

Development of New Digester Technologies for Improved Adoption and Cost Reduction

C. Frear, W. Liao, Z. Wang, J. Ma, U. Zaher, T. Ewing, C. Li, L. Yu and S. Chen

Chapter Introduction

Previous chapters within the Climate Friendly Farming (CFF) anaerobic digestion (AD) section have focused on the development of new auxiliary technologies that work in conjunction with the core digester. These technologies allow for the production of numerous co-products that enhance project economics and improve sustainability. In addition to these important technology developments, there is an equally strong need for improving digester design to reduce project capital costs and to allow for application of the core technology to waste materials other than scrape manure. Improved digester designs could accelerate the adoption of AD technology generating benefits for air and water quality and the climate.

CFF research on new AD designs has focused on three areas:

- Development of a vertical, mixed, plug-flow reactor capable of biogas yields and operation reliability equivalent to its original horizontal form but at reduced capital costs and land footprint;
- Development of a high-rate reactor tailored for treatment of dilute manure streams using fibers in the manure as natural biomass carriers for improved kinetics and project costs;
- Development of a new high-solids reactor designed for treatment of the organic fraction of municipal solids waste and/or industrial food processing wastes.

This chapter focuses on the research efforts within these three design areas and the accomplishments to date in regard to science, engineering and commercialization.

Vertical Mixed Plug-Flow Reactor

Introduction

The mixed, plug-flow reactor evaluated within the CFF project is, because of its unique mixing, capable of being applied to a variety of manures and wastes containing a wide range of solids and volatile content. In particular it has become an industry standard in the US for treatment of scraped dairy manure. Several design and operating elements within the mixed plug-flow system allow for the design to compare favorably with common complete-mix reactors, particularly in regard to reduction of short-circuit flows, more reliable volatile and pathogenic destruction, tolerance to a variety of solid and fixed solid inputs, and lowered parasitic electrical loading (US-EPA, 2005). Despite this, concerns do exist in regard to the capital costs for any commercial AD which is limiting the market development for ADs, particularly on smaller dairy farms (Figure 11.1). Simply constructing a less

expensive system should not be the goal as the system's resulting poor performance could make an even less financially feasible system. From a financial viewpoint, finding a construction method that would decrease the capital cost without conceding performance objectives is the primary goal.

To that end, the CFF project has teamed with Andgar Corporation and GHD, Inc., to design, operate and test a mobile, small-scale vertical version of their patented plug-flow design (Dvorak, 2008). The primary objective of the study was to validate the vertical plug-flow concept in regard to maintaining effective performance while potentially reducing capital and material costs, especially for smaller farms. Another purpose for constructing the small vertical plug flow reactor is the opportunity it provides to research different operating temperatures, feedstock variances, loading rates, and retention times without affecting a large-scale system. With a reactor size of 4,500 gallons, operating parameters can be adjusted to determine the point where biological failure occurs, then drained and filled with new material to begin a new test. With commercial systems having a reactor size of millions of gallons, draining the reactor and starting a new test is a considerably more cumbersome and economically unviable as customer bottom-lines are dependent upon its continuous operation. The small vertical plug flow reactor is also sized small enough to be transported and become a demonstration piece for on-site waste handling.

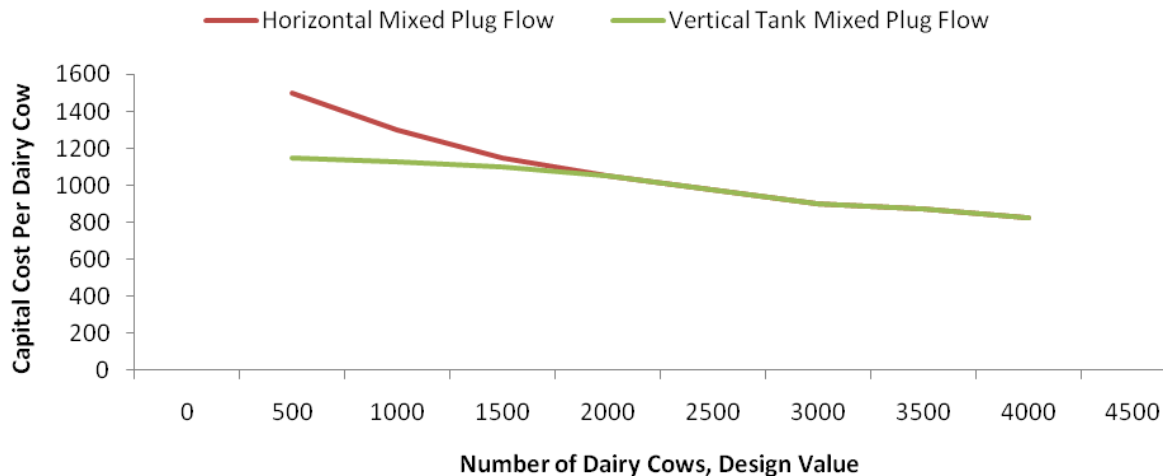


Figure 11.1: Comparative estimated capital costs for vertical tank mixed plug flow and horizontal mixed plug flow.

Design, Construction and Operation

The design of the vertical tank mixed plug flow reactor was intended to be large enough to replicate a commercial project but at the same time be small enough to be easily transported. The tank is constructed of mild steel and coated to prevent deterioration from the biogas. Internal equipment is designed and constructed to mimic the scale of the larger commercial systems. The tank has automated

temperature controls to maintain the specified temperature and has the ability to measure the gas volume produced to determine performance. The tank is operated similar to commercial sized systems with influent added throughout the day. The volume of effluent added per day can be adjusted to change the retention time within the tank. Effluent removed from the tank is sent to a lab for third party testing to determine the destruction of volatile solids and pathogen reduction.

Conclusion

As of December 2009, construction of the mobile unit was just nearing completion, with scheduled testing of the vertical concept, analysis of comparative material and capital costs as well as its use as a permanent, mobile pilot-scale test-bed to begin and continue throughout 2010 and 2011. Besides its continued use as a test-bed for new feedstocks and determining optimal loading rates and retention times, it is hoped that a new generation of mixed plug-flow digesters of vertical design can be marketed and implemented in the US, allowing for continued effective performance but at a reduced footprint and lower capital cost, opening the door to installations at smaller CAFO dairies.

High-Rate Reactor for Use with Dilute Waste Streams

Introduction

Presently only a hundred or so ADs are operating on commercial dairies in the US serving only 3.8% of targeted dairy CAFOs (confined animal feeding operations) and 1.7% of the total cow population in the U.S. (US EPA, 2007). This poor adoption rate has been attributed to high capital costs, past concerns regarding reliability of operation and performance, and the recalcitrance of the high fiber content within dairy manure (US EPA, 2007). Another important concern is the ability of AD technology to effectively work within the climate zones where dairies are located, and to accept the types of manure produced by dairies, mainly scraped manure (6-10% total solids) and flushed (2% total solids) (Burke, 2001).

The two primary industry-standard AD technologies being utilized on farms today are heated plug-flow and non-heated covered lagoon. Heated plug-flow systems can be installed in any climate zone and handle manures with relatively high solids content, including scrape manure. Non-heated covered lagoon technologies work only in warm climate zones and are ideal for dilute manures (US EPA, 2007). Notably, neither technology is ideal for large cold-climate dairies that use flushed handling systems (Burke, 2001).

Flushed systems have low solids content because stored lagoon water hydraulically moves the manure through the farm. The hydraulic force and subsequent dilution reduces labor requirements and mechanical failures but negatively impacts potential downstream AD treatment. The increased volume of manure is difficult to heat economically, and the AD system generally must be larger to accommodate the high volume of manure and water. As a result of these concerns, farms wishing to

implement AD have either been forced to switch, at additional cost, to a scrape system or to utilize one of two modified digester technologies, both of which only partially address the problem.

The first modification settles and concentrates the manure prior to digestion in a typical plug-flow reactor while leaving the liquid portion untreated. Several modified plug-flow digesters (modified GHD mixed plug-flow with pre-settling tank for concentration of solids) have been built in the US for this purpose, resulting in a concentrate at the desired level of 6-10% total solids (TS). The drawback to this approach is that a considerable portion of readily digestible organic matter is actually dissolved in the liquid phase of flushed dairy manure (Mackie et al., 1998), and its untreated discharge reduces the volume of recovered methane gas, with negative impacts on greenhouse gas mitigation as well as odor concerns and air and water quality (Wilkie et al., 2004), although use of a closed-loop flush alley approach has minimized some of these concerns.

For this reason, a second approach has been proposed, to separate out the majority of solids and treat only the liquid fraction in a biofilm-enabled, high-rate reactor operated at psychrophilic temperatures (temperatures that require organisms that thrive at relatively low temperatures, in our case, below 27°C). To date this approach has only been commercialized on a single small dairy at the University of Florida (Wilkie et al., 2004). Since the liquid portion still represents a significant volume, the system must rely on biofilm growth to enhance microbial activity and reduce reactor volume as well as rely on low temperatures to secure an effective energy balance (Vartak et al., 1997). Support media with high specific area have been used to provide a surface for biofilm growth (Vartak et al., 1997). These media could be clogged by dairy manure containing solids, reducing biomass retention and short-circuiting flow around the medium; to prevent this, a strict screening process (mechanical separation and/or settling) has been placed in front of the biofilm digester to exclude manure solids (Wilkie et al., 2004). This approach therefore also results in incomplete treatment and a potentially substantial reduction in methane production with previous studies reporting as much as 54 to 80% of methane production found in excluded manure solids (Chastain et al., 2001; Hills and Kayhanian, 1985).

Studies in rumen ecology show that fibrous solids function as substrate and support media for microbial organisms to grow and be retained in the rumen environment (McAllister et al., 1994), which implies that fibrous solids in dairy manure should have the potential to play the same role within a biofilm digester. If successful, this strategy offers the possibility of digesting the entirety of flushed manure simultaneously in one digester. However, to date, very limited information has been available with regard to the biofilm support role dairy manure containing fibrous solids can play. The objectives, then, of this study were to use both laboratory and pilot-scale experiments to: (1) better ascertain the properties and biogas potential of flush dairy manure; (2) confirm the potential of using fibrous particles as a

bacterial support; and (3) confirm the viability of digesting the entire flush stream in a new high-rate reactor utilizing the fibrous material as support media

Biogas Potential and Microbial Population Distributions in Flushed Dairy Manure

Three Zone Formation in Flushed Dairy Manure

After thirty minutes, homogenous fresh flushed dairy manure settles into three distinct zones (top, middle and bottom), each with specific particle size and property characteristics (Figure 11.2). In general, the large fibrous solids settled to the bottom while fine particles remained suspended in the top liquid zone giving a visible turbidity. The middle section formed a transition zone formed in between the top and bottom zones with a graduated small to large particle distribution pattern. Volumetric fractions of these three zones were about 85%, 5% and 10% from the top to the bottom, indicating an over eight-fold dilution of fresh manure with flush water during the flush handling process. Bottom zone solids demonstrated a 20-fold faster settling velocity than those in the middle zone while, by contrast, there were nearly 11 g L^{-1} fine particles that remained suspended in the top zone throughout the tested duration (Table 11.1). Sludge volume index (SVI) is a commonly used parameter for evaluating how readily mixed liquor will settle (e.g. a high SVI indicates poor settling). Measured SVIs showed that solids in the middle and bottom zones were four times more likely to settle than those in the top zone (Table 11.1). Organic content (OC) as defined by a ratio of volatile solids/total solids (VS/TS) and shown in Table 11.1 reveals that the top zone contained only a 41% OC, quite a bit lower than the 71 to 87% OC of the middle and bottom zones, respectively. This indicates that many of the inorganic nutrients within the manure are retained within the fine particles that stay suspended in the top zone — an important nutrient management conclusion that has also been commented on by Wright (2005) and Meyer et al. (2007).

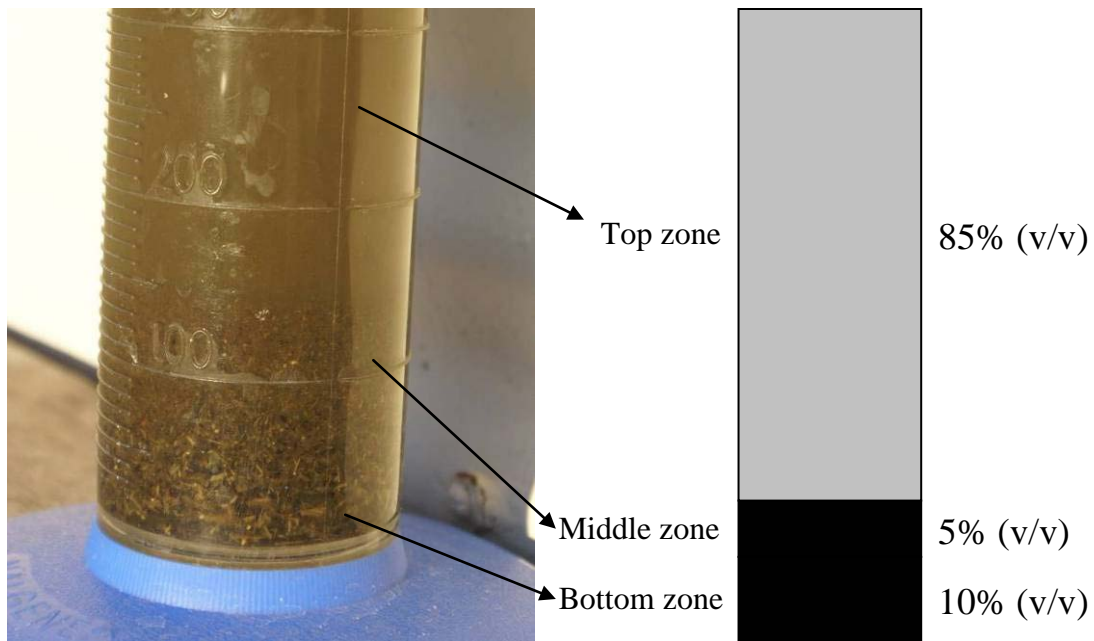


Figure 11.2: Three fractionated zones of flush dairy manure.

Table 11.1: Intrinsic properties of three zones of flushed dairy manure

| Intrinsic properties | Three zones in 30 min settled flushed dairy manure | | |
|--|--|---------------------|---------------------|
| | Top zone | Middle zone | Bottom zone |
| Specific gravity | 1.0019 ± 0.0012 | 1.0072 ± 0.0030 | 1.0087 ± 0.0141 |
| Zone settling velocity (m h^{-1}) | 0.00 ± 0.03 | 2.33 ± 0.06 | 45.34 ± 1.68 |
| Water content (g g^{-1}) | 0.99 ± 0.02 | 0.96 ± 0.01 | 0.90 ± 0.05 |
| SVI (mg g^{-1}) | 102.02 ± 3.35 | 24.67 ± 2.45 | 9.32 ± 0.54 |
| TS (g L^{-1}) | 11.30 ± 0.51 | 38.05 ± 1.10 | 105.11 ± 7.44 |
| VS (g L^{-1}) | 6.66 ± 0.41 | 26.93 ± 0.14 | 91.91 ± 7.11 |
| Organic content (%) | 41.47 ± 0.69 | 71.39 ± 1.88 | 86.94 ± 1.29 |
| COD (g L^{-1}) | 8.95 ± 0.45 | 47.47 ± 0.86 | 70.69 ± 0.99 |
| Volumetric fraction | $85\% \pm 2\%$ | $5\% \pm 1\%$ | $10\% \pm 1\%$ |

SVI = sludge volume index; TS = total solids; VS = volatile solids; Organic content = VS/TS; COD = chemical oxygen demand

Particle Size Distributions

The three zones in Figure 11.2 were separated and homogenized for particle size distribution analysis. As can be seen from Figure 11.3, the top zone is dominated by small particles ranging in diameter from 0.04 to 0.10 mm while the middle zone is uniformly distributed with solids between 0.04 and 2.00 mm in size, and the bottom zone has mainly 0.59 to 5.00 mm solids.

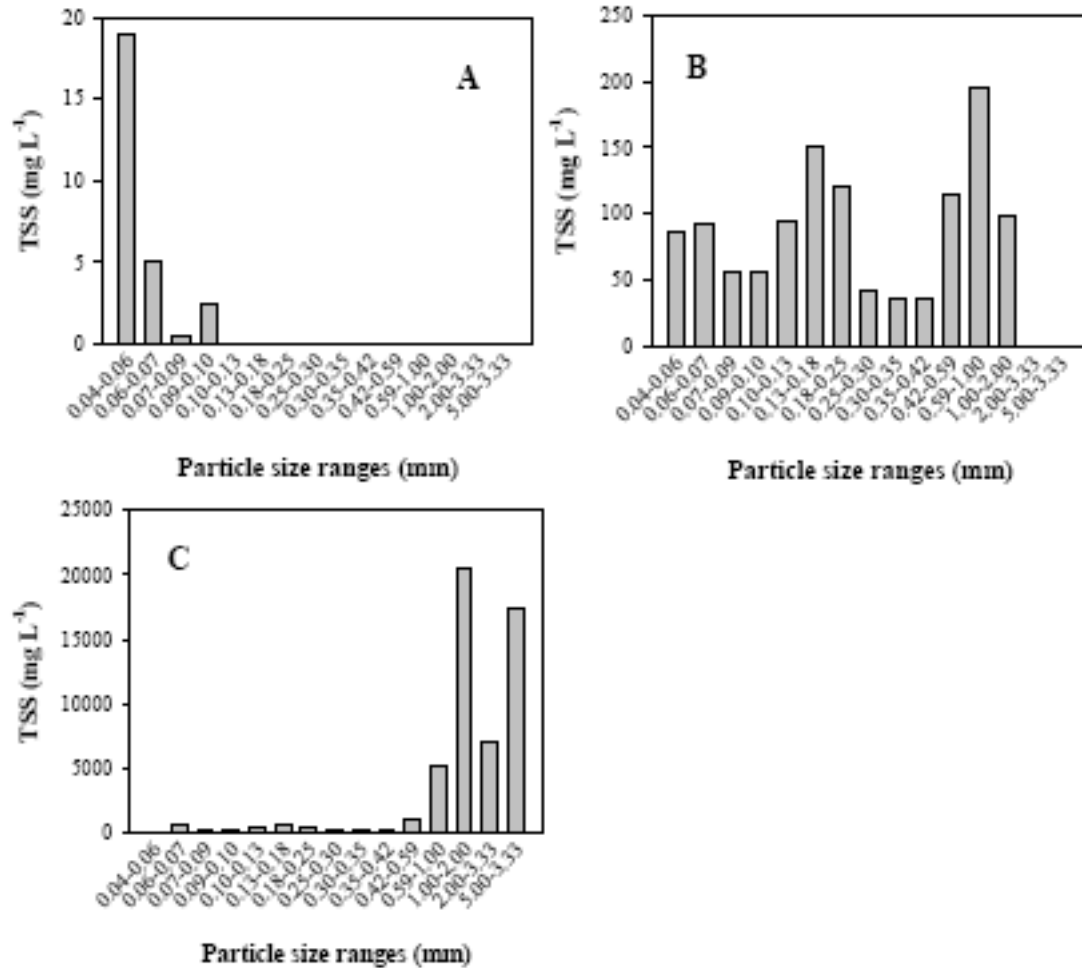


Figure 11.3: Particle size distribution in three zones (A: top, B: middle; C: bottom) of flushed dairy manure.

It should be pointed out that the solids concentration in the bottom zone is hundreds of times the concentration in the middle zone, implying that the majority of the solid mass is concentrated in large size particles. This finding is in line with results from Wright (2005) who showed that 0.63 mm particles and larger predominate in dairy manure solids. Observation of the particles clearly shows that the majority of the large particles contained in the middle and bottom zones are forage-like plant fibers resulting from undigested roughage and/or bedding materials.

Biogas Production Capacity Distribution

Samples from each of the three zones were tested for biogas production capacity. Results showed that unit volumes of mixed liquor from the middle and bottom zones generated more than 4 and 6 times the biogas than that from the top zone, indicating that greater biogas production capacity is held in the manure solids (Figure 11.4). This observation might be associated with the relatively high VS and COD contents held in the solid phase of the middle and bottom zones (Table 11.1).

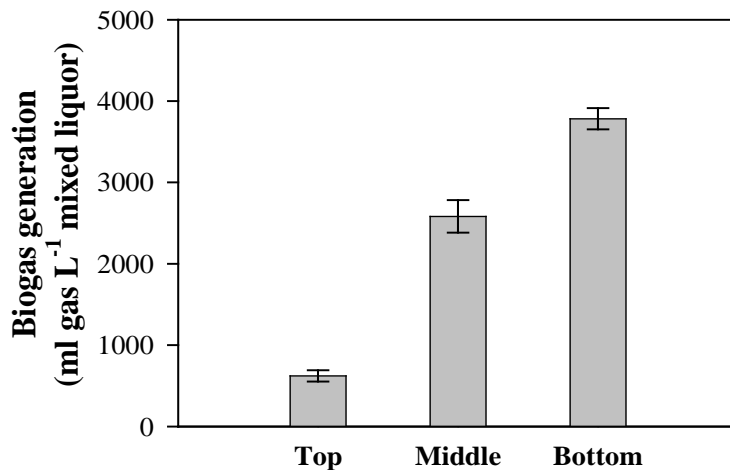


Figure 11.4: Biogas generation from unit mixed liquor volume in three zones of flushed manure

Adjusting the unit volume biogas measurements by the respective volumetric fractions for each zone allows for an overview of the total biogas generation capacity from each of the three zones (Figure 11.5). Interestingly, the biogas generation capacities for the top liquid layer and the combined two solid layers are very comparable, being split almost equally between each other. Figure 11.5 indicates that roughly 50% of readily recoverable bioenergy might have been excluded in existing dairy AD processes that selectively treat either the solids or liquid phases of flushed dairy manure.

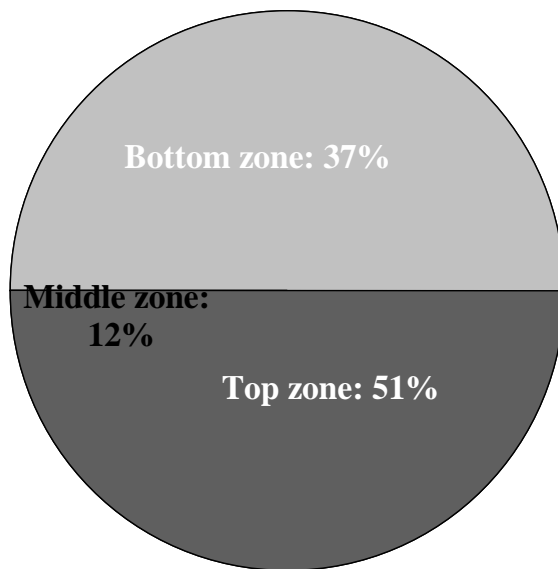


Figure 11.5: Distribution of biogas production capacity from the three zones of flushed dairy manure.

Visualization of Microbial Distribution in Flushed Dairy Manure

Confocal laser scanning microscope (CLSM) images were used to explore microbial distributions in the flush dairy manure. Figure 11.6 shows the microbial distribution labeled with SYTO 9 (green), with the majority of microbial organisms living in an attached state on the dairy manure fiber, even though some are interspersed in the bulk liquid.

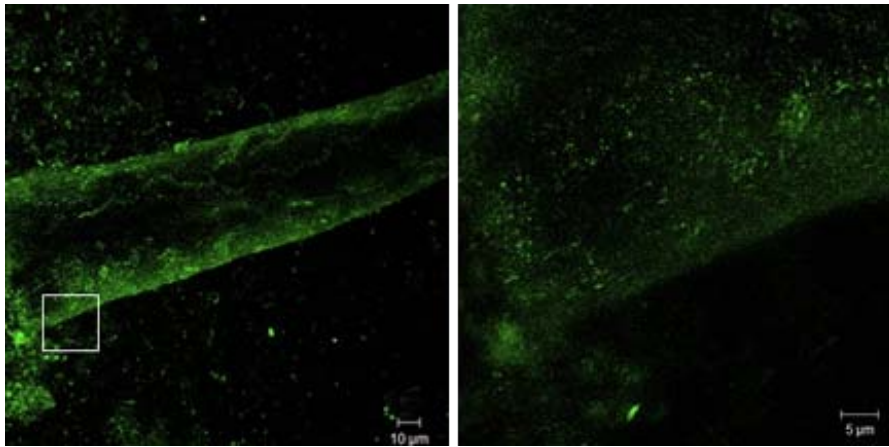


Figure 11.6: CLSM visualization of microbial distribution on flushed dairy manure fiber (left), and a zoom into rectangle area on the left image (right).

A magnified image of the area in the white rectangle in Figure 11.6 (left) further reveals microorganisms immobilized on the fiber surface (Figure 11.6 right) and in particular their unevenly distributed and pitted array on the fiber surface. This observed microbial distribution is analogous to those visualized in rumen environment; rumen microorganisms were always found randomly colonized on fiber surfaces with notable hydrolytic pitting into the surface of the fiber (Dinsdale et al., 1978; Mcallister et al., 1990; Shinkai and Kobayashi, 2007; Weimer et al., 2006).

Methanogen Distribution in Flushed Dairy Manure

The level of activity of acetate-utilizing methanogens was measured in each of the three zones of flushed manure via anaerobic respirometry with sodium acetate as the carbon source. As shown in Figure 11.7, three very different methane generation profiles were demonstrated. All samples were initiated with prolonged lag phases which might be due to the presence of low dissolved oxygen (DO) levels in the flushed manure, which could have suppressed methanogen activity (Gerritse and Gottschal, 1993) (Table 11.2). Figure 11.7 seems to suggest that dairy manure methanogens are able to overcome this inhibition effect after 50 to 90 hours of incubation.

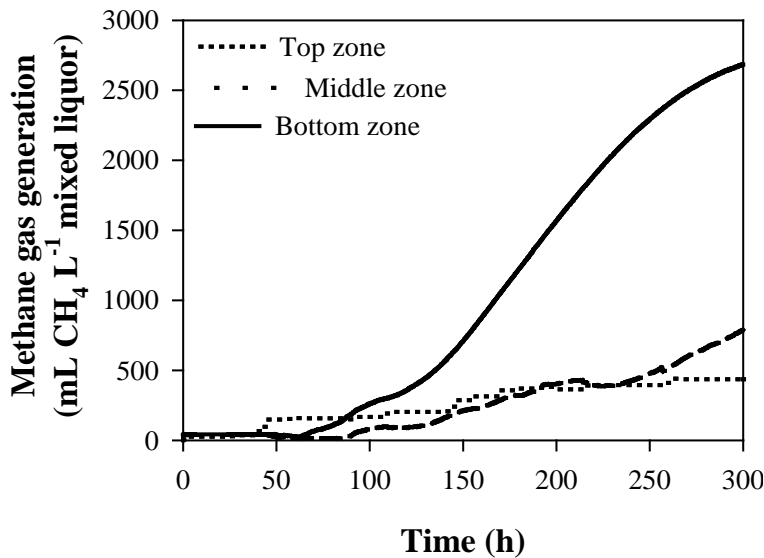


Figure 11.7: Respirometer-measured methane production capacity from three zones of settled dairy manure samples.

After the lag phase, methane gas was generated in all three zones with a maximum methane generation achieved in the sample from the bottom zone, implying that a considerable amount of methanogens are contained in this zone. In comparison, less than one third of the methane gas generation was measured in the middle and top zones, even though the same amount of acetate was provided as a carbon source. Zero order methanogenic activity showed that methanogenic activity in the bottom zone was about 3 and 34 times those in the middle and top zones, respectively (Figure 11.8).

Table 11.2: Overview of flush manure system at WSU dairy center

| Parameters | Mean | SDV |
|---|-------|--------|
| Cow heads | 180 | ± 15 |
| Water used (L cow ⁻¹ d ⁻¹) | 428 | ± 76 |
| Wastewater (L cow ⁻¹ d ⁻¹) | 473 | ± 95 |
| Flushing times per day | 2 | - |
| TS (g L ⁻¹) | 18.88 | ± 4.29 |
| VS (g L ⁻¹) | 12.57 | ± 3.00 |
| Total COD (g L ⁻¹) | 17.05 | ± 6.97 |
| Soluble COD (g L ⁻¹) | 5.36 | ± 1.59 |
| Dissolved oxygen (mg L ⁻¹) | 0.81 | ± 0.02 |
| Alkalinity (g CaCO ₃ L ⁻¹) | 4.41 | ± 0.70 |
| Manure temperature (°C) | 11.20 | ± 5.90 |
| pH | 7.78 | ± 0.20 |

Evaluation of these values against the volumetric fractions of each of the three zones showed that 70% of the acetate-utilizing methanogens were associated with the 10% volume of the bottom zone, while methanogens suspended in the liquid phase only accounted for 17% of the total population (Figure 11.9) It seems very likely that the majority of the acetate-utilizing methanogens in flushed dairy manure might therefore have been lost in existing AD processes that exclude the solids phase. This conclusion is supported by findings from McGarvey et al. (2004) who found that bacterial numbers and diversity in flushed dairy manure dropped to marginal level after solids removal in a separator pit.

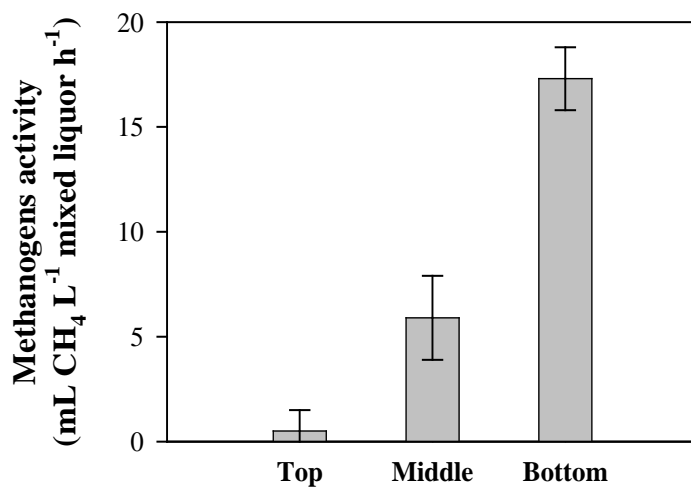


Figure 11.8: Maximum methane production rates from three zones of flushed dairy manure samples.

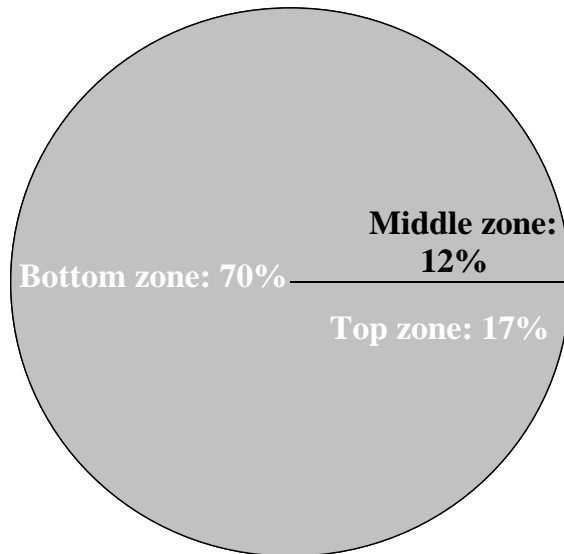


Figure 11.9: Acetate-utilizing methanogens distributions in the three zones of flushed dairy manure

Mechanism of Microbial Immobilization on Dairy Manure Solids

Flushed dairy manure consists of a very large volume of liquid phase containing suspended inorganic fine particles and a solids phase dominated by large fibrous solids. Most of the microorganisms within the dairy manure are immobilized on those undigested fibrous solids (Figure 11.6). This distribution pattern should be closely associated with the mechanism for biodegradation of insoluble substrates. It has been understood that effective biodegradation of insoluble substrates such as those in cattle fed forage requires functional microorganisms to remain in stable juxtaposition to the substrate surface as well as to additional cooperative microorganisms (Costerton, 1992). Biofilm visualized in Figure 11.6 appears to re-create this juxtaposition in the solids portion of flush dairy manure. Kinetic evidence from Figures 11.7 and 11.8 supports the conclusion that dairy manure methanogens are closely associated with the manure solids phase, even after the intensive hydraulic-action during the flushing process.

Implications for Dairy AD Process Design

A high rate AD process driven by high biomass retention instead of high temperature appears to be an economical approach for methane recovery from flushed dairy manure. The high affinity of microbes to dairy manure fibrous solids seems to suggest that the fibrous solids can act as a natural biofilm support medium for high biomass retention, thus eliminating the need for external media that might clog and add cost. Forage-like straw fibers have previously been evaluated as excellent support medium for methanogenic biofilm establishment, demonstrating the highest methane production rate in a comparative study on straw, glass and plastic materials (Andersson and Bjornsson, 2002). Likewise, straw materials have also been found to act as effective medium for immobilization of other types of

biomass (Svensson et al., 2007; Yoon et al., 2007). It is possible that fibrous solids could function as both substrate and support medium for biomass growth and retention in a bioreactor, like the AD mechanism functioning in the rumen (Figure 11.10). If so, it would be possible to achieve methane production from both solids and liquid phases of flushed dairy manure in one digester.

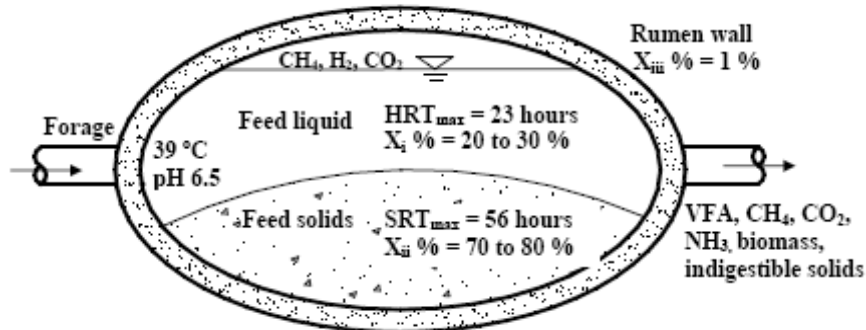


Figure 11.10: Schematic demonstration of cattle rumen ecology.

Studies of rumen ecology show that rumen microorganisms can be categorized into three groups depending on their location, i.e. i) microorganisms that inhabit liquid; ii) microorganisms that inhabit solids, and iii) microorganisms that inhabit the rumen wall (Cheng et al., 1995). It has been estimated that about 70 to 80% of rumen microorganisms reside in the rumen solids phase, with only 20 to 30% associated with liquid (Craig et al., 1987; Forsberg and Lam, 1977). These findings appear to be in direct accordance with our results showing the distribution of the acetate-utilizing methanogens in dairy manure as shown in Figure 11.9. It is worth mentioning that the majority of microorganisms in the liquid phase in rumens are believed to be detached from rumen solids (Latham, 1980). It is these detached microorganisms that initiate attachment to newly ingested solids, but microorganisms that inhabit the rumen solid phase are responsible for principal feed forage digestion (Latham, 1980).

For slow growing ruminal microbes like methanogens to be sustained in the rumen, their rumen retention time must be greater than their generation time. As most rumen microbial organisms are closely associated with ingested solids, the microbial retention time should be close to the feed forage retention time in the rumen. Dairy cattle rumen usually contain forage with a solids concentration ranging from 10 to 15% (Bath et al., 1966), and their rumen retention time depends on feed loading and particle size (Owens and Goetsch, 1986). Rumen studies have confirmed that the solids retention (SRT) time in the rumen is about 2 to 3 times that of the feed liquid hydraulic retention time (HRT), and the maximum rumen SRT and HRT are 56 and 23 hours, respectively (Owens and Goetsch, 1986). When compared with minimum generation times of typical AD anaerobes listed in Table 11.3, it is evident that the rumen maximum HRT is actually shorter than the minimum generation time of fermentative and methanogenic microorganisms. In

other words, there is no chance for ruminal microorganisms to sustain themselves in the liquid phase of the rumen environment. Hence, only by attachment to ingested solids could rumen microbes survive.

The uncoupled rumen HRT and SRT appear to provide a hydraulic selection pressure, favoring a microbial ecology that is capable of and in need of attachment on feed solids. In fact, the same mode of selection pressure has been utilized in bioreactors for successful microbial immobilization. For instance, sequencing batch reactors (SBR) and upflow anaerobic sludge beds (UASB) have typical operations capable of uncoupling HRT and SRT for cell immobilization into the form of biogranules (Hulshoff Pol et al., 1988; Liu et al., 2005). Analogous to the rapid feed liquid passage rate through the rumen is the fast critical settling velocity ($V_s = \text{SBR discharge height/settling time}$) of a SBR (Wang et al., 2006) and the quick liquid upflow rate (V_{up}) of a UASB (Alphenaar et al., 1993), in that each of these processes functions as a selection pressure that drives microbial immobilization.

The potential of hydraulic selection pressure for this specific application is supported by an analysis of available data related to acetate-utilizing methanogens. Although feed solids provide a prolonged retention time for rumen microorganisms, Table 11.3 reveals that the minimum generation time of acetate-utilizing methanogens are still greater than the maximum rumen SRT, indicating that acetate-utilizing methanogens are not able to subsist in cattle rumen and acetate is not be utilized in cattle rumen.

Table 11.3: Typical values of minimum generation time (θ_{min}) for AD microorganisms

| Organism types | Substrates | θ_{min} (hours) | References |
|----------------|---------------------------------|------------------------|------------------------------|
| Fermenters | Sugars | 20 | (Rittmann and McCarty, 2001) |
| Methanogens | H ₂ /CO ₂ | 48 | (Rittmann and McCarty, 2001) |
| Methanogens | Acetate | 80 | (Rittmann and McCarty, 2001) |

Others have come to similar conclusions (Madigan et al., 2003) and additional evidence is seen in the high acetate and volatile fatty acids (VFA) levels in flush manure, 0.92 g L⁻¹ and 1.05 g L⁻¹, respectively. So far, only the Methanosarcinales, specifically *Methanosarcina* and *Methanosaeta*, have been shown to convert acetotrophic substrates such as acetate to methane. It is worthy of pointing out that *Methanosarcina* is the only methanogen that is capable of utilizing all three forms of substrate (CO₂-type, methyl and acetotrophic) for methane production (Madigan et al., 2003); however it will preferentially utilize H₂/CO₂ when both H₂/CO₂ and acetate are present in culture (Ferguson and Mah, 1983). The apparently high Gibbs

free energy change of H_2/CO_2 to methane ($\Delta G^\circ = -131$ kJ per mol) over that of the acetate ($\Delta G^\circ = -36$ kJ per mol) might account for *Methanosarcina*'s preference in utilizing the former to the latter (Schink, 1997). This implies that the acetate conversation to methane detected in the respirometer should be attributed to a high concentration of *Methanosarcina*, an inference that is supported by the minor amount of *Methanosaeta* detected in dairy manure as compared to that of *Methanosarcina* (Griffin et al., 1998).

Conclusions

The liquid and solid phases of flushed dairy manure have equal biogas generation, indicating the importance of developing a combined AD process for flushed dairy manure. Microscopic and kinetic studies reveal that microbial organisms in dairy flush manure indeed prefer attached growth on dairy manure fibrous solids, most likely due to hydraulic selection pressure within the rumen. These findings agree well with the microbial distribution in cattle rumen and suggest the potential of dairy manure fiber to act as a natural and economical biofilm support medium for high rate AD that digests both the liquid and solid phases of flushed dairy manure in one digester. SBR and UASB appear to be the appropriate means for this purpose.

A Hybrid Anaerobic Digestion System Treating Flush Dairy Manure

Introduction

Given our attempt to develop a high rate AD that digests both the liquid and solid phases of flushed dairy manure, it is worth considering the two alternatives for digesting flushed dairy manure in more detail. The modified scrape plug-flow technology first settles and concentrates the flushed manure to a higher concentration (6-10% TS) and then treats this concentrate in a typical plug-flow reactor while leaving the liquid portion untreated. The negative of this approach is that roughly 50% of the biogas potential resides in the liquid phase of flushed dairy manure (Frear et al., 2009). This untreated fraction seems likely to lead to a reduction in recoverable methane gas, with consequent impacts greenhouse gas mitigation as well as odor and air/water quality (Wilkie et al., 2004).

The alternative approach, fixed-film technology, overcomes the slow growth kinetics of methanogens (Masse et al., 1993) by using certain methanogens' predilection for biofilm attachment (Meier-Schneiders et al., 1993) on high specific area support media (Vartak et al., 1997). As such, it has the potential to create the enhanced biomass concentrations and reaction kinetics necessary to efficiently digest the prodigious volumes of liquid present in flush dairy manure; thereby requiring only limited HRT and reactor volumes. Unfortunately, in order to ensure positive energy balances, the approach must rely on ambient or psychrophilic temperatures to secure an effective energy balance (Vartak et al., 1997). In addition, since no tested types of biofilm support media tolerate influent solids, dairy manure containing solids could potentially clog the biofilm support media, reducing biomass retention and short-circuiting the flow around the medium. Hence, a strict screening

process (mechanical separation and/or settling) has been placed in front of the biofilm digester to exclude manure solids (Wilkie et al., 2004).

Recognizing the need for a technological option capable of treating the full flush manure flow, the unique characteristics regarding fiber-bacterial attachment learned in earlier work was tested in an adapted, hybrid fixed-film pilot-scale reactor (Figure 11.11). In short, the flush flow was sent across a gravity settler aimed at retaining the liquid portion as well as the solid portion containing solids of < 1mm, allowing theoretically for nearly 70% of the biogas potential to enter the hybrid high-rate packed reactor (Frear et al., 2009). Meanwhile, solids not intercepted were treated for pathogens in a modified high temperature leaching-bed (Liao et al., 2009), allowing for their use as animal bedding. Upon entry into the hybrid reactor, flow was induced through a series of packing chambers filled with 1.5 inch Pall Rings, while a slurry pump recycled flow from the bottom of the reactor back to the top through a heat exchanger to maintain desired temperature. An internal baffle ensured no short-circuit of the flow prior to exit from the effluent port. Objectives of the pilot work were to: (1) devise mechanisms that could allow for the majority of the flush liquid and solids to enter a fixed-film reactor without inducing clogging so as to improve biogas production; (2) ascertain the role of artificial media and/or fiber media in the biological process for next-stage engineering design; and (3) determine overall mass and energy balances so as to determine whether near complete treatment of the entire flush flow would be economically viable in cold climates. This section summarizes some of the key results and conclusions regarding the pilot work.

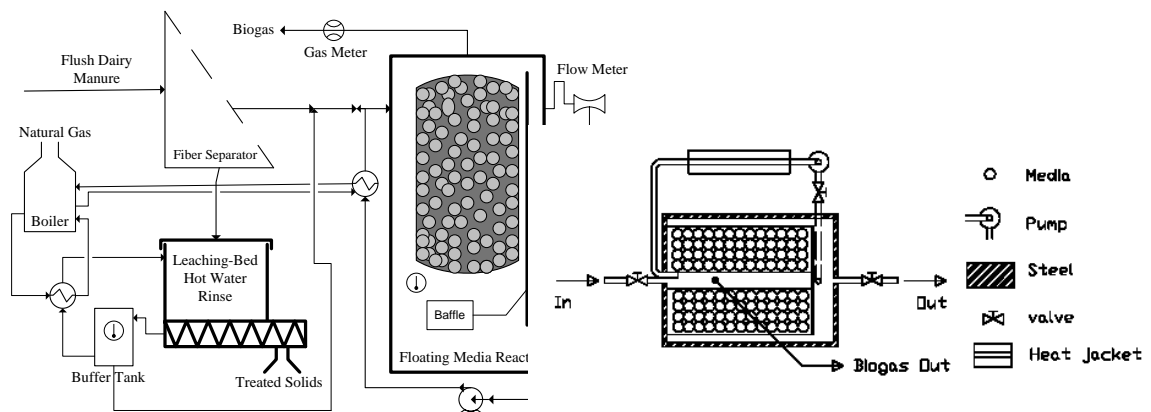


Figure 11.11: Hybrid Pilot-Reactor, flow chart and pan view of packed reactor.

Hybrid AD Performance

Digester performance was tested over the course of a year at various HRT and organic loading rate (OLR) using a pilot reactor at the WSU Dairy Center (Pullman, WA?), designed for treatment of flows equivalent to 25 cows or 12m³ manure/day. Figures 11.12 and 11.13 summarize the performance of the hybrid digester across those treatments. At 27°C, both specific methane and volumetric productivity yielded optimal results at OLR and HRT between 3-4 kg COD/m³ day or 3-4 days;

pointing to preferred operating conditions for a future engineering design. Productivity at these operating conditions reached $0.12 \text{ m}^3 \text{ CH}_4/\text{kg COD}_{\text{in}}$ and $0.40 \text{ m}^3 \text{ CH}_4/\text{m}^3 \text{ reactor}$, respectively. Previous long-term batch digestion by the authors (Frear et al., 2009) using the same manure feed and a 10% by volume wastewater inoculation resulted in a specific methane productivity of $0.06 \text{ m}^3 \text{ CH}_4/\text{kg COD}_{\text{in}}$ or $\frac{1}{2}$ of the productivity of the hybrid reactor. The doubling of the productivity suggests that the hybrid reactor develops a bacterial population capable of not only enhanced kinetics but also improved capacity to biodegrade solids. The improvements are presumably made possibly through a combination of increased biomass concentration, acclimation to inhibitors, and/or generation of a preferred bacterial population more efficient at hydrolysis.

Table 11.4 summarizes the reduction potentials of the hybrid digester with 31.9, 40.9, 47.7, 60.2, and 93.4% reductions in TS, VS, COD, soluble chemical oxygen demand (SCOD), and VFA, respectively while operating in the previously determined optimal OLR and HRT ranges at 27°C. These reductions (and our measured methane productivity) are similar to results reported by Wilkie et al., while digesting flush dairy manure on their bench and demonstration-scale fixed-film reactors (40% VS and 48% COD reductions, and $0.40 \text{ m}^3 \text{ CH}_4/\text{m}^3$ performance) (Powers, 1997; Wilkie, 2004; Wilkie et al., 2004).

A troubling concern of any hybrid or fixed-film reactor is the potential for organic or inorganic accumulations within the digester that can eventually clog and fail the digester but also can cause inaccurate reduction predictions. Typical indicators for accumulation within reactors are fixed solids (FS), total Kjeldahl nitrogen (TKN), and total phosphorous (TP) with little to no change in these parameters during the digestion process indicating accumulation. Notably, we found no statistically significant reductions in the organic indicators (TKN and TP). The mean FS concentration of the effluent was statistically lower than the effluent indicating potential accumulation of inorganic material, but upon completion of the year-long testing, this accumulation was deemed to be sporadic or of a small nature as the reactor was opened and little to no significant accumulation of inorganic material was noted inside the reactor.

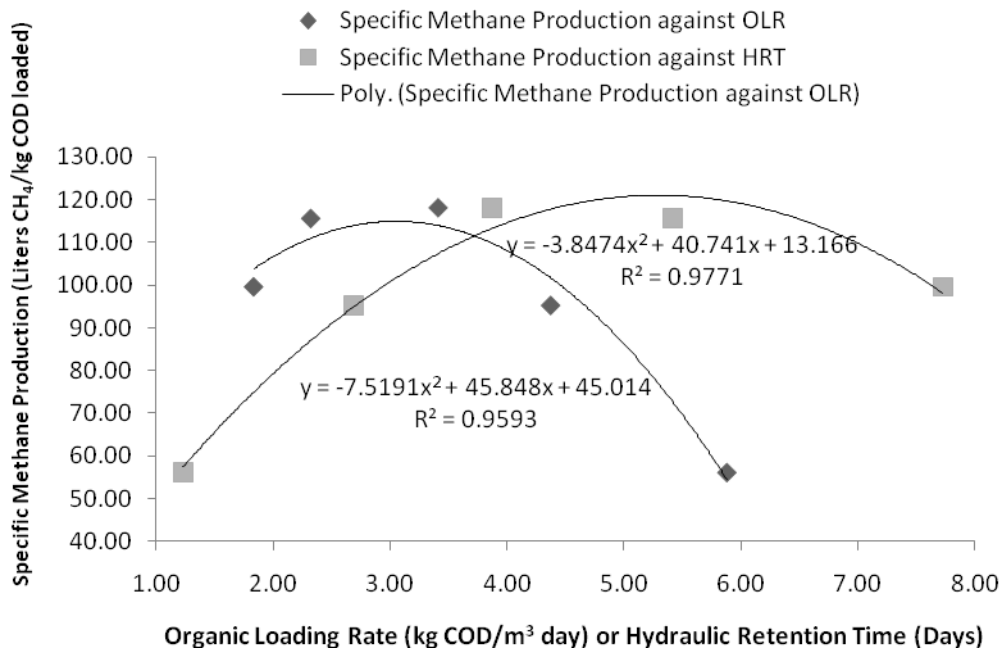


Figure 11.12: Specific methane production against both OLR and HRT at 27°C.

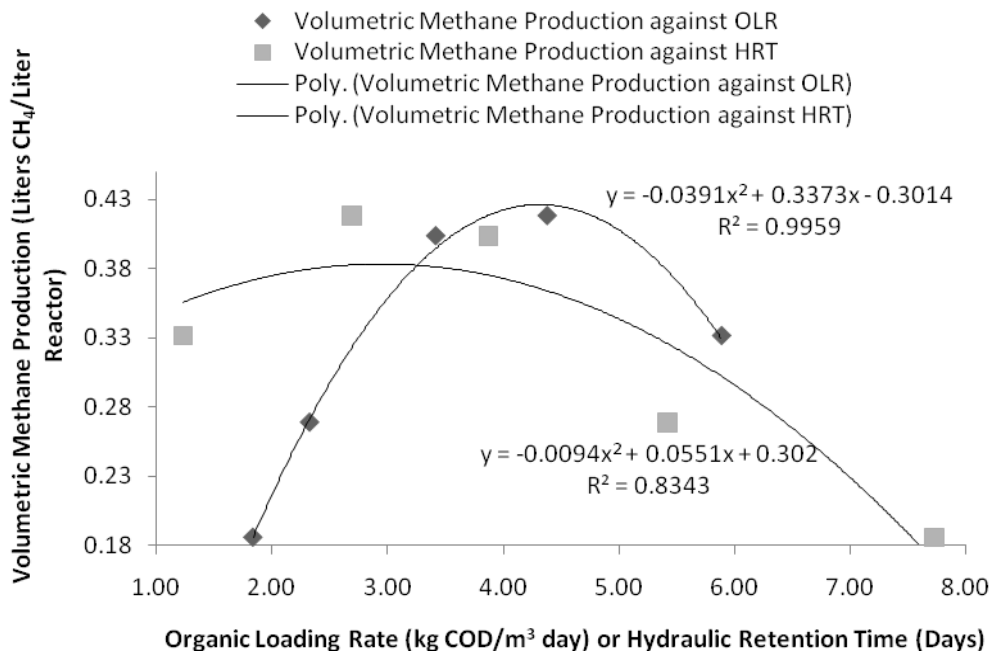


Figure 11.13: Volumetric methane production against OLR and HRT at 27°C

Table 11.4: Reactor performance at 27°C, HRT of 3.87 days and OLR of 3.42 kg COD/m³ day

| Parameter (g/L) | Influent | Effluent | % Reduction |
|-----------------|--------------|-------------|-------------|
| TS | 11.43 ± 0.61 | 7.78 ± 0.19 | 31.9 |
| VS | 7.50 ± 0.61 | 4.43 ± 0.16 | 40.9 |
| FS | 3.93 ± 0.29 | 3.35 ± 0.10 | 14.8 |
| COD | 13.22 ± 0.96 | 6.91 ± 0.34 | 47.7 |
| SCOD | 5.70 ± 0.25 | 2.27 ± 0.11 | 60.2 |
| VFA | 0.91 ± 0.05 | 0.06 ± 0.04 | 93.4 |
| TKN | 0.86 ± 0.27 | 0.76 ± 0.27 | NA |
| TAN | 0.63 ± 0.02 | 0.64 ± 0.04 | NA |
| TP | 0.10 ± 0.06 | 0.09 ± 0.01 | NA |
| pH | 7.63 ± 0.20 | 7.79 ± 0.24 | NA |
| Alkalinity | 4.28 ± 0.16 | 4.70 ± 0.18 | 9.8 |

NA refers to mean reduction parameters not statistically relevant as determined by General Linear Model (GLM) ANOVA analysis with Statistical Analysis System program 9.0 (SAS Institute Inc. NC) at $\alpha=0.05$ with n=20 samples. All reductions were with calculated p-values <0.0001 except for TKN (0.6371), TAN (0.5465), TP (0.0729), and pH (0.4863).

In anticipation of concerns regarding generation of positive energy balances, several temperature treatments (27, 21, and 18°C) were studied. Figure 11.14 shows a comparison of biogas productions at the three different temperatures, each with consistent ORL, in an ln biogas vs 1/T graph. The linear fit confirms other study findings (Metcalf and Eddy, 2003; Safley and Westerman, 1990) that the reactor and digestion processes occur according to principles identified by the van't Hoff-Arrhenius Equation. As might be predicted, beyond lowering the biogas production in a defined way, decreasing the reaction temperature resulted in similar vector reduction decreases at a given OLR and HRT.

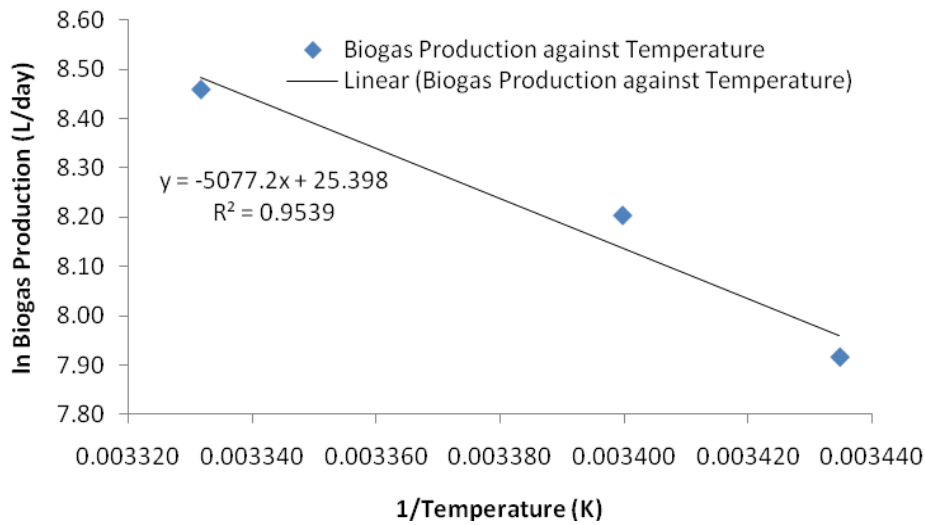


Figure 11.14: Biogas production against reactor temperature.

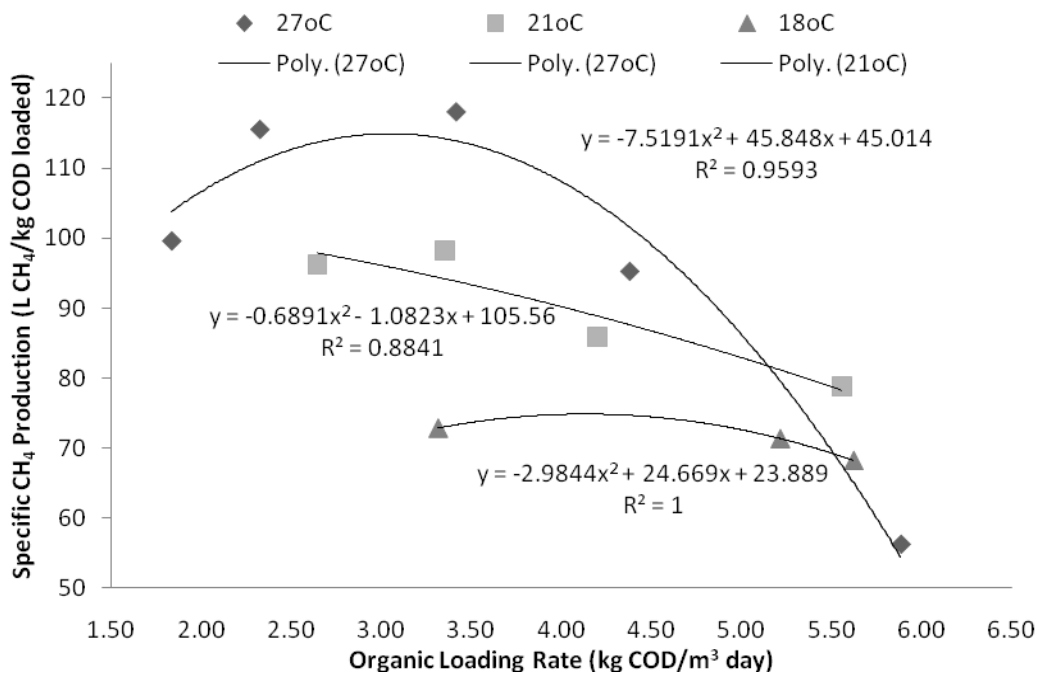


Figure 11.15: Specific methane production at various temperatures and OLR.

Calculated methane productivities for the three temperature treatments at the identified optimal OLR and HRT range of 3-4 were 0.12, 0.08 and 0.07 m³ CH₄/kg COD_{in}, respectively for 27, 21 and 18°C. Interestingly, optimal operating conditions

changed for the lower temperatures; showing either greater methane productivity or greater tolerance as the OLR increased (Figure 11.15).

Biofilm Development and Significance to Performance and Engineering Design

Upon completion of the testing of the hybrid reactor, the digester was opened up and inspected for development of biofilm. As can be seen in Figure 11.16, a significant amount of biofilm material existed, however the biofilm was only loosely attached to the plastic packing rings, so loosely, in fact, that only a very small amount of agitation induced the material to completely fall from the support. Further analysis showed that the biofilm was actually numerous small fibrous particles cemented together by extra-cellular polymers with consortia of bacteria residing within and among the polymers and fibers (Figure 11.17).



Figure 11.16: Plastic support media with loosely attached biofilm sludge

Completion of this long-term digestion study reinforces the original hypothesis, that the observed biofilm matrix between microorganisms and the fibrous particles originated in the cow rumen as the results of selection pressure induced by the HRT and SRT within the cow rumen (Frear, 2009). It also extends the concept by asserting that this original affinity and biofilm development is preferred within the given reactor environment thus producing the observed non-attached biofilm sludge. Observation of non-attached biofilm sludge has been noted previously. Young and Dahab (1983) showed a strong correlation between COD removal and media type, size and shape, but found that a media's ability to entrap and prevent washout via redistribution of interstitial flow was more important than unit surface area of the media. Anderson et al, (1994), meanwhile, showed that when using PVC rings the most common occurrence was the presence of unattached clumps of biomass lodged within the void space of the media as opposed to true interstitial biofilm growth. Given these additional observations we think that it is most likely that under the existing reactor operating conditions, the plastic floating media

served not as an attachment material but merely as a mechanism for entrapping fibrous particles so as to ensure a higher SRT and further biofilm production within the fibrous matrix.

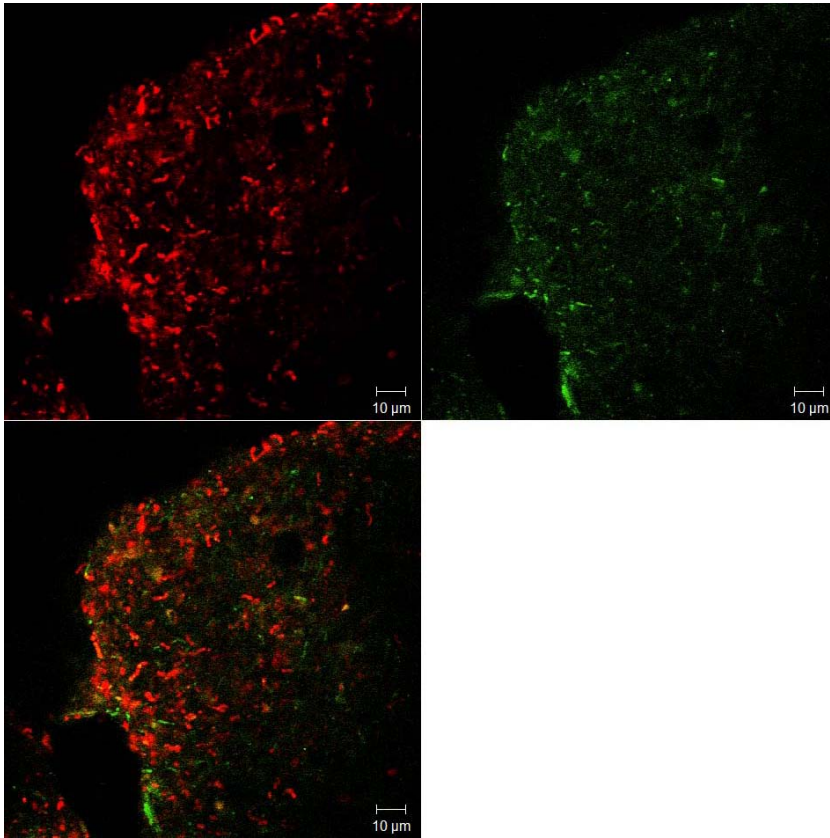


Figure 11.17: FISH image of biofilm sludge on surface of plastic support media (Red: Eukaryotic Bacteria; Green: Archae; Combined Color: Both Eukaryotic Bacteria and Archae).

Esterl et al., (2003) pointed out that packed-bed reactors typically produce biofilm with a branched, loose architecture as opposed to the compact and dense architecture found in other wastewater reactors and pipes. Theoretical explanation for their observation fits in nicely with the influent and fluid-flow regime present in the hybrid reactor. Applying their original reasoning to the hybrid reactor, the highly soluble and biodegradable nature of the flush manure influent most likely yields a Damköhler ratio much greater than unity (Equation (1)):

$$D_a = \frac{V_{\max}}{K_s C_o} \gg 1 \quad (1)$$

such that the maximum conversion rate (V_{\max}) exceeds the maximum mass transfer rate ($K_s C_o$) wherein K_s represents the mass transfer coefficient and C_o is the influent substrate concentration. Such a case is transport-limited and the biofilm

growth and architecture thus becomes strongly dependent upon either flow-induced mechanical stress, i.e. Reynolds number, and/or inflow concentration. Because the flow rate through the plastic media and the reactor as a whole is non-turbulent and of quite low velocities, the result is a continuing transport limitation and subsequent growth rate inhibition. In an attempt to overcome the inhibition, the microbes increase the transport and nutrient transfer by other means. The formation of a loosely structured biofilm allows for greater surface area and therefore improved transfer while adherence to substrates capable of supplying high nutrient concentrations, i.e. the fibrous particles, also increases nutrient transfer. Thus, a combination of unique characteristics present in flushed manure digestion within the hybrid reactor have resulted in the observed biofilm phenomenon: (1) a non-turbulent, low velocity flow pattern; (2) highly biodegradable and soluble influent substrates; and (3) existing rumen-induced fiber/microbial interaction.

This explanation for the observed biofilm phenomenon suggests several promising strategies for how to better engineer future hybrid reactors. If the biofilm sludge is not attached and the biofilm growth is linked to fibrous substrates already present in the feed, then plastic media is not required for effective operation. A preferred reactor would thus involve either a simple sludge-bed or sequencing-bed reactor whereby the fibrous substrate is retained through feed and/or wasting protocols instead of expensive and potentially clog-inducing plastics. The engineering result could be a reactor design which maintains the effective methane yields and vector reduction potentials already noted while reducing capital costs and improving system operation and reliability. Such a design would be particularly beneficial if it were economically-viable for smaller dairy operations. Discussion of energy balances and system economics for a potential future commercial application is discussed in more detail in the next section.

As noted earlier, an interesting discovery in the pilot research was the ability of the hybrid reactor to perform effectively at higher loading rates, even when stressed with temperature reductions. Studies have shown that acetoclastic *methanosaeta*, with their unique filamentous and biofilm producing traits, are normally present in biofilm reactors and that induced stresses, be it temperature or loading rates, shift the microbial population towards hydrogenotrophic methanogens (McHugh et al., 2004; McHugh et al., 2006). In the case of increased loading rates, the shift was attributed to *methanosaeta*, known to have low acetate tolerances, not being able to adequately adjust and biodegrade the new higher acetate levels. In the case of temperature reductions, it has long been known that temperature reductions cause elevations in propionate concentrations, inducing a shift towards a more diverse, synergistic microbial community composed of both acetoclastic and hydrogenotrophic methanogens capable of controlling the propionate levels (McHugh et al., 2004; McHugh et al., 2006).

As can be seen from Figure 11.18, the biofilm generated in the hybrid reactor operation contained much more *methanosarcina* than *methanosaeta*, a ratio that

was maintained throughout all operation treatments including high OLR and temperature reduction (data not shown). It is proposed here that flush dairy manure, which has a high initial level of *methanosarcina* inoculums due to rumen conditions (Frear et al., 2009), remains concentrated in *methanosarcina* (as opposed to shifting towards *methanosaeta*) as a result of the discussed unique flow regimes and high acetate concentrations within the reactor. This unique reactor population might in itself partly explain the loosely-attached biofilm observed, as *methanosarcina* are known to not be as adept at biofilm or granular production as is *methanosaeta*. In addition, it helps explain why an increase in OLR with a simultaneous decrease in temperature resulted in continued effective. Our hypothesis is that at the lowered kinetics brought on by the temperature shift, the simultaneous increase in feed rate was beneficial to maintenance of the existing *methanosarcina* population, whereas a *methanosaeta* population would be incapable of tolerating the elevated acetate levels.

One conclusion from this analysis is that the hybrid reactor, as operated, and with its *methanosarcina* sludge population, is a more effective reactor for the flush dairy manure feed being input and better suited for operation at psychrophilic temperatures and high acetate loadings that might occur with high liquid volume co-digestion with cheese whey.

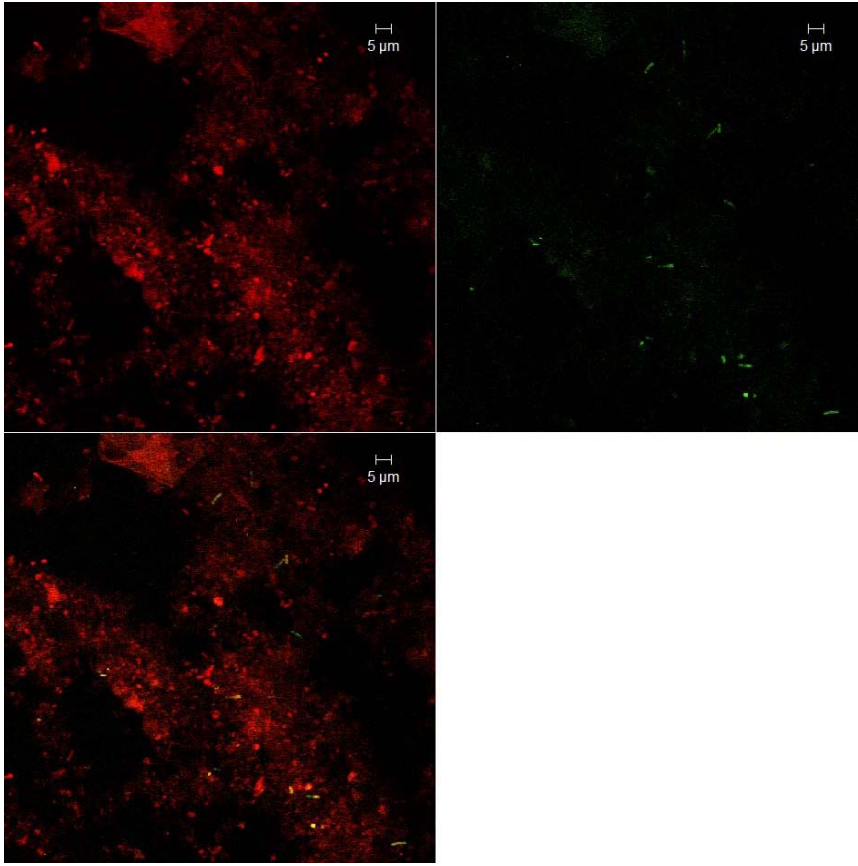


Figure 11.18: FISH image of biofilm sludge (Red: *methanosarcina*; Green: *methanosaeta*; Combined Colour: Both *methanosarcina* and *methanosaeta*)

Energy Balance

The optimal production parameters determined during plot testing were used to determine whether or not effective energy balances and project economics could be developed for a small flush dairy treating manure from 100 wet-cow equivalents in a cold-climate such as experienced at the WSU Dairy Center. Figure 11.19 summarizes the daily, seasonal and yearly mean air and manure temperatures for the WSU Dairy Center during 2007. The annual mean manure temperature of 11.2°C was used as a baseline for operation of an assumed 27°C AD reactor and a 50°C fiber-wash process (Liao et al., 2009).

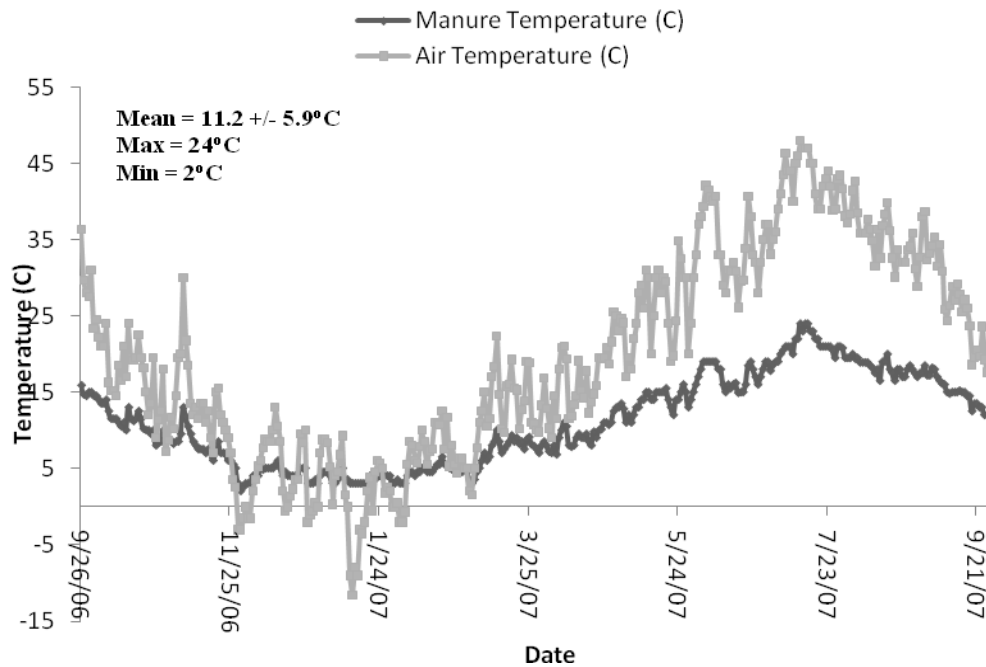


Figure 11.19: Manure and air temperature profiles for WSU Dairy Center (Pullman, WA) in 2007

Table 11.5 summarizes the parameters used to determine energy balances for the system under these temperature conditions. As can be seen from Figure 11.20, digestion of flush manure alone does not result in a positive energy balance for operation within this cold of a climate. Thus, co-digestion with high strength liquid food processing waste such as milk, cheese whey, egg breakage, etc. will be necessary.

Co-digestion could not be incorporated in the pilot system due to infrastructure concerns, but data was available from a separate co-digestion study for scrape manure using a plug-flow reactor. In that study, a 15-20% volumetric supplementation of dairy manure with high-strength industrial co-digestion substrates (~ 200 g/L COD) resulted in a doubling of methane production. For purposes of this analysis, it was assumed that highly biodegradable liquid substrates added at relatively low volumes would not overwhelm or negatively impact the operation of the digester, particularly given the high alkalinity supplied by the flush manure (4 g CaCO_3/L) which is well within the 2-5 g CaCO_3/L preferred alkalinity identified for effective and stable digester operation (Metcalf and Eddy, 2003). It was also assumed that the supplementation would result in an overall 40 g/L COD feed to the digester and a doubling of methane production, similar to the results of the plug-flow study. Under these assumptions, the yearly energy balance becomes positive as long as necessary changes in substrate loading are made to operate in particular seasons.

Table 11.5: Energy Balance and Project Economics Parameters

| Parameter | Units | Flush Manure | Co-Digestion ^a |
|---|--|--------------|---------------------------|
| <i>AD Reactor</i> | | | |
| CH ₄ Productivity | m ³ CH ₄ /kg COD _{in} | 0.12 | 0.07 |
| COD Concentration | kg/m ³ | 11.72 | 40.00 |
| Waste Flow Rate | m ³ / cow* day | 0.45 | 0.52 |
| CH ₄ Heat Value ^b | BTU/m ³ | 35,315 | 35,315 |
| Heat Capacity | BTU/kg C | 3.96 | 3.96 |
| Δ Temperature | C | 15.8 | 15.8 |
| Boiler Efficiency ^c | % | 80 | 80 |
| Heat Losses ^d | % | 10 | 10 |
| <i>Fiber Wash Reactor</i> | | | |
| Mass Flow Rate | kg/cow day | 18 | 18 |
| Heat Capacity ^e | BTU/kg C | 2.38 | 2.38 |
| Δ Temperature | C | 38.8 | 38.8 |

^a Manure and liquid substrate digestion at 85/15 (v/v) ratio using COD loading and methane production values as determined by Frear *et al.*, (2009b)

^b Metcalf and Eddy (2005)

^c Parker hot water boiler with biogas modification, model # T-5700

^dLiu *et al.*, (2009)

^eSpecific Heat Capacity of light fiber board (Engineering Toolbox)

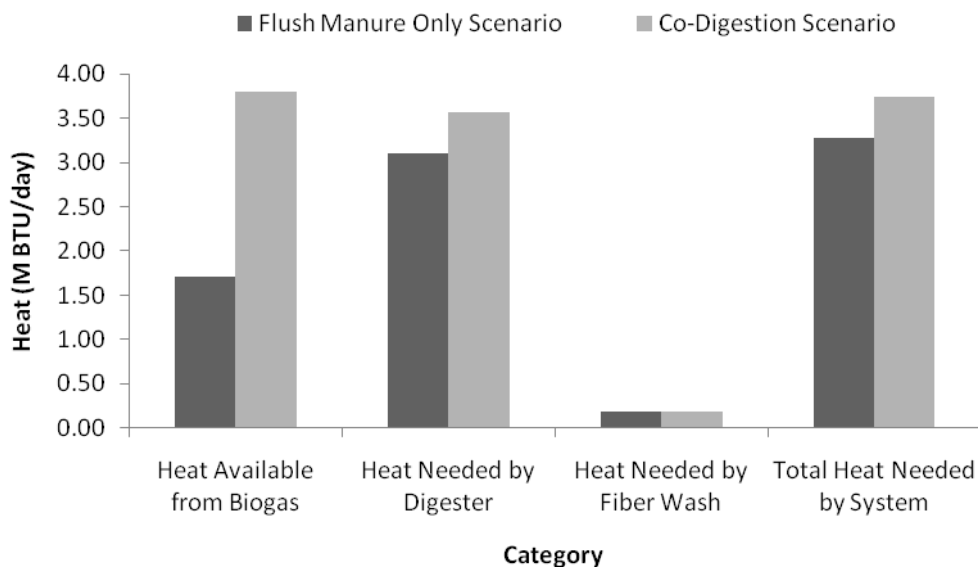


Figure 11.20: Energy balances for manure-only and co-digestion scenarios

Project Economics

An attempt has been made to determine whether or not a positive economic scenario can be produced for co-digestion on a 100-cow dairy. Table 11.6 details the main capital costs of a projected 100-cow flush facility with co-digestion at a 15% volumetric rate. This facility would process 45 metric tons of flush manure per day with an additional 6.75 metric tons of industrial food processing waste per day. At an estimated HRT of 4 days and an OLR of 4 kg COD/m³ day, this would require a reactor with a working volume of approximately 210 m³ and total volume of roughly 250 m³ with at least 15% volumetric headspace. Assuming a 10% by volume production of separated fibrous solids, the associated leaching-bed reactor would require a volume of roughly 5 m³.

Table 11.6: Estimated capital costs for 100-cow system ^a

| Material | Specifications | \$ |
|--------------------------------|---|------------------|
| Insulated Digester Tank | h=22 ft; r=11ft; V=8,400ft ³ | \$105,000 |
| Leaching-Bed Tank w/ Augur | Insulated, 250 ft ³ | \$20,000 |
| Heat Exchanger, Mixing | Plus miscellaneous pipes and valves | \$45,000 |
| Gas Collection | Blower, piping, gas prep | \$10,000 |
| Insulated Boiler (200 KBTU/hr) | Storage tank, pumps, valves, piping | \$35,000 |
| Excavation and Concrete | Digester, leaching-bed, building | \$30,000 |
| Building | 20 ft by 20 ft, for boiler, controls, etc | \$25,000 |
| Control Panel | Automated, probes, casings, meters | \$20,000 |
| Safeties | Flame arrester, relief valve, flare | \$10,000 |
| Other | Permits, travel, freight, rentals | \$55,000 |
| Total | | \$355,000 |

^aPrice quotes for individual materials and overall project cost are from Andgar Corporation (2009).

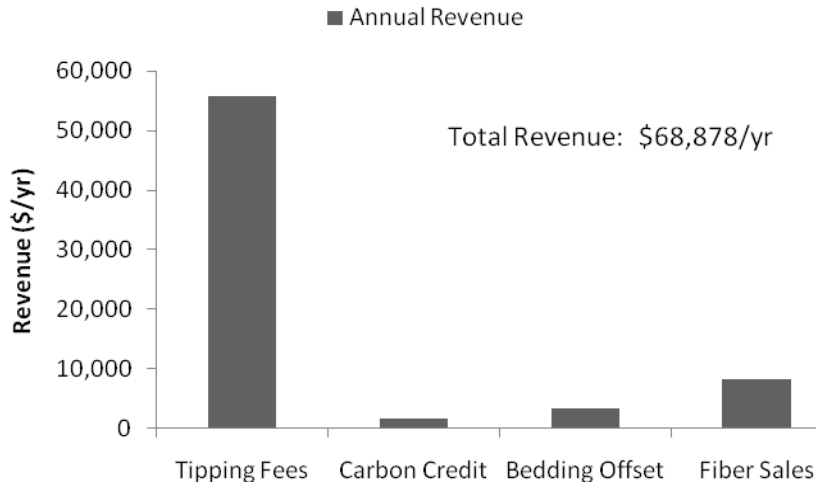


Figure 11.21: Estimated Revenue from 100-cow system (tipping fees at \$20/ton; bedding offset at \$10/ton; fiber sales at \$25/ton; carbon credits for manure only at \$10/ton as per (Frear et al., 2009).

Figure 11.21 summarizes the potential revenue that could result from these treated flows, assuming no electricity production and near-complete use of boiler heat for reactor temperature maintenance. As can be seen from the projected capital costs and revenues, the project yields a capital cost to revenue ratio of just over 5 for a potential payback period of 5 years, assuming no debt maintenance.

Conclusions

A newly engineered system for AD of dilute, flush dairy manure has been proposed and tested at pilot-scale. Biogas production rates and mass/energy balance calculations show that co-digestion is needed to maintain a positive energy balance so as to sustain reactor operation at the proposed 27°C and 50°C reactor temperatures. Reactor volumes and required reaction times for treatment of both the liquid and fibrous fractions are significantly reduced, resulting in potential cost savings to the farmer and operator. Biological imaging suggests the proposed high-rate reactor requires no artificial media for generation of active biomass, thus reducing complexity and capital and operating costs significantly while also improving upon process stability through development of the unique *methanosarcina*-dominated population. For a small dairy, projected project economics and overall viability are strongly dependent upon co-digestion and tipping fees for received substrates. From a farm-level perspective, dependence on co-digestion is potentially problematic because of instability of available substrates and prices received as well as the potential regulations related to industrial or commercial solid waste digestion on-farm, particularly with a psychrophilic digester and its associated reductions in pathogens. Further testing of the reactor concept without artificial media for solids entrapment and development of a self-sustaining

selection-pressure driven mechanism for fiber retention and biomass concentration is still required.

Effects of Settling Time on Active Biomass Retention in Flushed Dairy Manure

Introduction

For a given feedstock, the rate of AD increases with the temperature and the amount of active biomass retained in the digester (Batstone et al., 2001; Lettinga et al., 2001). Active biomass retention provides a cost-effective way to facilitate AD at lower temperatures (Connaughton et al., 2006; Lettinga et al., 2001). Gravity settling is a conventional method for biomass retention with no need of supporting materials. It takes advantage of a prolonged settling time to separate bacterial cells from the supernatant. Based on Equation (2), a gravity settling theory (GS) was established,

$$V_c = \frac{L_d}{t_s} \quad (2)$$

in which V_c represents a critical settling velocity created by the depth of discharge zone (L_d) and the settling time (t_s) (Vesilind, 2003). According to the gravity settling theory, all particles with settling velocity $V_p > V_c$ will settle beneath the discharge zone and get retained (Vesilind, 2003). Due to the minor settling velocity of bacterial mass, a rather long t_s , typically 1 to 3 hours, is normally needed to retain bacterial cells at a given L_d (Lee, 2000; Wilderer et al., 2001). On the other hand, influent solids, e.g. undigested cattle manure fibers, may possess settling velocities greater than bacterial cells, and thus applying GS into an AD environment may retain not only active but also inactive biomass (Lott et al., 1994). Obviously, inactive biomass retention is unwanted as it takes extra reactor volume.

Selection pressure (SP) driven cells immobilization is another theory developed in recent years for active biomass retention without need of external media (Liu et al., 2005). SP theory is founded on an equation similar to Equation (2), but unlike GS theory, it requires an extremely short t_s , typically less than 5 min, to create a large V_p to drive cells towards self-immobilization and retention (Qin et al., 2004). According to SP theory, microorganisms are able to make active responses to short t_s to avoid being washed out of a reactor (Qin et al., 2004). Using this theory, successful bacterial retention has been achieved in solids-containing dairy wastewater (Schwarzenbeck et al., 2005). SP theory takes advantage of bacterial initiative and thus has the potential to selectively favor bacteria that are capable of active biomass retention over inactive bacteria. Such a possibility has not yet been explored for AD for wastes including solids. This study was designed to test and compare the ability of GS and SP theories to promote active biomass retention in AD of flushed dairy manure by using sequencing batch reactors (SBR) as a platform. For this purpose, a wide spectrum of t_s ranging from 0.5 to 60 min was employed. The

mechanism behind the reverse settling time roles in GS and SP theories was also investigated. It is expected that this work will offer innovative ideas for active biomass retention technology in mixed solid/liquid waste AD.

Process of Active Biomass Retention in SBRs Running at Various Settling Times

Laboratory experimentation has shown that volumetric biogas production rate is associated with the amount of active biomass retention in a bioreactor. The microbial retention process can be divided into four phases based on the trends in biogas production rates (Figure 11.22). The first phase (about 30 days) is characterized by decreasing biogas production rate, indicating a continuous washout of inoculums. After this is a transitional phase of about 10 days, in which microbial washout was offset by growth. From the 40th day on, remaining inoculums seem adapted to SBR operation. Thus begins a quick growth phase with steep increase in volumetric biogas production rate. In comparison with the washout phase, this growth phase indicates successful active biomass retention in SBR serum bottles. After 65 days, a stationary phase is achieved in all SBRs with stabilized biogas production rate at different levels, indicating settling time is playing a role on the amount of active biomass retention.

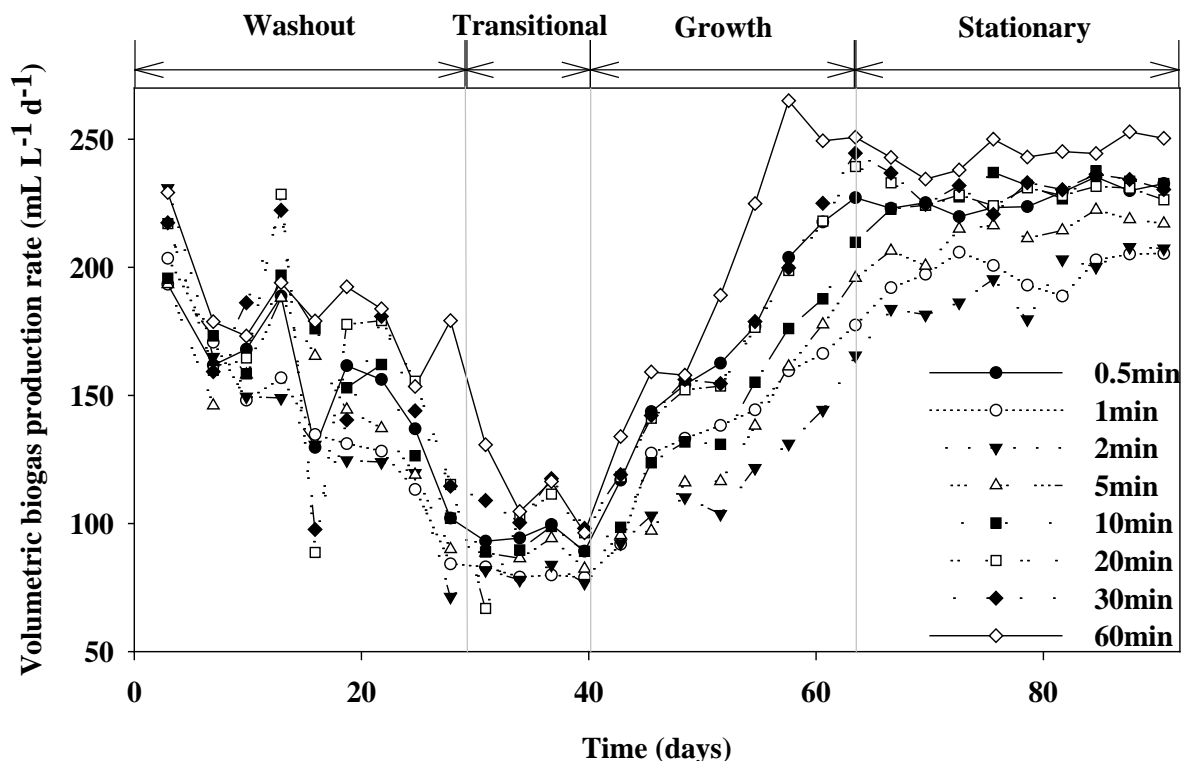


Figure 11.22: 90-day volumetric biogas production profiles in SBRs operated at different settling times.

Effect of Settling Time on Active Biomass Retention

So far, there is no standard method available for measuring active biomass concentration in solid-containing wastes, especially for complicated samples like dairy manure (Grady et al., 1999). In order to make a best estimation, three parameters were employed in parallel in this study to assess the actual level of active biomass retention: i) volumetric biogas production rate; ii) ATP concentration and iii) methanogenic activity. Volumetric biogas production rate strongly relies on the amount of fermentative and methanogenic microorganisms retained in a bioreactor. Its relationship with settling time shows a “tick” curve bottoming out at 2 min settling time and peaking at both the shortest- and longest-settling times (Figure 11.23). This “tick” curve implies there exists a critical settling time $(t_s)_c$ at which an SBR retains the minimum amount of active microorganisms. It is interesting to see that the active biomass retention kept increasing as settling time increased or decreased in either direction away from this $(t_s)_c$.

Since ATP is the primary energy donor for life processes and only exist in living cells (Lundin and Thore, 1975), ATP concentration was used here as an indicator of active biomass. It can be regarded as particularly suited for applications in dairy manure that is full of dead biomass debris. The ATP profile in Figure 11.24 appears to be in line with Figure 11.23, i.e., a “tick” curve with $(t_s)_c$ at 2 min settling time with maximum retention at both extremes. This consistency seems to indicate bipolar effects of settling time on active biomass retention on both sides of the $(t_s)_c$.

Methanogens are the slowest growing organisms in an anaerobic digester. Their retention can therefore be regarded as a good sign of successful active biomass retention. Mixed liquor methanogenic activity is shown in Figure 11.25. Once again, its profile exhibits a “tick” curve with $(t_s)_c$ at 2 min settling time. It seems certain from the figures that there exists a $(t_s)_c$ on both sides of which are favorable settling times for active biomass retention, i.e., not only a long- but also a short- settling time would be favorable for active biomass retention in SBRs.

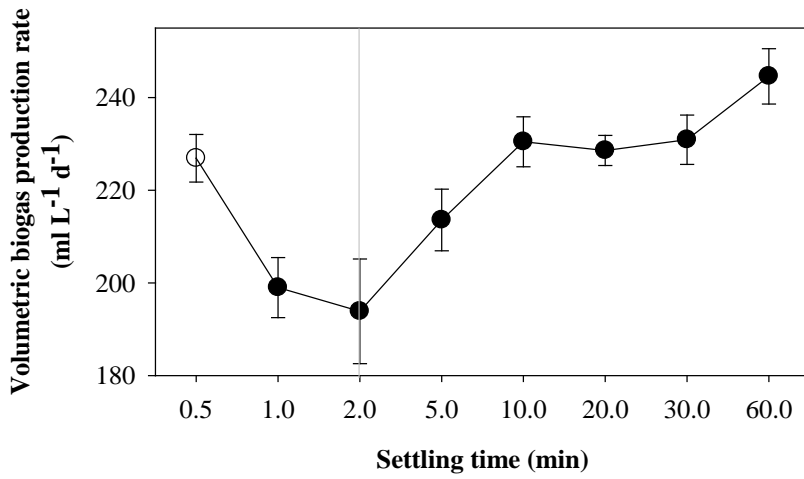


Figure 11.23: Effect of settling time on steady state volumetric biogas production rate.

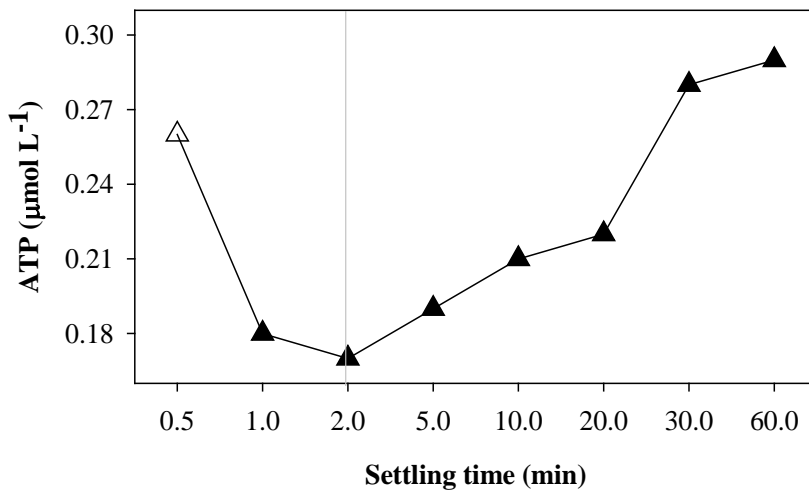


Figure 11.24: Effect of settling time on mixed liquor ATP concentration.

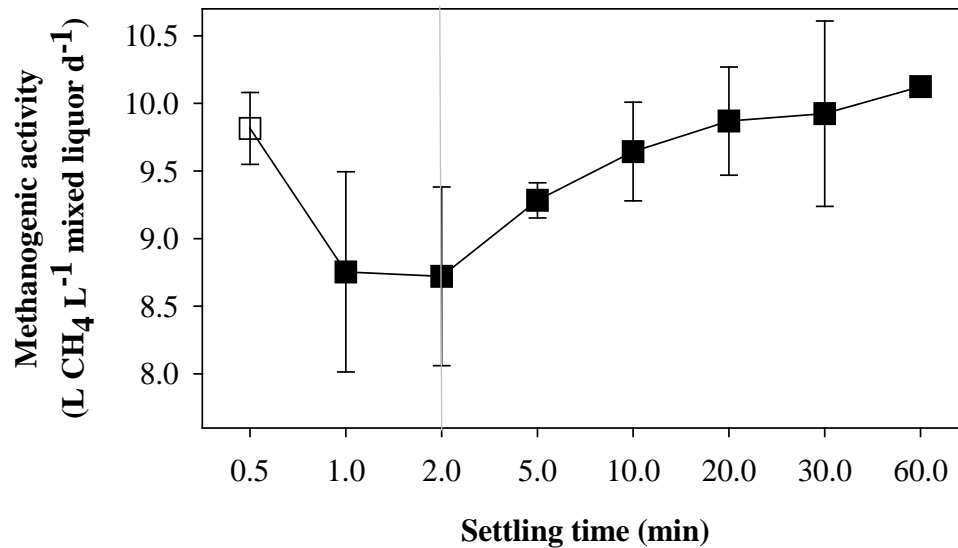


Figure 11.25 Effect of settling time on methanogenic activity.

Microbial Active Response to Settling Time

According to GS theory, a long settling time is favorable for biomass retention because it physically retains whatever materials settle beneath the effluent zone. However, the active biomass retention at very short settling time in Figures 11.23-11.25 implies a possible microbial response to settling time in favor of their own retention. In order to look into the difference between biotic and abiotic particle response to settling time, a washout coefficient was defined as Equation (3),

$$\varepsilon_w = \frac{C_e}{C_i} \quad (3)$$

in which C_i and C_e stand for particle concentrations in SBR mixed liquor and effluent supernatant, respectively. Technically, ε_w represents washout strength imposed by settling time on settling particles. Since biotic solids concentration can be considered minor in relation to abiotic solids concentration in dairy manure, TSS is used here to stand for abiotic particle concentration. As for the biotic washout coefficient, the methanogen activity measured in mixed liquor and effluent supernatant was employed. Results in Figure 11.26 demonstrate that abiotic ε_w decreases with settling time. In other words, there are always more abiotic particles washed out of the SBRs at shorter settling time, which is in line with GS theory prediction. Biotic particles demonstrate a reversed trend on each side of the 2 min $(t_s)_c$. Moreover, as can be seen, both biotic and abiotic values overlap at 2 min $(t_s)_c$. Considering the “washout strength” meaning of ε_w , $(t_s)_c = 2$ min appears to be a threshold value at which both biotic and abiotic particles were washed out at an

equivalent percentage. This therefore explains the observed minimum active biomass retention at $(t_s)_c = 2$ in Figures 11.23-11.25.

Biotic ϵ_w on the left-hand-side of $(t_s)_c$ is the only increasing trend in Figure 11.26. This increase suggests less washout of active biomass at shorter t_s , which is in opposite to the dropping abiotic ϵ_w trend in the same region. This suggests that biotic particles, unlike their abiotic counterparts, are able to make active response to settling time, i.e., more active biomass is actually going to be retained at stronger abiotic washout strength.

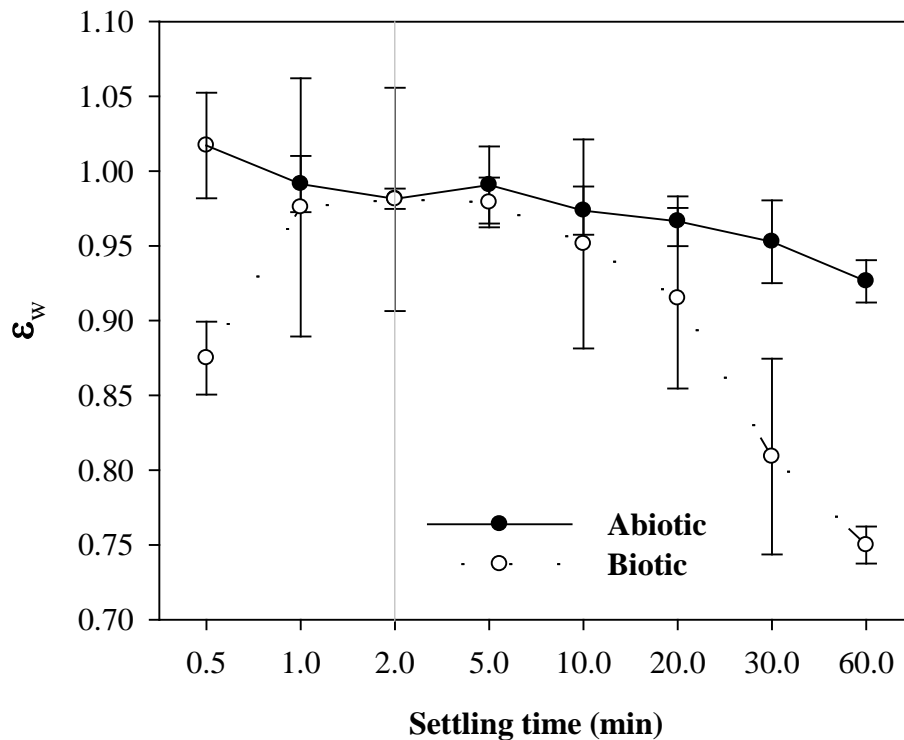


Figure 11.26: Effect of settling time on washout coefficients for abiotic and biotic particles.

Effects of Settling Time on Active Biomass Retention

It is probable that this study shows the first example where settling time is shown to have bipolar effects on microorganism retention in SBRs, i.e., both extremely long- and short- term settling time are able to help retain active microbial biomass. The major difference between the long- and short-settling times lies in the forms of those retained microorganisms: the long-settling time retains bioflocs whereas the short-settling time is dominated with biofilm. For decades, settling time has normally been set at greater than 45 minutes in conventional SBR applications in biological wastewater treatment (Irvine et al., 1977; Lo et al., 1985; Woolard and Irvine, 1995). Like other gravity settling devices, the design of SBR settling time is guided by Equation (2), according to which SBR discharge height (L_d) and settling

time (t_s) create a designed critical settling velocity (V_c) (Figure 11.27). Particles with settling velocity $V_p > V_c$ will be completely retained in the SBR (Vesilind, 2003). Accordingly, a smaller V_c (which implies a longer t_s) should be favorable for particle retention. This holds true for both biotic and abiotic particles. At least two important points are conveyed from Equation (2), i.e., i) t_s actually plays its particle retention role via V_c ; and ii) t_s should be positively related to particle retention, and a maximum retention would be achieved at $t_s \rightarrow +\infty$. This description seems in line with the $t_s > (t_s)_c$ side in Figures 11.23-11.25, but it does not explain the retention downturn at $t_s < (t_s)_c$ in those same figures.

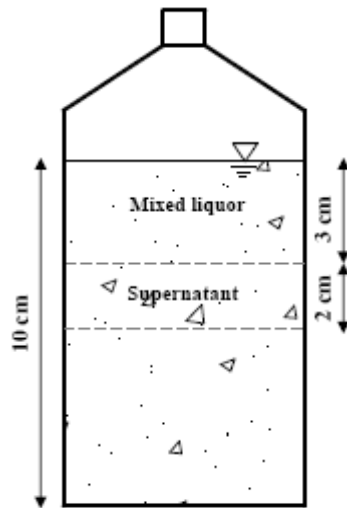


Figure 11.27: Schematic of SBR operation in a serum bottle.

Extremely short settling times began to be applied in SBRs in 1999 (Beun et al., 1999). It was found that microorganisms can be retained despite strong washout strengths, but they were only retained in the form of biofilm (Beun et al., 1999). Later studies indicated that short settling time also plays a retention role via V_c defined in Equation (2) (Wang et al., 2006), but this time it shows a totally reversed relationship with reference to GS theory, i.e., short t_s turns out to be a positive factor for microbial retention in this case. Based on this, the SP theory was established to interpret this microbial active response to washout selection phenomenon, i.e., a selection pressure will be created by V_c as defined in Equation (2) to wash out slow-settling bioparticles. Only those capable of immobilizing themselves in the form of biofilm as represented in Figure 11.28a can be retained and become dominant at short-settling time. As a matter of fact, this is also the same by which fermentative microorganisms are retained in animal rumen operating under fairly short hydraulic retention time (McAllister et al., 1994). Thus, it seems that SP theory can be used to give a satisfactory explanation for observations on the $t_s < (t_s)_c$ side of Figures 11.23-11.25. It should be emphasized that the SP theory only applies to active bioparticles capable of self-retention in response to selection pressure, but

not to abiotic particles. Instead, abiotic particles should presumably always follow GS theory no matter the range of t_s . This integrated interpretation of GS and SP sheds important light on the observations illustrated in the previous figures.

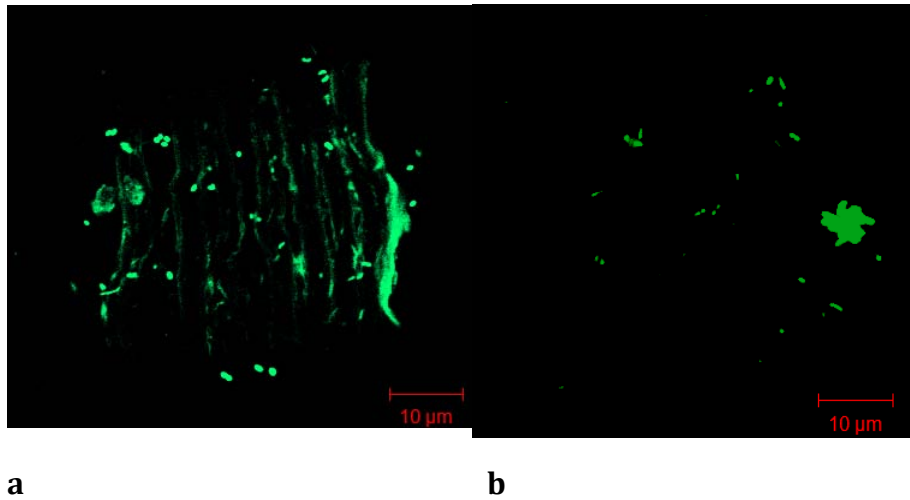


Figure 11.28: Confocal visualization of active microbial organisms in steady-state SBR running at settling times of 0.5 min (a) and 60 min (b).

Conclusions

For the first time, this study reveals that both short- and long- settling times are able to retain high active microbial concentration, though the microbes retained are different. Settling time plays its role via a combination of ideal settling velocities for gravity settling and selection pressure theories, theories that exhibit reverse correlation. A better understanding of these relationships has important implications for the operation of fiber-based SBR-type reactors

Overall Conclusions for High-Rate Reactor for Use with Dilute Waste Streams

Numerous laboratory and pilot-scale experiments have led to significant findings in regard to design of a new reactor tailor-made for high-rate treatment of dilute flush manure, particularly in colder climates. Unlike previous high-rate reactors, which required fastidious removal of solids within the flush manure so as to not interfere with or clog the artificial media employed for biomass retention and high-rate operation, research done here has shown that effective settling based on particle size identification ($> 1\text{mm}$) can lead to a feed influent with a considerably higher VS and COD concentration, representing nearly 70% of the biogas potential (Frear et al., 2009). Importantly, the fibrous material remaining in the influent can serve as a natural support media for bacteria, a fact suggested by rumen theory and confirmed in laboratory and pilot testing of operating reactors. A high-rate reactor without need for artificial media reduces system costs and operating concerns, particularly those regarding clogging and organic or inorganic accumulation, compared to other available designs. In addition, proper operation of the system, via flow patterns, mixing and feedstock addition regimes, can generate optimized selection pressures

capable of inducing desired biomass activities, biofilm structures and bacterial species. In regard to bacterial species, evidence exists that it is possible to maintain a *methanosarcina*-dominated population, providing greater reactor resiliency in the face of upsets or changes in loading and/or temperature. This resiliency is particularly advantageous for this application as energy balances show that even given the high-rate efficiency of the reactor design economical operation at very cold temperatures will require both higher psychrophilic temperatures and artificially-elevated feed concentrations accomplished through co-digestion of substrates. Pilot-scale study of the lessons and approaches learned is on-going as of the writing of this chapter. A 90-gallon pilot SBR process flow diagram devised and now being used in studies is shown in Figure 11.29.

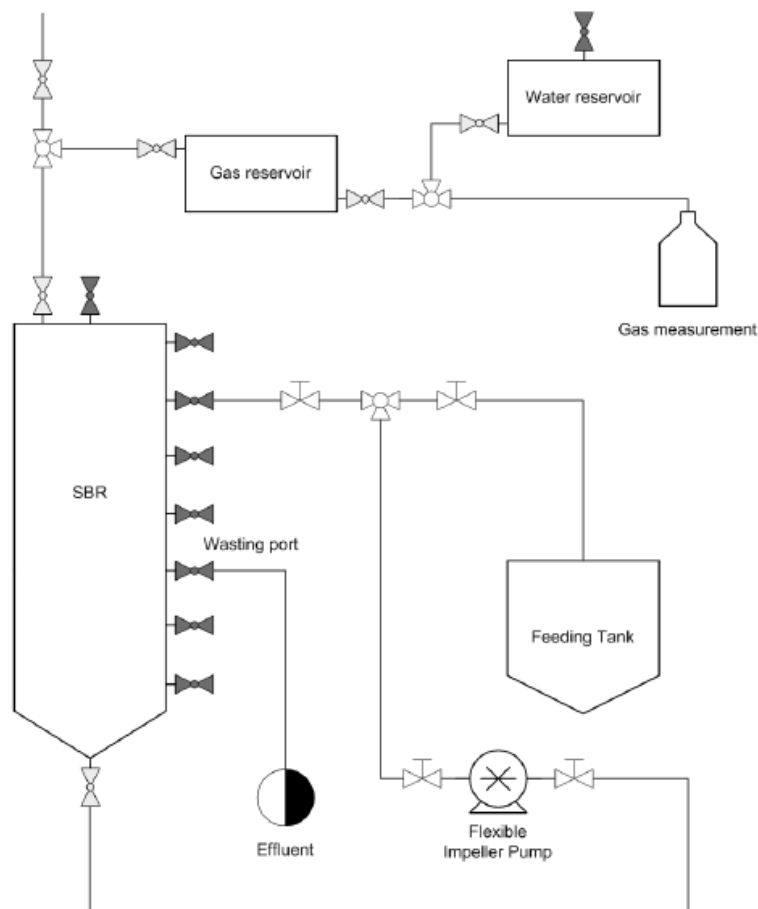


Figure 11.29: Pilot SBR process flow diagram

A Dual-Reactor Anaerobic Digestion System for Treating the Organic Fraction of Municipal Solid Waste

Introduction

As Washington continues its efforts to more sustainably utilize organic wastes, new and better technologies to accomplish this will need to be developed. Washington

State currently produces over 16 million dry tons of underutilized biomass, according to the Biomass Inventory and Bioenergy Assessment (Frear et al., 2005) conducted by Washington State University (WSU) and the Washington State Department of Ecology (Ecology). Of that amount, approximately 750,000 tons of post-consumer wastes (food waste, yard waste, yellow and brown grease, other miscellaneous organics) and 360,000 tons of food processing and packing wastes are potentially suitable for digestion, many of which can be co-digested with manures on farm AD facilities. In many cases, though, location, regulations, and economics dictate that treatment of these wastes occurs outside of the farm environment and without co-digestion with manures and their buffering stability. Presently, industry predominantly chooses to not digest the waste materials instead opting for other options such as landfill, compost or incineration, technologies that do not provide as much renewable energy and sustainability benefits as AD. One reason for this choice against AD is the difficulty in digesting highly volatile organic waste solids in an economical and stable manner. Clearly, new technological options must be made available to industry in order to increase the adoption rate of AD for these particular high-strength solids.

Several different types of AD technologies exist to accommodate high-strength solid wastes. These technologies can be broadly classified as those appropriate for low solid concentrations (less than 15% of TS) or high solid concentrations (greater than 15%). High solids anaerobic digestion (HSAD) is a relatively new application of conventional AD technology and can be accomplished through three basic forms of technology:

- Wet systems—approach that dilutes the high solids to low TS capable of being pumped and mixed in typical plug-flow and/or complete mix designs;
- Dry systems—approach that maintains the high solids content in a stackable form that is not actively mixed, but simply uses liquid leachate return as a mechanism for mass transfer;
- Phased systems—approach that breaks the AD process into acidification and methanogenesis steps, each with their own dedicated reactors and units processes—notably, one reactor is making primarily methane while the other reactor produces mostly CO₂ and H₂.

A review of these existing commercial HSAD designs shows that scientific and engineering concerns still exist within each of these approaches. In the wet system, dilution with potentially valuable, costly and scarce water resources makes little engineering sense as larger and more expensive reactors are required to handle the diluted waste stream. In addition the mechanical mixing and solids recycling that occurs to maintain effective bacterial mass transfer and inoculation to protect the system from inhibition are costly from both a capital and operating energy sense. In the dry system, purposeful non-mixing reduces capital and operating energy costs but biological kinetics are severely hampered by the loss in mass transfer efficiency, resulting in less than impressive biogas production and performance. In the phased system, the separation of biological processes results in added complexity in regard

to flow patterns and number of reactors, with consequent capital and operating cost increases. Goals of this study were to develop a new engineering approach towards high solids digestion that combines the best concepts of existing approaches and formulates a new design capable of reducing capital and operating costs while maintaining effective biogas production performance and stability.

New HSAD Design

This research project developed, tested, and modeled an innovative design for a mesophilic (35°C) HSAD system for the biological treatment of biomass consisting primarily of the organic fraction of municipal solid waste (MSW) (Figure 11.30). This system utilizes an innovative dual-chamber digester design to efficiently inoculate high solids waste with a recycled leachate containing a dense concentration of anaerobic organisms. The leachate is separated from the solids chamber, treated in a modified high rate upflow anaerobic sludge blanket (UASB) digester (seed chamber), and recycled back to the high solids chamber to provide mixing, pH control, and seeding of anaerobic microorganisms. At the same time recycling the leachate provides a convenient pathway for nutrient removal and recovery from the digester.

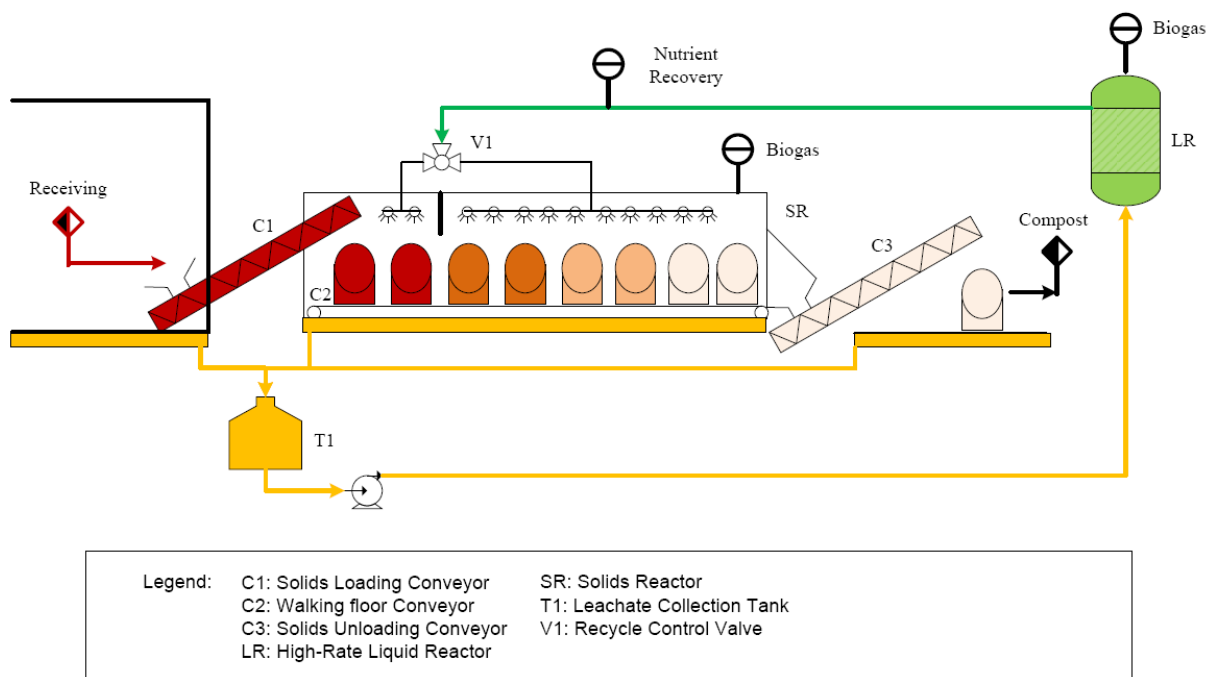


Figure 11.30: New HSAD process flow diagram

This hybrid system utilizes dual reactors but is not phased in that both reactors are operating under near neutral pH conditions and producing an effective methane concentration within both reactor headspaces. In addition, this system is in many ways a typical dry system in that a stackable pile is digested without active mechanical mixing, only liquid leachate return, but the biological kinetics and

stability are enhanced over typical dry systems in that the attached high-rate reactor digests the high VFA liquid leachate--returning a pH neutral liquid to the pile. Advantages of this high-rate liquid reactor and neutral pH leachate return are:

- Improved biogas production kinetics through the use of a high-rate liquid reactor;
- Enhanced and more cost-effective bacterial inoculation resulting from the release of bacteria from the high-rate reactor to the solids reactor as opposed to using sludge or solids recycle;
- Greater system stability in that high VFA liquids are quickly reacted prior to entry back to the pile, removing a notable product inhibition threat plaguing many digesters;
- Use of VFA removed leachate as a means for mass transfer throughout the system allows for more sustainable use of limited water resources, reduction in reactor sizes and importantly, a means for inducing nutrient recovery as the majority of the mineralized nutrients reside within the liquid leachate.

Proof of System Capabilities and Viability

Preliminary modeling based on bench-scale experimental results has indicated that this system compares favorably to the reported performance of current dry digester technologies. The bench-scale results for this system also compare favorably to existing AD technologies in several areas. Digester loading rate and biogas production rate are improved by about 50%, while achieving comparable chemical oxygen demand and total solids reduction. This compares a bench scale experimental design to actual facility performance. At full scale, the system will require optimization to achieve similar or enhanced performance. In addition to the waste treatment benefits of this system, the potential to integrate a nutrient removal and recovery system increases the overall economic value of the system. It is estimated that integrating the leachate recycle loop into a nutrient removal and recovery system would produce 2.1 kg/ton of nitrogen and 3.72 kg/ton of phosphorus from food waste. Based on the bench-scale results, the cost of treating organic waste with this system is estimated to be \$1.08/kW-h compared to \$1.55/kW-h calculated for an existing technology. These values account for capital and operational costs amortized over the predicted operating life of the facility. This system has potential to lower both capital and operational costs compared to existing technologies.

Conclusions

With proof-of-concept and initial modeling of the new system validated, large bench-scale testing of the system is about to begin through the cooperation of industrial partners interested in eventual commercialization of the technology. If scaled testing proves valid as well it is hoped that the design will further advance AD adoption within an entirely new sector of municipal solid wastes, both at a large municipal scale, i.e. industrial compost yards, or at smaller industrial operations, i.e. food processors.

Overall Chapter Conclusions

Although the road to commercialization for new engineering solutions and technologies is long and cumbersome, the first important steps to specific commercialization for three distinct technologies are well along the path. The vertical plug-flow mobile pilot is constructed and do for evaluation and testing this Spring of 2010 while small-pilot, floor-scale testing of both the dilute, flush manure SBR and novel HSAD are also do to begin operation and testing this Spring of 2010. Beyond, the progress towards commercialization is the important fact that WSU researchers and its industrial partners now have access to two large pilot-scale AD facilities for continued and long-term testing of AD concepts. These facilities are the 12 m³/day pilot at the WSU Dairy Center and the mobile vertical plug-flow digester just completed. These facilities provide infrastructure which can test not only this immediate generation of new technologies but future ones as well while also providing a platform for important scientific studies in general AD.

Key Project References Related to Chapter

The majority of the work presented in this chapter has been previously published as:

- Frear, C., Zhi-Wu, W., Li, C.-L., Chen, S., 2009. Biogas potential and microbial population distributions in flushed dairy manure and implications on anaerobic digestion technology, *Biomass and Bioenergy*, Submitted.
- Frear, C., 2009. Anaerobic digestion strategies for dairy manures. PhD thesis, Department of Biological Systems Engineering, Washington State University, August 2009.
- Wang, Z., Ma, J., Chen, S., 2010. Bi-polar effects of settling time on active biomass retention in anaerobic sequencing batch reactors digesting flushed dairy manure, *Water Research*, Submitted.
- Zaher, U., Li, C., Yu, L., Ewing, T., Chen, S., 2009. Producing energy and fertilizer from organic municipal solid waste—final report, Washington State Department of Ecology, Olympia, Washington.

References

- Alphenaar, P., Visser, A., Lettinga, G., 1993. The effect of liquid upward velocity and hydraulic retention time on granulation in UASB reactors treating wastewater with a high sulphate content. *Bioresource Technology*, 43, 249-258.
- Anderson, G.K., Kasapgil, B., Ince, O., 1994. Comparison of porous and non-porous media in upflow anaerobic filters when treating dairy wastewater. *Water Research*, 28, 1619-24.
- Andersson, J., Bjornsson, L., 2002. Evaluation of straw as a biofilm carrier in the methanogenic stage of two-stage anaerobic digestion of crop residues. *Bioresource Technology*, 85, 51-56.

- Bath, D.L., Ronning, M., Lofgreen, G.P., Meyer, J.H., 1966. Influence of Variations in Ruminal Contents upon Estimates of Body Weight Change of Dairy Cattle during Restricted Feeding. *J. Dairy Sci.*, 49, 830-834.
- Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H., Vavilin, V.A., 2001. The IWA Anaerobic Digestion Model No 1 (ADM1) 9th World Congress on Anaerobic Digestion, Antwerp, Belgium, pp. 65-73.
- Beun, J.J., Hendriks, A., Van Loosdrecht, M.C.M., Morgenroth, E., Wilderer, P.A., Heijnen, J.J., 1999. Aerobic granulation in a sequencing batch reactor. *Water Research*, 33, 2283-2290.
- Burke, D.A., 2001. Dairy waste anaerobic digestion handbook. Environmental Energy Company, 6007 Hill Street, Olympia, WA 98516.
- Chastain, J.P., Vanotti, M.B., Wingfield, M.M., 2001. Effectiveness of liquid-solid separation for treatment of flushed dairy manure: A case study. *Applied Engineering in Agriculture*, 17, 343-354.
- Cheng, K.J., McAllister, T.A., Costerton, J.W., 1995. Biofilms of the ruminant digestive tract. in: H.M. Lappin-Scott, J.W. Costerton (Eds.), *Microbial Biofilms*. Cambridge University Press, New York, USA, pp. 221 - 232.
- Connaughton, S., Collins, G., O'Flaherty, V., 2006. Psychrophilic and mesophilic anaerobic digestion of brewery effluent: A comparative study. *Water Research*, 40, 2503-2510.
- Costerton, J.W., 1992. Pivotal Role of Biofilms in the Focused Attack of Bacteria on Insoluble Substrates. *International Biodeterioration & Biodegradation*, 30, 123-133.
- Craig, W.M., Broderick, G.A., Ricker, D.B., 1987. Quantitation of Microorganisms Associated with the Particulate Phase of Ruminant Ingesta. *J. Nutr.*, 117, 56-62.
- Dinsdale, D., Morris, E.J., Bacon, J.S.D., 1978. Electron Microscopy of the Microbial Populations Present and Their Modes of Attack on Various Cellulosic Substrates Undergoing Digestion in the Sheep Rumen. *Applied and Environmental Microbiology*, 36, 160-168.
- Dvorak, S., 2008. Permanent access port. in: U.S.P.a.T. Office (Ed.) 2008/0277336. GHD, Inc, USA.
- Esterl, S., Hartmann, C., Delgado, A., 2003. On the influence of fluid flow in a packed-bed biofilm reactor. in: S. Wuertz, P.L. Bishop, P.A. Wilderer (Eds.), *Biofilms in wastewater treatment*. International Water Association, London, England, pp. 89-116.

- Ferguson, T.J., Mah, R.A., 1983. Effect of H₂-CO₂ on Methanogenesis from Acetate or Methanol in *Methanosarcina* spp. *Appl Environ Microbiol*, 46, 348-355.
- Forsberg, C.W., Lam, K., 1977. Use of Adenosine 5'-Triphosphate as an Indicator of the Microbiota Biomass in Rumen Contents. *Appl Environ Microbiol*, 33, 528-537.
- Frear, C., Zhao, B., Fu, G., Richardson, M., Chen, S., 2005. Biomass inventory and bioenergy assessment: An evaluation of organic material resources for bioenergy production in Washington state. in: W.S.D.o. Ecology (Ed.), Olympia, Washington.
- Frear, C., Zhi-Wu, W., Li, C.-L., Chen, S., 2009. Biogas potential and microbial population distributions in flushed dairy manure and implications on anaerobic digestion technology *Biomass and Bioenergy*, Submitted.
- Gerritse, J., Gottschal, J.C., 1993. 2-Membered Mixed Cultures of Methanogenic and Aerobic-Bacteria in O₂-Limited Chemostats. *Journal of General Microbiology*, 139, 1853-1860.
- Grady, C.P.L., Daigger, G.T., Lim, H.C., NetLibrary Inc., 1999. Biological wastewater treatment. 2nd edn ed. Marcel Dekker, New York.
- Griffin, M.E., McMahon, K.D., Mackie, R.I., Raskin, L., 1998. Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids. *Biotechnology and Bioengineering*, 57, 342-355.
- Hills, D.J., Kayhanian, M., 1985. Methane from settled and filtered flushed dairy wastes. *Transactions of the ASAE*, 28, 865-869.
- Hulshoff Pol, L.W., Heijnekamp, K., Lettinga, G., 1988. The selection pressure as a driving force behind the granulation of anaerobic sludge. in: G. Lettinga, A.J.B. Zehnder, J.T.C. Grotenhuis, L.W. Hulshoff Pol (Eds.), *Granular anaerobic sludge: microbiology and technology*. Wageningen, Netherlands, pp. 153-161.
- Irvine, R.L., Fox, T.P., Richter, R.O., 1977. Investigation of fill and batch periods of sequencing batch biological reactors. *Water Research*, 11, 713-717.
- Latham, M.J., 1980. Adhesion of rumen bacteria to plant cell walls. in: R.C.W. Berkeley, J.M. Lynch, J. Melling, P.R. Rutter, B. Vincent (Eds.), *Microbial Adhesion to Surfaces*. Ellis Horwood, West Sussex, U.K., pp. 339.
- Lee, C.C., 2000. *Handbook of environmental engineering calculations*. McGraw-Hill, New York.
- Lettinga, G., Rebac, S., Zeeman, G., 2001. Challenge of psychrophilic anaerobic wastewater treatment. *Trends in Biotechnology*, 19, 363-370.

- Liao, W., Frear, C., Oakley, K., Chen, S., 2009. Production of a Pretreated Fibrous Manure Solid as a Soil Amendment Bedding Replacement using A Leaching-Bed Reactor. *Bioresource Technology*, Submission.
- Liu, Y., Wang, Z.W., Qin, L., Liu, Y.Q., Tay, J.H., 2005. Selection pressure-driven aerobic granulation in a sequencing batch reactor. *Appl Microbiol Biotechnol*, 67, 26-32.
- Liu, Y., Wang, Z.W., Qin, L., Liu, Y.Q., Tay, J.H., 2005. Selection pressure-driven aerobic granulation in a sequencing batch reactor. *Applied Microbiology and Biotechnology*, 67, 26-32.
- Lo, K.V., Bulley, N.R., Kwong, E., 1985. Sequencing aerobic batch reactor treatment of milking parlor wastewater. *Agricultural Wastes*, 13, 131-136.
- Lott, S.C., Loch, R.J., Watts, P.J., 1994. Settling characteristics of feedlot cattle feces and manure. *Transactions of the American Society of Agricultural Engineers*, 37, 281-285.
- Lundin, A., Thore, A., 1975. Comparison of Methods for Extraction of Bacterial Adenine Nucleotides Determined by Firefly Assay. *Applied and Environmental Microbiology*, 30, 713-721.
- Mackie, R.I., Stroot, P.G., Varel, V.H., 1998. Biochemical identification and biological origin of key odor components in livestock waste. *J.Anim.Sci.*, 76, 1331-1342.
- Madigan, M.T., Martinko, J.M., Parker, J., 2003. Brock biology of microorganisms. Upper Saddle River, NJ: Prentic Hall/Pearson Education.
- Masse, D., Droste, R., Kennedy, K., Patni, N., Munroe, J., 1993. Psychrophilic Anaerobic Treatment of Swine Manure in Intermittently Fed Sequencing Batch Reactors ASAE International Winter Meeting, Chicago, IL.
- McAllister, T.A., Bae, H.D., Jones, G.A., Cheng, K.J., 1994. Microbial Attachment and Feed Digestion in the Rumen. *Journal of Animal Science*, 72, 3004-3018.
- Mcallister, T.A., Rode, L.M., Major, D.J., Cheng, K.J., Buchanansmith, J.G., 1990. Effect of Ruminant Microbial Colonization on Cereal Grain Digestion. *Canadian Journal of Animal Science*, 70, 571-579.
- McGarvey, J.A., Miller, W.G., Sanchez, S., Stanker, L., 2004. Identification of bacterial populations in dairy wastewaters by use of 16S rRNA gene sequences and other genetic markers. *Applied and Environmental Microbiology*, 70, 4267-4275.
- McHugh, S., Carton, M., Collins, G., O'Flaherty, V., 2004. Reactor performance and microbial community dynamics during anaerobic biological treatment of wastewaters at 16-37 DegC. *FEMS Microbiology Ecology*, 48, 369-378.

- McHugh, S., Collins, G., O'Flaherty, V., 2006. Long-term, high-rate anaerobic biological treatment of whey wastewaters at psychrophilic temperatures. *Bioresource Technology*, 97, 1669-78.
- Meier-Schneiders, M., Busch, C., Diekert, G., 1993. The attachment of bacterial cells to surfaces under anaerobic conditions. *Applied Microbiology and Biotechnology*, 38, 667-73.
- Metcalf and Eddy, 2003. Wastewater engineering: treatment and reuse. 4th edn ed. McGraw-Hill, Boston.
- Meyer, D., Ristow, P.L., Lie, M., 2007. Particle size and nutrient distribution in fresh dairy manure. *Applied Engineering in Agriculture*, 23, 113-117.
- Owens, F.N., Goetsch, A.L., 1986. Digesta passage and microbial protein synthesis. in: L.P. Milligan, W.L. Grovum, A. Dobson (Eds.), Control of Digestion and Metabolism in Ruminants. Prentice-Hall, Englewood Cliffs, NJ, pp. 196.
- Powers, W., Wilkie, A., Van Horn, H., Nordstedt, R., 1997. Effects of Hydraulic Retention Time on Performance and Effluent Odor of Conventional and Fixed-Film Anaerobic Digesters Fed Dairy Manure Wastewaters. *Transactions of the ASAE*, 40, 1449-1455.
- Qin, L., Liu, Y., Tay, J.H., 2004. Effect of settling time on aerobic granulation in sequencing batch reactor. *Biochemical Engineering Journal*, 21, 47-52.
- Qin, L., Tay, J.H., Liu, Y., 2004. Selection pressure is a driving force of aerobic granulation in sequencing batch reactors. *Process Biochemistry*, 39, 579-584.
- Rittmann, B.E., McCarty, P.L., 2001. Environmental biotechnology: principles and applications. McGraw-Hill, Boston.
- Safley, L.M., Jr., Westerman, P.W., 1990. Psychrophilic anaerobic digestion of animal manure: proposed design methodology. *Biological Wastes*, 34, 133-48.
- Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews*, 61, 262-280.
- Schwarzenbeck, N., Borges, J.M., Wilderer, P.A., 2005. Treatment of dairy effluents in an aerobic granular sludge sequencing batch reactor. *Applied Microbiology and Biotechnology*, 66, 711-718.
- Shinkai, T., Kobayashi, Y., 2007. Localization of ruminal cellulolytic bacteria on plant fibrous materials as determined by fluorescence in situ hybridization and real-time PCR. *Applied and Environmental Microbiology*, 73, 1646-1652.
- Svensson, L.M., Bjornsson, L., Mattiasson, B., 2007. Enhancing performance in anaerobic high-solids stratified bed digesters by straw bed implementation. *Bioresource Technology*, 98, 46-52.

- US-EPA, 2005. An evaluation of a mesophilic, modified plug-flow anaerobic digester for dairy cattle manure. United States Environmental Protection Agency.
- US EPA, 2007. Guide to Anaerobic Digesters Anaerobic Digester Database.
- Vartak, D.R., Engler, C.R., McFarland, M.J., Ricke, S.C., 1997. Attached-film media performance in psychrophilic anaerobic treatment of dairy cattle wastewater. *Bioresource Technology*, 62, 79-84.
- Vesilind, P.A., 2003. Wastewater treatment plant design. IWA Pub, London.
- Wang, Z.W., Liu, Y., Tay, J.H., 2006. The role of SBR mixed liquor volume exchange ratio in aerobic granulation. *Chemosphere*, 62, 767-771.
- Weimer, P.J., Price, N.P.J., Kroukamp, O., Joubert, L.M., Wolfaardt, G.M., Van Zyl, W.H., 2006. Studies of the extracellular glycocalyx of the anaerobic cellulolytic bacterium *Ruminococcus albus* 7. *Applied and Environmental Microbiology*, 72, 7559-7566.
- Wilderer, P.A., Irvine, R.L., Goronszy, M.C., 2001. Sequencing Batch Reactor Technology. IWA, London, UK.
- Wilkie, A.C., 2004. Fixed-film digesters. in: E. Agstar (Ed.) AgStar National Conference.
- Wilkie, A.C., Castro, H.F., Cubinski, K.R., Owens, J.M., Yan, S.C., 2004. Fixed-film anaerobic digestion of flushed dairy manure after primary treatment: Wastewater production and characterisation. *Biosystems Engineering*, 89, 457-471.
- Woolard, C.R., Irvine, R.L., 1995. Treatment of hypersaline waste-water in the sequencing batch reactor. *Water Research*, 29, 1159-1168.
- Wright, W.F., 2005. Dairy manure particle size distribution, properties, and implications for manure handling and treatment 2005 ASAE annual international meeting. Paper Number: 054105, Tampa, Florida.
- Yoon, B.T., Kim, G.Y., Kim, S.B., Choi, M.J., 2007. Manufacture of the fluidizing media using rice straw and paper wastewater treatment. *Palpu Chongi Gisul/Journal of Korea Technical Association of the Pulp and Paper Industry*, 39, 9-16.
- Young, J.C., Dahab, M.F., 1983. Effect of media design on the performance of fixed-bed anaerobic reactors. *Water Science and Technology*, 15, 369-83.