between acetate-producing and consuming organisms, reducing the acetate utilization rate of the methanogenic biomass and leading to acetate accumulation in the reactor. Alternatively, as suggested by Vavilin (Vavilin et al. 2008), the higher mixing intensity may have acted to break up particulate matter and increase the hydrolysis and VFA production rates. If these rates were slow to change and remained elevated during the days after mixing was restored to the original level, it would provide an explanation for the continued VFA accumulation that was observed. Returning to a lower mixing intensity would have decreased the hydrolysis rate over time and encouraged the reestablishment of the close proximity syntrophic relationship between acidogenic bacteria and hydrogenotrophic

methanogens. Temporary suspension of feeding would have allowed the microbial consortium to quickly reverse the VFA accumulation and restore balanced conditions in the digester.

It is of interest to compare the estimated degree of mixing intensity in AD3-b with standard design guidelines, which are frequently based on the concept of the velocity gradient (Tchobanoglous et al. 2003):

$$G = \sqrt{\frac{P}{\mu * V}}$$

where G = velocity gradient (s^{-1})

P = power delivered to the fluid (W)

μ = fluid dynamic viscosity ($N*s/m^2$)

V = reactor volume (m^3)

The recommended velocity gradient range for mixing anaerobic digesters given in *Wastewater Engineering: Treatment and Reuse* is between 50 s^{-1} and 80 s^{-1} (Tchobanoglous et al. 2003). If the input power is not known, it is possible to estimate it using the empirically calculated impeller number and the following equation (Tchobanoglous et al. 2003):

$$P = N_i * \rho * n^3 * D^5$$

where N_i = impeller number (dimensionless)

ρ = fluid density (kg/m^3)

n = impeller shaft rotational speed (s^{-1})

D = impeller diameter (m)

The impeller number is provided by the manufacturer, although ranges of typical impeller numbers for common impeller types are available in engineering texts. Using this approach, the velocity gradient in AD3-b was estimated to be 240 s^{-1} during balanced steady-state

operations and 440 s^{-1} during the mixing-induced upset. These estimates are based on the measured shaft rotational speed and the assumption that the digestate has the same viscosity and density as water. Although this assumption is not entirely valid, it is not without precedent in the literature (Meroney and Sheker 2014), and the instruments required to directly measure digestate viscosity were not available. The impeller number was estimated based on values for 3-bladed propellers (values were not available for 2-bladed propellers) found in *Water Treatment: Principles and Design* (Crittenden et al. 2012). Due to the fact the actual viscosity, fluid density, and impeller number were not known, some uncertainty is present in these estimates. However, because the equations are most sensitive to the rotational speed and impeller diameter, both of which were known, the estimates are likely to be reasonably close to the actual values. Thus it would appear standard design guidance is quite conservative with respect to mixing intensity, as stable AD performance was achieved at a velocity gradient approximately 3 to 5 times greater than recommended (the higher intensity was necessary in order to prevent temperature fluctuation associated with heterogeneous conditions), while that required to induce upset was about 5.5 to 9 times greater.

Intense mixing should be avoided as it provides no benefit, increases energy consumption, and will result in severe and completely avoidable impairment of digester operations. This is especially true during startup of a new digester, when it could interfere with establishment of a robust microbial consortium, particularly with respect to the degradation of propionate. The design of an anaerobic digester should provide the operator with the means to assess mixing intensity and effectiveness in order to avoid treatment impairment caused by under-mixing (formation of dead zones and development of heterogeneous conditions) and over-mixing (interference with syntrophic microbial relationships).

The concentration of VFAs was the most sensitive indicator of impending digester upset. Propionate was the first VFA to accumulate, and the speciation provided by gas chromatography therefore allows for more accurate process troubleshooting than would be possible using titrations. Biogas production was a good indicator of upset as well, but in the

absence of VFA data it would be difficult to differentiate the onset of an actual process upset from random fluctuations due to the inherently variable biogas potential of the feedstock. Biogas methane content was the next most sensitive indicator, although its usefulness is limited by the dependence of the gas phase carbon dioxide concentration on temperature and the carbonate equilibrium system. The high alkalinity and buffering capacity of the dairy manure made pH measurements ineffective as a performance indicator, but pH would be useful when treating lower alkalinity substrates such as domestic wastewater sludge. VS destruction was too insensitive to process changes to be used effectively as an indicator of upset. Ideally, all of these parameters would be monitored by the operator on a regular basis to avoid digester imbalance, overloading, and to ensure efficient process operation.

Appendix A—Additional Digester Performance Data

A.1 Digester Configurations

Several different operational and mechanical variations of the AD systems described previously in this thesis were operated during the two-year period in which this research was conducted. A secondary goal of this research was to collect performance data, primarily in the form of methane yields on a gram VS applied basis, for each variant. This data, in combination with that of previous system variants AD1, 2, and 4 reported in (Coats et al. 2012) and (Coats et al. 2013), could be used to guide the design of a full-scale AD-PHA production facility, as well as to estimate the impact on digester methane production of diverting different quantities of liquid fermenter effluent to the PHA production process. Five different digester system configurations were operated in addition to the AD6-c system described in Chapter 4, referred to as AD6-a, -b, and -e, AD3-d, and AD3s-AD6s. The operation and configuration of these digesters differed from that of AD6-c as follows:

AD1, AD2, AD4, and AD6-a: The impeller used was a 4.25” diameter pitched-blade turbine. Digester temperature was not actively controlled, but was maintained within about two degrees of the 35 °C setpoint by cycling of hot water through the heating coils every 80 minutes. Biogas production was quantified using a wet-tip gas meter manually calibrated to record in 100 mL increments using a 150 mL gas-tight syringe. The organic load applied to the fermenter was 216 g VS/d. AD1 was operated without an upstream fermenter as a conventional single-phase process, in contrast to the two phase configuration used with all other digesters. The biogas measurements from this digester include methane and carbon dioxide which would have been produced in the fermenter in the phase separated systems. Fermenter biogas production in the two-phase systems was not quantified.

AD6-b: Digester heating was accomplished using the passive control scheme of AD6-a. Biogas production was quantified as in AD6-a.

AD6-d: The digester SRT was reduced to 10 days from 20 days.

AD3-d: After the conclusion of the direct comparison experiment (Chapter 4), the solids separation method used to batch the feed to this digester was switched from screening to the centrifugation approach used in the AD6-series digesters. The active volume was maintained at 30 L and the SRT reduced to 10 days.

AD3S – AD6S: In order to examine the effect of operating digesters in series, AD6-d was drawn down to a 30 L active volume and fed the effluent from AD3-d. The total active volume of the resulting system was 60 L, with a 20 day SRT.

In each of these configurations, samples were collected and analyzed for TS/VS, VFAs, and biogas production and methane content. The resulting methane yields on a gram VS applied basis for each system variation operated during the course of this research, as well as three additional variations operated prior to this research (AD1, 2, and 4) are summarized in Table 17. The system yield (L CH_4 @ STP/g VS applied) was calculated based on the VS mass applied to the fermenter, while the digester yield was based on the VS mass reaching the digester. VS reduction occurring in the fermenter and diversion of fine suspended solids and VFAs to the PHA process was not accounted for by the digester yield metric. All adjustments of methane production volumes to STP were based on the assumption that the average temperature and pressure observed in the lab during the comparison experiment of Chapter 4 was representative of average conditions during previous experiments, so that the same correction factors were applied to each data set. AD1 was operated as a single-phase digester, and consequently the system and digester yields are similar.

Table 17. Summary of average digester performance.

Reactor	Volume (L)	System OLR target (g VS/d)	SRT (d)	CH4 Volume (L/d @ STP)	Digester OLR (g VS/d)	System CH_4 Yield	Digester CH_4 Yield
AD1	40	144	20	23.68	148	0.164	0.160
AD 2	40	216	16	23.77	168	0.110	0.142
AD 4	40	216	30	19.64	122	0.091	0.162
AD3-d	30	175	10	22.72	128	0.134	0.186
AD 6a	40	216	20	22.82	144	0.106	0.158
AD6b	40	175	20	20.35	128	0.116	0.159
AD6c	40	175	20	22.69	123	0.130	0.184
AD6-d	40	175	10	24.31	135	0.139	0.180
AD3S - AD6S	60	175	20	28.00	114	0.167	0.232

A.2 Comparison of Digester Yields

Ideally the data of Table 17 would have been collected using one reactor design that was as representative of a future full scale plant as possible. However, this research was conducted by several different individuals over a period of several years using different designs, a factor which has likely introduced some variability into the yield values. However, it is still possible to extract useful relationships between methane yield, digester SRT, and the fraction of fermenter effluent diverted to PHA production from an analysis of the data. The linear regression equations presented in the following sections were developed using the linear regression tools in Microsoft Excel, with the slope of the regression line declared significant at $p < 0.05$.

A high yield AD process is capable of generating more methane from a given quantity of substrate than a low yield process, and will consequently be more profitable to operate. AD methane yield is a function of digester SRT, with higher yields occurring at longer SRTs (Grady et al. 2011, Speece 2008). If the methane yields from Table 17 are used to develop a yield vs. SRT plot on both the digester and system basis (Figure 23), a relatively strong linear relationship emerges for system yield as a function of SRT ($R^2 = 0.721$, $p = 0.016$ for slope), while the regression has no predictive value ($R^2 = 0.159$, $p = 0.33$ for slope) for digester-based yield as a function of SRT.

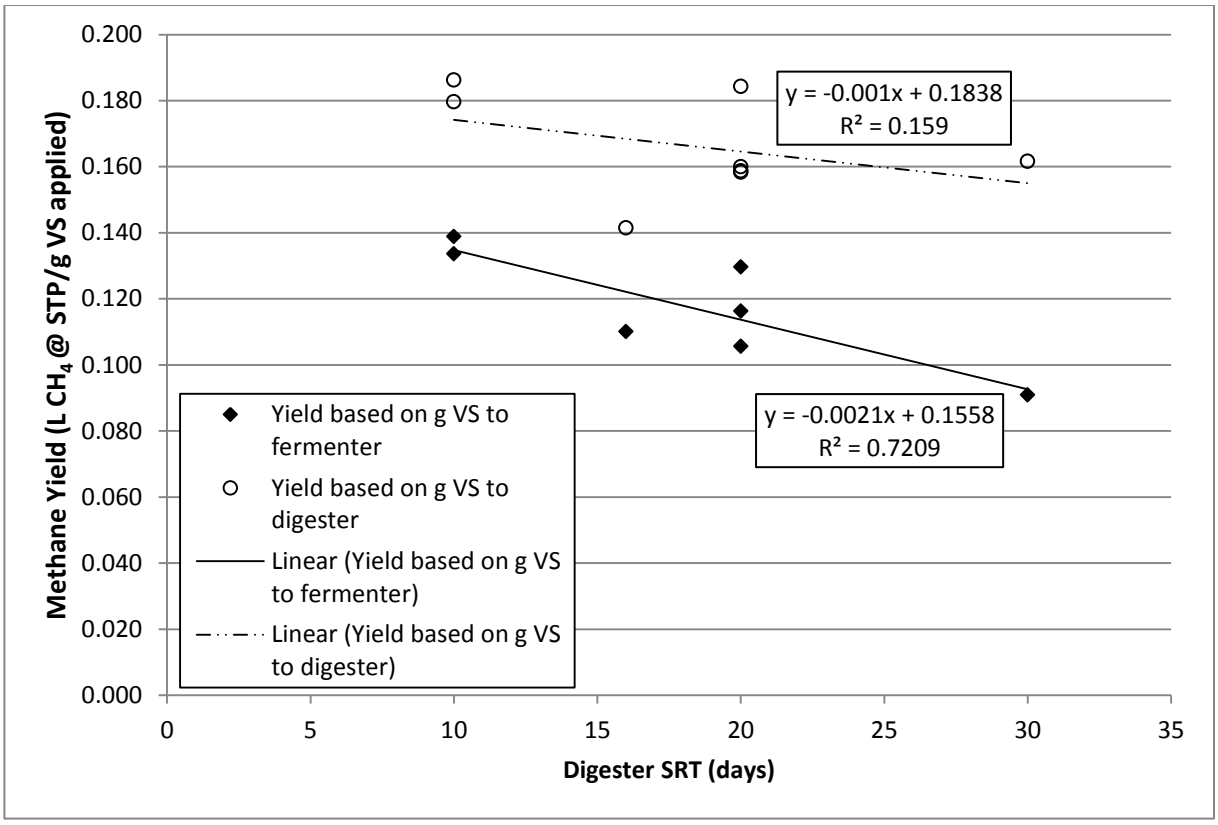

Figure 23. Yield-SRT relationships on a g VS applied to fermenter and g VS applied to digester basis.

However, the decreasing linear nature of the system yield-SRT curve and lack of correlation between digester SRT and yield is not what would be expected. As seen in Figure 24, typical yield-SRT curves are composed of a positive exponential phase at low SRT values that transitions to a gradually increasing linear phase at higher SRTs (Grady et al. 2011).

The form the system yield-SRT curve of Figure 23 takes is specific to our two-phase, PHA production coupled system. In this system, the fermenter was operated to produce 5 L of effluent per day regardless of digester operating parameters, while the volume of liquid effluent diverted to PHA production from the digester was controlled by varying the digester SRT through either the active volume or the feed/waste flow rate. Thus the feed of 20 day SRT digester would have a higher solids concentration than that of a 10 day SRT digester, but would also receive less of the VFA-rich liquid fraction of the fermenter effluent. As the SRT increased, the loss of methane production resulting from VFA diversion offset the gain in production achieved through increased VS reduction associated with the longer retention

time. This explains the lack of a clear association between digester yield and SRT, and the negative correlation between system yield and SRT shown in Figure 23.

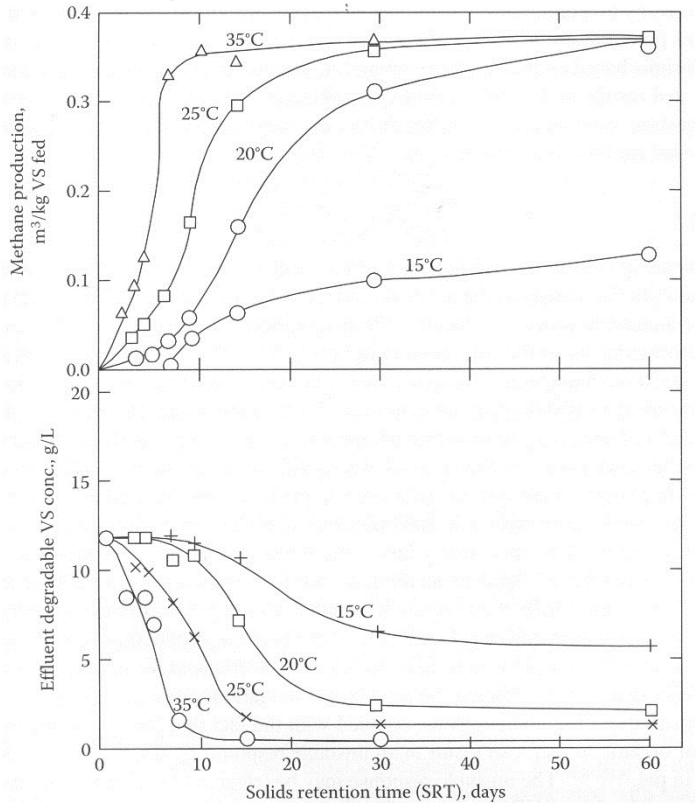

Figure 24. Typical plots of methane yield and effluent biodegradable VS concentration as functions of digester SRT in the 15-35 °C temperature range (Grady et al. 2011).

In a full-scale PHA/methane production plant, the size of the reactors would be fixed and the fractions of substrate directed to PHA and methane production will depend on which commodity generates more revenue. As commodity prices fluctuate, it would be desirable from an operational standpoint to be able to predict the impact on methane production of diverting different quantities of liquid fermenter effluent to PHA synthesis. Based on Figure 23, the dominant factor affecting methane production is the fraction of liquid effluent diverted. The data of Table 17 can be used to calculate the percentage of the 5 L of fermenter effluent produced daily that was diverted to PHA production for each digestion system (Table 18). The diversion fraction of AD1 is zero because it was operated as a single-phase digester with no PHA production load. If the system yield is plotted as a function of the diversion percentage, the strength of the linear relationship increases (Figure 25, R^2 =

0.82, p = 0.0018 for slope), confirming the impact of the degree of diversion on yield. Although scatter exists around replicate values of diversion percentage, likely due to differences in system operation and/or configuration (e.g. active temperature control, mixing intensity, etc.), the resulting regression equation could still be used to predict the impact of changes to the PHA process on methane production volumes with a reasonable degree of confidence, allowing revenue estimates to be made for a range of operational conditions. The linear regression applies only to the single stage CSTR data due to differences in AD system volume and improved efficiency associated with the series configuration, as discussed in Appendix A.3.

Table 18. Feed diversion fractions and methane yields used to generate Figure 25.

Digester	Feed fraction (% of 5 L) diverted to PHA reactors	Yield (L CH4/g VS fed (system))
AD1	0.0%	0.164
AD 2	50.0%	0.110
AD 4	73.3%	0.091
AD3-e	40.0%	0.134
AD 6a	60.0%	0.106
AD6b	60.0%	0.116
AD6c	60.0%	0.130
AD6-e	20.0%	0.139
AD3s-AD6s series	40.0%	0.167

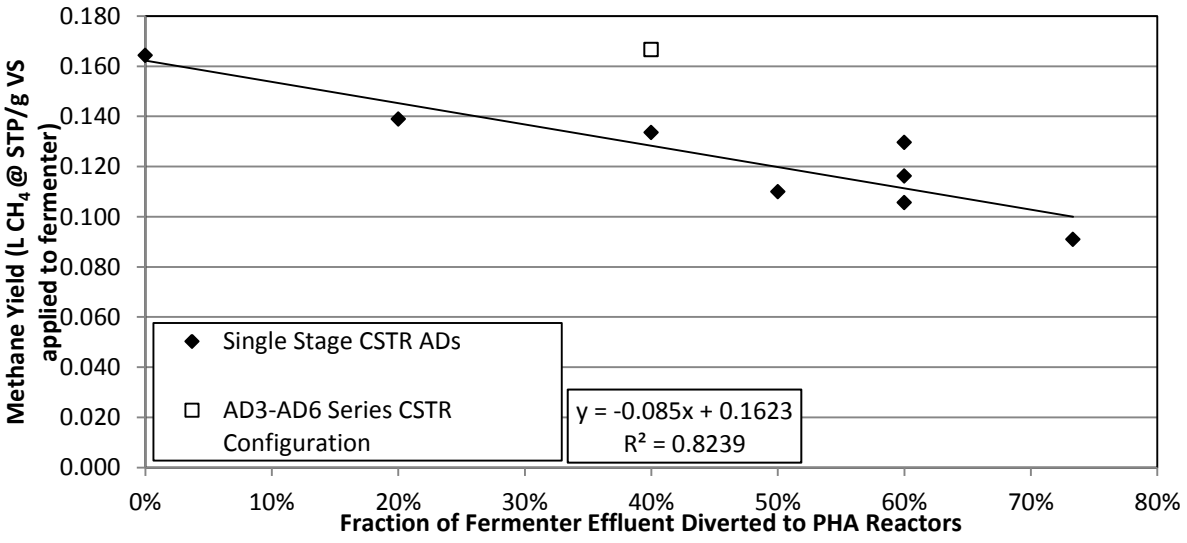

Figure 25. Methane yield per gram VS applied to fermenter.

A.3 Series Digestion

It is well established that a series of CSTRs will provide better process efficiency for a given net reactor volume than a single CSTR, and that if enough CSTRs are placed in series, the behavior of the system of reactors will approach that of a plug flow reactor (Crittenden et al. 2012). This effect is due to the increased concentration of reactants in each series CSTR relative to a single CSTR, which increases the overall rate of reaction, and the fact the actual hydraulic retention time more closely approaches the theoretical retention time as the number of reactors is increased. Although the plug flow system is a common configuration for manure digesters in the US (EPA 2010), the lack of mixing in plug flow digesters has been associated with operational problems, including solids retention and crust formation (Artrip et al. 2013), making it difficult to utilize the kinetic advantage of the process. In Germany, a world leader in biogas production from agricultural sources, the most common digester configuration used for agricultural waste treatment is the CSTR (Weiland 2010), probably due to the operational problems inherent in plug flow systems. Therefore, it might be expected a series of CSTRs could be utilized in manure digestion to increase both treatment efficiency and methane production. Others have examined this possibility (Boe and Angelidaki 2009, Kaparaju et al. 2009, Massey and Pohland 1978, Wen et al. 2007), although the research in these papers focused on using two reactors to separate the acidogenic and methanogenic phases (the two phase system), or operation of multiple methanogenic reactors in series. This preliminary research focused on coupling a fermenter to two methanogenic reactors in series (i.e., a two-phase system utilizing methanogenic reactors in series), which to our knowledge has not been previously examined.

The research was conducted using one of the fermenters and the two large digesters described in Chapter 4.2.1. The fermenter was operated at a 20 L active volume and 4 day SRT, while each digester (AD3-S and AD6-S) was operated at a 30 L active volume and a 10 day SRT. The organic load applied to the fermenter was 175 g VS/d. Two liters of liquid fermenter effluent was directed daily to PHA production (40% diversion), and solids separation was accomplished using centrifugation. The total system SRT (fermenter and digesters) was 24 days, or 20 days for the digesters only. All other operational conditions for

the three reactors were unchanged from those of System 1 described Chapter 4.2.1. Biogas production volumes and methane and carbon dioxide content were measured daily, while sampling for TS/VS and VFAs was conducted approximately once per week. Analytical techniques were the same as described in Chapter 4.2.2.

The primary goals of the series digestion experiment were:

1. Quantification of average daily methane production and yield
2. Comparison of performance with that of other lab AD systems
3. Demonstration of the feasibility of operating methanogenic reactors in series in a two-phase AD configuration

Methane production by both digesters was stable during the 42-day data collection period (Figure 26), and the average total production volume was 29.2 L/d, which was the highest observed in any of the lab AD systems. Effluent VFA concentrations were similar to those observed in AD6-c (~50 mg/L as COD on average in both systems). Scum formation in both digesters was minimal, there were no signs of foaming, and digester stability was exceptional overall, confirming the viability of the two-phase serial digestion approach.

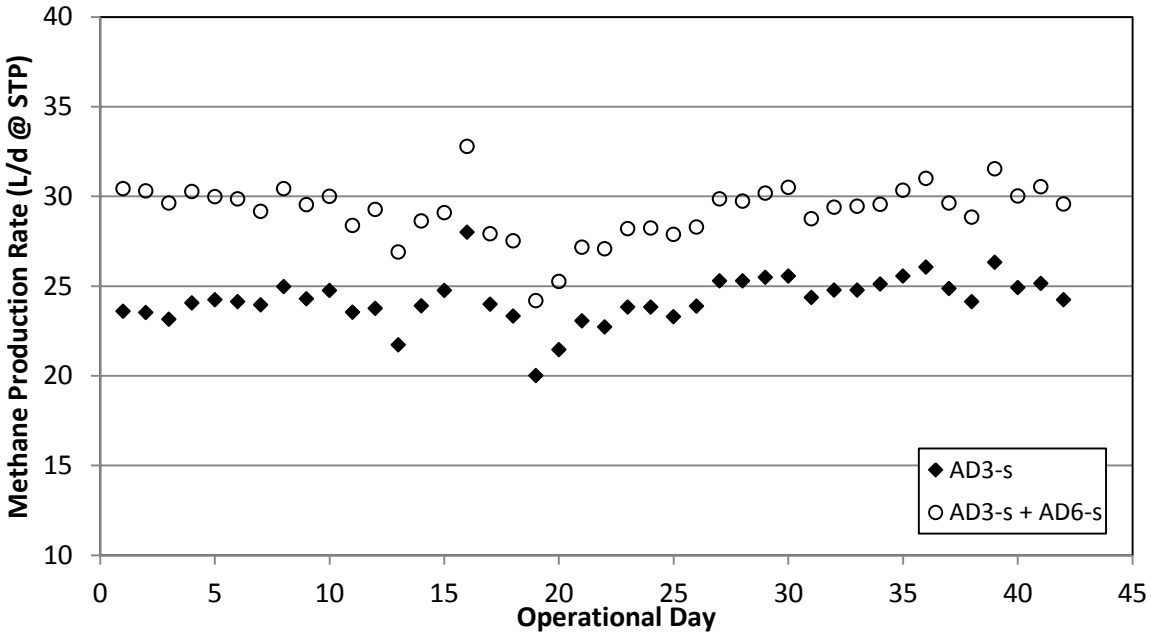

Figure 26. Methane production of serial AD configuration.

The methane yield of the combined system was the highest observed in any of the lab AD configurations (Table 17, Figure 25). Some of the increase in methane production was due to increasing the SRT at the 40% diversion level, with the remainder due to the greater efficiency of the series configuration. All single stage digesters operated at a 20-day SRT were limited to 60% (i.e. 3 L of effluent to PHA reactors) diversion due to the fact the maximum individual digester volume was 40 L. As this was preliminary research, operation of two AD systems simultaneously to isolate the effect of the series configuration was not performed, and the impact was instead estimated using previously collected data.

In order to make the comparison, an estimate of the quantity of methane that would be produced by a single 60 L digester operated at a 20 day SRT is needed. The best performing 40 L, 20 day SRT digester was AD6-c, and the average methane production of this digester (22.7 L/d) can be used as the basis of the estimate if the assumption is made that the additional methane produced by the hypothetical 60 L system will be derived primarily from the VFAs present in the additional 1 L of digester feed. Digesters AD3-d and AD6-d (Appendix A.1) were used to test this assumption, with each operated at the same SRT but at different volumes so that one digester received an additional liter of feed. The average difference in methane production between the two digesters was divided by the average VFA as COD concentration in the liquid fraction of the digester feed, and the yield was found to be 0.34 L CH_4/g VFA COD, which is quite close to the theoretical value of 0.35 L CH_4/g COD (Speece 2008). This indicates it is reasonable to use the theoretical COD equivalent of methane to estimate the impact of different levels of fermenter effluent diversion on methane production for a given SRT. Using this approach in combination with the average VFA as COD concentration of all fermenter samples taken at the 175 g VS/d loading rate (4964 +/- 900 mg/L as COD, n = 98), the estimated methane production of a single 60 L digester is 24.4 L/d. Comparison of the difference in methane production between the theoretical 60 L single-stage digester and the observed methane production of the 60 L serial digester results in an estimated increase in yield due to the series configuration of approximately 16%. The improvement in AD performance using series digesters correlates with increased VS

destruction vs. AD6-c (38.2% vs. 34.9%) and AD1 (conventional single phase digester, 55.7% vs. 43.7%, including VS destruction occurring in the fermenter).

The methane yield of the AD3s-AD6s configuration on a system basis was comparable to that of AD1 (Table 17), which was operated as a conventional digester not subjected to the additional substrate demand exerted by PHA production. The system yield of AD3s-AD6s was approximately 70% of the average maximum methane yield of dairy manure as adopted by the IPCC (IPCC 1997). If the methane production occurring in the fermenter, as estimated in Chapter 4.3.1, is taken into account the expected yield would be about 85% of the maximum.

In summary, the data indicates a two-phase AD configuration utilizing two digesters operated in series after the fermenter would be capable of producing the same amount of methane from a given quantity of VS as a conventional single phase digester, despite the diversion of as much as 40% of soluble COD to PHA production. Alternatively, if operated as a stand-alone system without PHA production, a serial AD configuration would be capable of a substantially greater methane yield than a conventional AD system or a two-phase system utilizing a single digester. The initial capital cost of a serial configuration would be somewhat greater due to the need for additional reactors, pumps, piping, valves, etc., and an economic analysis should be conducted to determine which configuration will provide the greatest return on investment.

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