ANAEROBIC KINETIC STUDY OF

SWINE SLURRY

By

DAVID JOE WILLIAMS

Bachelor of Arts
The University of Tulsa
Tulsa, Oklahoma
1999

Master of Environmental Science
The University of Oklahoma
Norman, Oklahoma
2001

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December 2004

ANAEROBIC KINETIC STUDY OF

SWINE SLURRY

Thesis Approved:

William Clarkson, Ph.D.

William Clarkson, Ph.D., Thesis Advisor

Ken Ede, Ph.D.

Ken Ede, Ph.D., Committee Member

Dee Ann Sanders, Ph.D.

Dee Ann Sanders, Ph.D., Committee Member

A. Gordon Emslie, Ph.D.

A. Gordon Emslie, Ph.D., Dean of the Graduate College

ACKNOWLEDGEMENTS

The author expresses gratitude for the immeasurable laboratory assistance provided by Hector Cumba-Delrio, Biosystems and Agricultural Engineering, Oklahoma State University; attainment of research samples and data from Pius Ndegwa, Ph.D., Biosystems and Agricultural Engineering, Oklahoma State University; and most especially the overall guidance imparted by William Clarkson, Ph.D., School of Civil and Environmental Engineering, Oklahoma State University. This research was made possible in part by a two-year grant awarded by the Oklahoma State University Environmental Institute. Appreciation is extended to my parents, Thomas and Nedra Williams, for their encouragement and support.

For Lauren.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

NOMENCLATURE

ASBR	anaerobic sequencing batch reactor
CH_3COOH	acetic acid
CAFO	confined animal feeding operation
CH_4	methane
CO_2	carbon dioxide
COD	chemical oxygen demand
COD_S	soluble chemical oxygen demand
COD_T	total chemical oxygen demand
HRT	hydraulic residence time
NH_4^+-N	nitrogen measured as ammonium
PO_4^{3-}-P	phosphorus measured as phosphate
SBR	sequencing batch reactor
SRT	solids residence time
%TS	total solids, percentage
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acids
%VS	total volatile solids, percentage

I. INTRODUCTION

Increasing human populations have placed new, challenging demands on agriculture. Never in the history of man has the global population been as high as it is currently – over 6 billion. In the United States alone, nearly 300 million persons now reside. The world is hungry, and livestock have been grown at an ever-increasing rate in order to keep pace. Multinational corporations have steadily gained dominance in the market. These companies have implemented confined animal feeding operations (CAFOs) – large livestock facilities that handle millions of swine, poultry, or cattle at any given time. According to the American Society of Agricultural Engineers, swine produce an average of 84 kg manure per 1,000 kg live animal mass per day (ASAE, 1999). Thus, it is no secret why swine wastes can quickly accumulate and become problematic at small operations, let alone a large CAFO. Swine wastes present aesthetic and health concerns. Undesirable odors from a CAFO can waft through the air for miles (Dague and Pidaparti, 1992). Elemental nutrients that are transported from CAFOs into streams and tributaries pose risks associated with water eutrophication. Human health is also a major concern. Swine waste can contain pathogenic bacteria and viruses that threaten persons in contact with contaminated water (Pagilla et al., 2000).

Recognition of the threat that animal wastes, including swine waste, pose to the natural environment has spawned new research that embraces engineering techniques as a

method of mitigation. Specifically, technologies incorporating microbiology have been developed. Anaerobic processes – degradation occurring in systems devoid of oxygen – are currently popular subjects of study. Effective anaerobic digestion relies upon many distinct species of bacteria, each with different biological requirements for survival. However, if system stability can be achieved, anaerobic sequencing batch reactors (ASBRs) offer treatment of swine waste with solids residence times (SRT) decoupled from hydraulic residence times (HRT), yielding a high rate process and energy production.

Development of an effective ASBR requires knowledge of the initial properties of the specific influent. The kinetics of swine waste degradation are an indicator of influent behavior within the ASBR. Determination of microbiological performance (measured primarily as volatile fatty acid (VFA) production and consumption as well as biogas (CH_4 and CO_2) production in a kinetic study is critical to the development of an operational ASBR, because it establishes an expectation of actual performance. Capital and operational costs are associated with any engineered system, biological reactors included, and system failure can be financially devastating. The construction of an ASBR tailored to the kinetic properties of the influent is therefore critical.

II. HYPOTHESIS & OBJECTIVES

The objective of this research project is the measurement of the initial kinetic properties of swine waste that contribute to the process efficiency of an anaerobic sequencing batch reactor (ASBR). Specifically, the following parameters will be monitored:

- pH
- Alkalinity
- Volatile Fatty Acid (VFA) Concentration
- CH_4 Production, Concentration
- CO_2 Production, Concentration
- Chemical Oxygen Demand (COD_T and COD_S)
- Total Solids (%)
- Volatile Solids (%)
- Nitrogen (measured as NH_4^+-N)
- Phosphorus (measured as PO_4^{3-}-P)

The goal of this research project is to measure, record, and analyze the kinetic properties of swine waste slurry over a total solids loading range of 0.25-0.75% and at temperatures of 20°C and 35°C, respectively. Understanding of the initial swine slurry properties and subsequent response to biological action are vital to construction of a reactor tailored to receive and process the influent with maximum efficiency. Therefore, a short-term study of swine slurry kinetics within the proposed operating conditions of the ASBR under development at Oklahoma State University was desired. Ultimately, kinetic rates of the swine slurry will govern the temporal and dimensional design of the ASBR. A specific hypothesis of this research is that the most efficient startup kinetics will be observed in the highest initial %TS reactors operated at 35°C, due to the fact that the greatest amount of consumable substrate at the ideal mesophilic temperature will result in the most active collection of anaerobic bacterial populations involved in the study. Additionally, it is hypothesized that NH_4^+-N and PO_4^{3-}-P, known environmental pollutants that contribute to the degradation of water quality, are recalcitrant compounds that will exhibit little or no decrease in concentration within the short-term anaerobic experiment.

III. LITERATURE REVIEW

Anaerobic treatment of swine waste has been the subject of considerable recent research. Systems incorporating anaerobic digestion have been present in the natural environment since time immemorial, but substantial engineering advances have been realized during the past two decades. Current experimental technology implements digestion over a wide range of operating temperatures and residence times.

Species of bacteria responsible for anaerobic processes can be broadly defined according to the temperature range in which they are active. Psychrophiles are most efficient at low (0-20ºC) temperatures, mesophiles at moderate (20-40ºC) temperatures, and thermophiles at high (40-70ºC) temperatures (Figure 1) (Lettinga et al., 2001). Most anaerobic studies have been conducted within the mesophilic range (Dague and Pidaparti, 1992). Mesophilic operations offer fast-rate reactions while requiring less energy input as compared to psychrophilic and thermophilic regimes (Lettinga et al., 2001). Anaerobic reactors are generally operated at a temperature of 35ºC (Dague and Pidaparti, 1992).

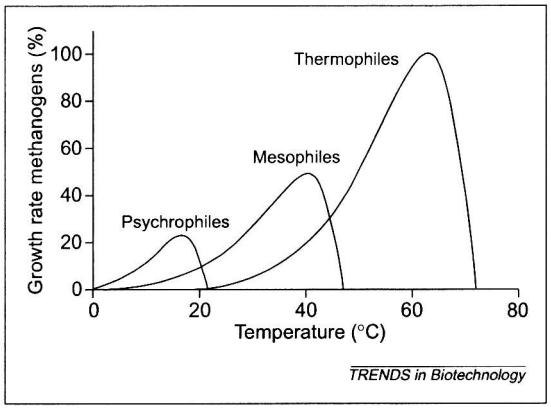


FIGURE 1: Temperature ranges of anaerobic bacteria (Lettinga et al., 2001)

Anaerobic digestion is a process that requires symbiosis between several distinct microbial populations. Four categories of bacteria are at work: hydrolytic-fermentative, acetogenic, aceticlastic methanogens, and hydrogenotrophic methanogens (Griffin et al., 1998). The anaerobic process ultimately converts complex organic compounds to methane and carbon dioxide (Pullammanappalil et al., 2001). Hydrolysis, the initial phase of the anaerobic sequence, involves the transformation of proteins and carbohydrates to amino acids and sugars. Similarly, lipids are degraded to fatty acids and alcohols. During fermentation, a hydrolysis co-process, the products of hydrolysis are further degraded to intermediary volatile fatty acids, including propionate, butyrate, and valerate. These acids are short lived and quickly transition to acetate. Carbon dioxide and hydrogen are also produced as a result of anaerobic oxidation. Finally, methanogens become the dominant microbiological force in the final phase of anaerobic digestion, appropriately known as methanogenesis, generating the end products carbon dioxide and methane (Figure 2) (Pavlostathis and Giraldo-Gomez, 1991).

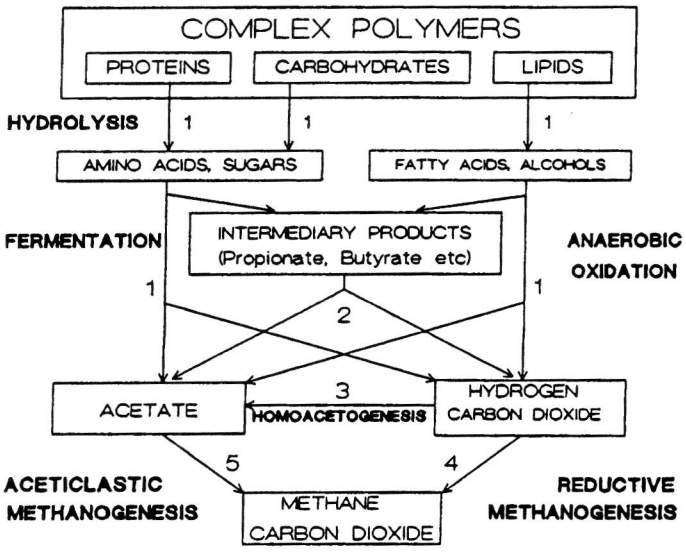


FIGURE 2: Anaerobic degradation sequence of complex organic compounds (Pavlostathis and Giraldo-Gomez, 1991)

Hydrolysis, the aforementioned preliminary phase of anaerobic digestion (Figure 2, phase 1), involves the conversion of complex polysaccharides, lipids, and proteins to short chain fatty acids, alcohols, carbon dioxide, and ammonia (Pavlostathis and Giraldo-Gomez, 1991). Acidogens – fermentative microbes responsible for the production of the intermediary products – rapidly grow and outnumber the other species of bacteria during hydrolysis, creating an initial abundance of volatile fatty acids (Bagley et al., 1999; Massé et al., 2001). Solubilization of complex polymers allows the transfer of resultant products across the cell membrane. Protein sequences that have undergone hydrolysis transform initially to amino acids and are subsequently fermented to VFA products and H_2 (Pavlostathis and Giraldo-Gomez, 1991). Hydrolysis can be stoichiometrically demonstrated with gelatin, a protein that is initially converted to amino acids:

$$CH_{2.03}O_{0.6}N_{0.3}S_{0.01} + 0.3006H_2O \rightarrow 0.017013C_5H_7NO_2$$ (Angelidaki et al., 1999)

Carbohydrates (namely cellulose) are reduced to cellobiose and glucose as well as other compounds, including uronic acid (Pavlostathis and Giraldo-Gomez, 1991). Lipids (fats) reduce to long chain fatty acids (Pavlostathis and Giraldo-Gomez, 1991). For example, glycerol trioleate undergoes lipolysis and is converted to oleate and glycerol:

$$C_{57}H_{104}O_6 + 3H_2O \rightarrow C_3H_8O_3 + 3C_{18}H_{34}O_2$$ (Angelidaki et al., 1999)

Subsequently, these lipolysis products are converted to propionate, an intermediate VFA (Angelidaki et al., 1999). Because they initially transform complex polymers and thus control the hydrolysis phase, acidogens are considered to be the primary degrading bacteria of anaerobic digestion (Daffonchio et al., 1995). If the system temperature were reduced from the optimal mesophilic range, acidogen populations would decrease, as would removal efficiency (Cha et al., 1997; Lettinga et al., 2001).

During fermentation, a co-process with hydrolysis (Figure 2, phases 1 and 2), short and long chain fatty acids are converted to acetate and H_2. This process involves the anaerobic oxidation of intermediates by acetogenic bacteria (Pavlostathis and Giraldo-Gomez, 1991). Thus, acetogens are considered to be intermediate degraders (Daffonchio et al., 1995). Degradation rates vary little with respect to short chain and long chain acids – long chain fatty acids are fermented at roughly the same rate as acetic and propionic

acids (Pavlostathis and Giraldo-Gomez, 1991). Many short chain fatty acids are the result of carbohydrate transformation. If a simple carbohydrate, glucose, is considered, acetate is produced through the following reactions:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$ (Batstone et al., 2002)

$$3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH + 2CH_3COOH + 2CO_2 + 2H_2O$$ (Batstone et al., 2002)

Likewise, the primary acetate intermediate propionate is produced through the following reaction:

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$$ (Batstone et al., 2002)

Other intermediate short chain VFA compounds are produced through similar transformations. The presence of intermediates such as propionate, butyrate, and valerate stimulates another fermentative process – acetogenesis. Acetogenic bacteria utilize the intermediate acids, transforming them into acetate and hydrogen (Griffin et al., 1998). An example of acetate production is the following degradation reaction of propionate:

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2$$ (Batstone et al., 2002)

Similar acetogenic reactions result in acetate production from other intermediate acids. Even though it is a product of acetogenic bacteria, H_2 is inhibitory to propionate and butyrate degrading acetogens at high concentrations (Angelidaki et al., 1993).

Acetogenesis is therefore dependent on efficient H_2 removal (Pavlostathis and Giraldo-Gomez, 1991). Thus, oxidation of H_2 to acetate is critical:

$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$ (LeVan et al., 1998)

Once fermentative bacteria have generated significant quantities of acetate, anaerobic digestion shifts to its final phase – methanogenesis (Figure 2, phases 4 and 5). Methanogens are a low growth rate, primitive bacteria (Griffin et al., 1998). However, they effectively consume the products of fermentation, producing the end products of methane (CH_4) and carbon dioxide (CO_2):

$CH_3COOH \rightarrow CH_4 + CO_2$ (Batstone et al., 2002)

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ (LeVan et al., 1998)

Thus, methanogens are ultimate degraders (Daffonchio et al., 1995). Methangenesis is limited by substrate availability. Few compounds can be consumed by methanogens, highlighting the necessity for hydrogen and acetate formation in previous steps (Hwang et al., 2001). Acetate is responsible for 70% of CH_4 (Pavlostathis and Giraldo-Gomez, 1991). Figure 3 illustrates the decrease of VFA concentration in an anaerobic reactor as biogas is produced.

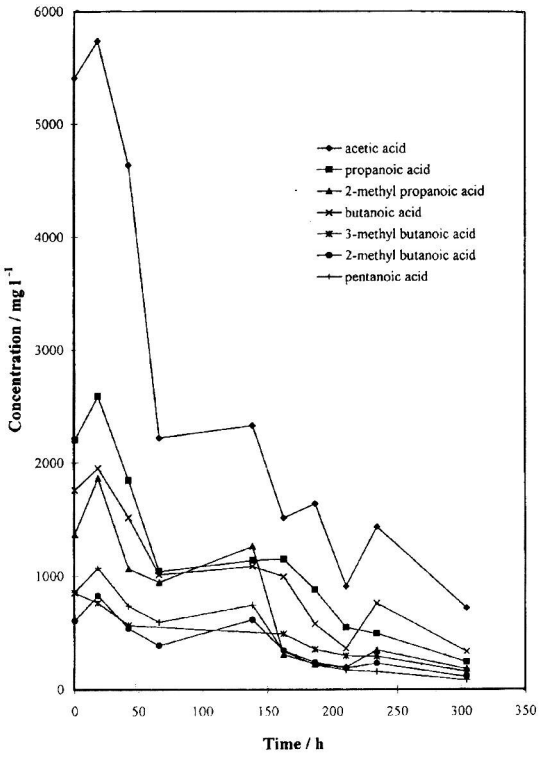


FIGURE 3: VFA consumption during methanogenesis (Hobbs et al, 1999)

Methane production efficiency is heavily influenced by temperature. Massé et al. (2003) found that methane production was as much as five times greater at 22ºC than at 11ºC. Competition from sulfate reducing bacteria for acetate, CO_2, and H_2 also impacts efficiency (Hansen et al., 1999). Species of methanogenic bacteria likely involved in anaerobic digestion include *Methanomicrobiales*, *Methanobacteriales*, *Methanococcales*, and *Methanosarcinales* (Zheng et al., 2000; Griffin et al., 1998; Angenent et al., 2002).

Of significant concern in any anaerobic study is system stability. Dependence upon several unique species of bacteria, particularly acetogens and methanogens that vary physiologically and require different parameters for maximum growth efficiency, dictates

the importance of equilibrium (Hwang et al., 2001). A primary indicator of system stability is volatile fatty acid (VFA) concentration (Ahring et al., 1995). Additionally, pH, alkalinity, and production of CH_4 and CO_2 can be used in tracking system performance (Lahav et al., 2000). Any type of imbalance within the anaerobic system is readily expressed by VFA accumulation – an indication that one or more of the bacteria populations are improperly functioning (Cha et al., 1997). An increase in VFA concentration can enhance any imbalance within an anaerobic reactor. Methanogens are sensitive to fluctuations in pH. If bicarbonate alkalinity is consumed, pH will subsequently fall, shifting the system to an acidic state and inhibiting methanogenesis (Griffin et al., 1998). However, even though imbalance can correspond to elevated VFA levels, high VFA concentrations should not be considered an absolute indication of system instability or failure. In fact, Pullammanappalil et al. (2001) found that propionic acid, an intermediary VFA, can be present in considerable concentration with no effect on system productivity. Methanogens utilize VFAs in biogas production, and they have been shown to reduce VFA concentrations rather quickly (Massé et al., 2001).

The physical properties of swine manure are another important consideration when measuring kinetic parameters. Generally, swine wastes are removed from holding pens through a system of slats or grates into pits that ultimately lead to lagoons. The waste is forced out of the pens with high-pressure water, significantly diluting the solids content and thus creating a slurry (Ndegwa et al., 2001). Even though swine slurry contains a low percentage of total solids, recalcitrant matter including undigested corn and hair

follicles remain in the waste. Large particles are not only resistant to biological action, but they interfere with experimentation because they clog valves. Screening the influent slurry effectively removes undesirable larger particles, but it may alter the chemical nature of the slurry in the process. Hill and Baier (2000) found that waste screening led to an increase in chemical oxygen demand (COD) because more readily degradable organic matter was confined to smaller particles. This research is supported by Rodriguez-Andara and Lomas-Esteban (2002), who observed an increase in organic degradation as particle size decreased, particularly below the supracolloidal threshold. Nitrogen and phosphorus are predominantly contained in the liquid fraction of swine slurry, and screening does not significantly reduce concentrations of either constituent (Hill and Baier, 2000).

A major consideration of commercial agricultural activities is the release of excess nutrients – namely nitrogen and phosphorus. When introduced in aquatic systems, both promote growth of phytoplankton and algae, represented by the empirical formula $C_{106}H_{263}O_{110}N_{16}P$ (Vezjak et al., 1998):

$106CO_2 + 16NO_3^- + HPO_4^{2-} + 122H_2O + 18H^+ \rightarrow C_{106}H_{263}O_{110}N_{16}P + 138O_2$ (Stumm and Morgan, 1981)

This process, known as eutrophication, is detrimental to aquatic diversity and stability. Algae and phytoplankton thrive in nutrient-rich waters, consuming available dissolved

oxygen. Ultimately, less tolerant fish species are replaced by those that are able to survive in oxygen depleted waters (Smith et al., 1999). Another consideration is geosmin, a chemical produced by blue-green algae that affects the taste and odor of eutrophic water (Lawton et al., 2003). Oxygen deficient streams and lakes are aesthetically undesirable and, in many cases, unhealthy (Smith et al., 1999).

Since eutrophication is a potential outcome of the release of agricultural wastes (including swine manure), accountability of nitrogen and phosphorus through the anaerobic digestion process is important. Swine manure is known to contain high concentrations of nitrogen. When calculated as ammonium-N, nitrogen in swine waste has been observed at concentrations as high as 8000 mg/L NH_4^+-N (Bernet et al., 1996; Obaja et al., 2002). Studies suggest that nitrogen can be effectively removed during combined aerobic-anaerobic processes, particularly during digestion within a sequencing batch reactor (SBR) (Tilche et al., 2001). Within this type of system, ammonia is oxidized to nitrate and nitrite through microbiological action, and is further reduced to atmospheric N_2 as the process advances (Brenner, 2000). However, nitrogen removal is slow and inefficient because the initial N concentration is very high, and the chemical oxygen demand (COD) ratio to N is low (Andreottola et al., 1997). Literature indicating nitrogen removal success within strictly anaerobic systems is limited. The presence of nitrogen within an anaerobic reactor poses a challenge to system stability because NH_4^+-N is toxic (Zhang et al., 1997; Obaja et al., 2002). Elevated concentrations of ammonia (NH_3-N) and ammonium correlate to a decrease in CH_4 production (Magbauna et al.,

2000). Elevated NH_3-N concentrations may lead to an "inhibited steady state" in which CH_4 is produced at restricted volumes (Angelidaki et al., 1993; Andelidaki et al., 1999).

Phosphorus also presents a challenge in anaerobic digestion. Measured as phosphate-P (PO_4^{3-}-P), it is released proportionally to the VFA concentration during anaerobic processes (Obaja et al., 2002). Generally, phosphorus concentrations are significantly lower than nitrogen in digester effluent (Obaja et al., 2002). However, because algae are capable of utilizing atmospheric nitrogen, phosphorus usually is the limiting factor of eutrophication (Ndegwa et al., 2001). During anaerobic digestion, polyphosphate accumulating bacteria release orthophosphate while consuming acetate (Brenner, 2000). Therefore, free phosphate concentration is a function of VFA concentration (Obaja et al., 2002).

One area of current research involving the treatment of swine waste focuses on the sequencing batch reactor (SBR). SBR technology, developed in the 1960s, aimed to develop a digestion process independent of temperature. The key was an increased microbial population that would compensate for decreased metabolic activity (Dague and Pidaparti, 1992). Other high rate process technologies include the upflow anaerobic sludge blanket (UASB) and the fluidized bed process (Sung and Dague, 1995). In anaerobic systems, solids digested at low temperatures require a longer solids retention time (SRT) (Dague and Pidaparti, 1992). Subsequently, in a SBR, the SRT has been

decoupled from the hydraulic retention time (HRT) (Sung and Dague, 1995). Liquid effluent is separated from settled biomass within the SBR at regular intervals (Zhang et al., 1997). A specific type of SBR, the anaerobic sequencing batch reactor (ASBR), has been the subject of intensive recent research. The ASBR was not considered to be a suitable treatment technique for swine waste because of the potential for rapid VFA accumulation, which could lead to system failure (Bagley and Brodkorb, 1999). Successful ASBR operation requires the presence of an active population of methanogens that are capable of VFA utilization and conversion (Sung and Dague, 1995).

The anaerobic sequencing batch reactor cycles through four phases: feed, react, settle, and decant (Figure 4) (Dague and Pidaparti, 1992; Zhang et al., 1997). The react phase is the cornerstone of the digestion process. During this phase, bacteria convert the waste stream to biomass, CH_4, and CO_2 (Bagley and Brodkorb, 1999). The react phase is the most time consuming process within the cycle (Dague and Pidaparti, 1992).

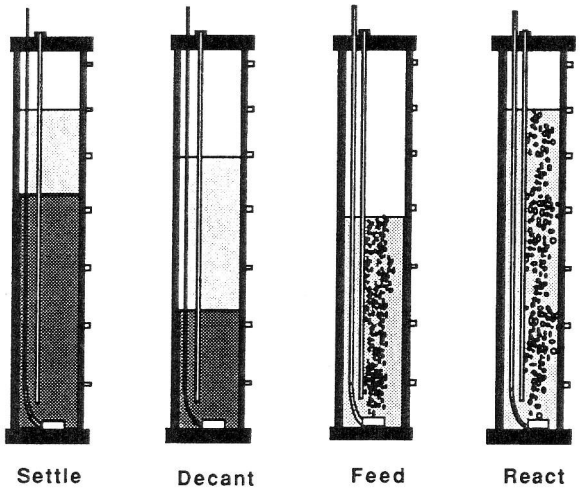


FIGURE 4: Four phases of the ASBR process (Dague and Pidaparti, 1992)

The food to mass ratio (F:M) is highest in an ASBR at the beginning of each cycle when influent is added, and it decreases exponentially through the process, reaching a minimum at the end of the decant phase (Figure 5) (Sung and Dague, 1995).

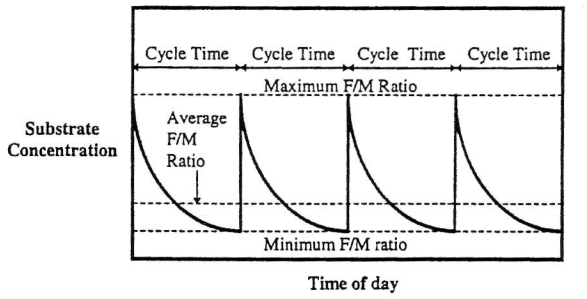


FIGURE 5: F:M response during ASBR cycling (Zhang et al., 1997)

Dague and Pidaparti (1992) concluded that ASBR technology can efficiently convert swine waste to biogas, and can do so over a wide range of temperatures. Operational ASBR systems function properly with a HRT of approximately 2-6 days, treating influent in a timely manner (Zhang et al., 1997). By comparison, a continuously stirred tank reactor (CSTR), a conventional treatment process, has a typical HRT of 15-30 days (Zhang et al., 1997).

Specific ASBR research studies have demonstrated appreciable removal of COD as well as significant CH_4 production. Dague and Pidaparti (1992) found that total gas production was a linear function of daily COD loading (Table 1).

TABLE 1: 12-Liter ASBR performance at 35ºC (Dague and Pidaparti, 1992)

COD Load (g/L/day)	COD Removal %	Gas Production (L/day)	VS Destruction %
1.005	78.40	3.99	87.40
2.167	71.50	11.30	78.80
3.283	67.00	16.50	77.20
4.372	65.10	20.20	74.00
5.436	64.60	30.30	73.60

Research conducted on swine waste by Sung and Dague (1995) also demonstrated the proportional increase in biogas (CH_4) production with increases in COD loading. Additionally, they showed that soluble COD removal efficiencies greater than 90% are practical for ASBR systems with varying HRT and COD loading values (Table 2).

TABLE 2: 12-Liter ASBR performance using 12-hour HRT (Sung and Dague, 1995)

COD Load (g/L/d)	Total COD Removal %	COD_S Removal %	CH_4 Production (L/d)
4	68.55	97.20	15.42
6	81.58	96.55	22.99
8	87.33	97.83	29.46
10	85.80	98.30	36.35
12	76.55	91.45	37.91

In order for the ASBR to operate efficiently without dependence on process temperature, the SRT must be adjusted accordingly. An ASBR functioning at a temperature of 25ºC may require an SRT twice as long as would be required at 35ºC (Dague and Pidaparti, 1992). Biomass granulation is an important feature of the ASBR. This process transforms biomass into settleable granules – the active microbial population of the

reactor that is retained. However, this is a time consuming component of reactor startup that may exceed 150 days (Wirtz, 1994).

IV. METHODS & MATERIALS

This research is an applied laboratory analysis of the initial kinetic properties of swine waste. Raw swine slurry was obtained from Pius Ndegwa, Assistant Researcher in the Department of Biosystems Engineering at Oklahoma State University. Animals at the Oklahoma State University swine facility were regularly fed a fortified corn-soybean meal ration. Physical and chemical measurements were taken on August 10, 2002 and are given in Table 3.

TABLE 3: Physical and chemical properties of swine slurry

Parameter	Value
%TS	12.2
pH	6.77
COD	102,000 mg/L
NH_4^+-N	4,807 mg/L
VFA	20,531 mg/L

The slurry was frozen and subsequently thawed. A significant amount of recalcitrant organic matter – undigested corn, hair follicles, etc. – was present in the swine slurry, and therefore a #10 (2mm) sieve was used to screen the waste. Organic matter that is not readily degradable is undesirable in an ASBR because of the potential for clogging. The screened slurry was then analyzed for total solids (%TS) content. Measurement was conducted using Standard Methods procedure 2540 B. The initial TS content of the swine waste was found to be 6.80%. Consultation with Dr. Ndegwa led to the

establishment of three dilution concentrations for kinetic research: 0.25%, 0.50%, and 0.75%. The ASBR under development at Oklahoma State University utilizes an influent with 0.25% TS in order to reflect field slurry conditions, thus the three concentrations allow for a range of data acquisition. Dilutions were calculated using the following equation:

[INITIAL TS (PPM)]/[FINAL TS (PPM)] = DILUTION FACTOR (1)

Final dilutions were prepared with a total volume of 1 liter, and influent volume was calculated in the following manner:

1000 mL / DILUTION FACTOR = mL SLURRY (2)

mL H_2O = 1000 – mL SLURRY (3)

Dilution volumes are given in Table 4.

TABLE 4: Dilution volumes prepared from initial slurry

%TS	mL Slurry	mL H_2O
0.25	36.8	963.2
0.5	73.5	926.5
0.75	109.9	890.1

Slurry samples were prepared on July 18, 2003 and stored at 4ºC.

Two kinetic research trials were conducted – one unseeded, and one seeded. Each trial included 24 reactors (100mL Pyrex Schott bottles). The reactors were fitted with a rubber stopper in which a capped Hungate tube was inserted for gas trapping and sampling with a 5ml syringe through a re-sealing serum stopper (see Figure 7). Each reactor was filled with 75mL of sample in order to leave 25mL free volume for gas production. Sampling was arranged in an interlaced design to maximize efficiency and minimize interference (Figure 6). The reactors, comprised of 0.25%, 0.50%, and 0.75% TS dilution concentrations, were exposed to a temperature of either 20ºC or 35ºC. These temperature settings were selected because 20ºC is an approximate room temperature, and 35ºC is the ideal mesophilic temperature. Temperature and mixing were controlled by two American YB-531 Shaking Water Baths. One of the shaking units continuously agitated 12 reactors at 20ºC, and the other continuously agitated 12 reactors at 35ºC.

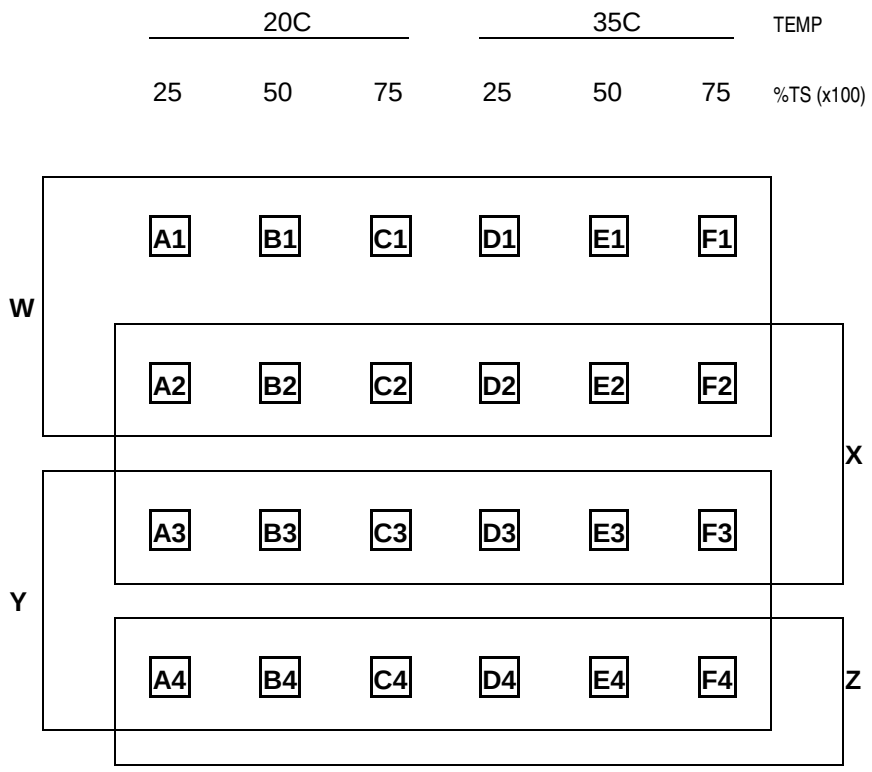


FIGURE 6: Experimental design, reactor bottles A_n – F_n grouped according to measurement parameter

Group W was monitored for biogas production, group X for VFA production and concentration, group Y for pH, and group Z for alkalinity. Alkalinity required the withdrawal of 5mL of non-replaceable sample, so it was limited to one set of reactors.

Biogas production (CH_4, CO_2) was measured every 48 hours once the methanogenic populations in the reactors were active. Volume measurement was achieved by using a 5mL syringe; positive pressure within the bottles equalized the syringe to bottle pressure. Thus, the equalization volume within the syringe was equivalent to the volume of biogas produced.

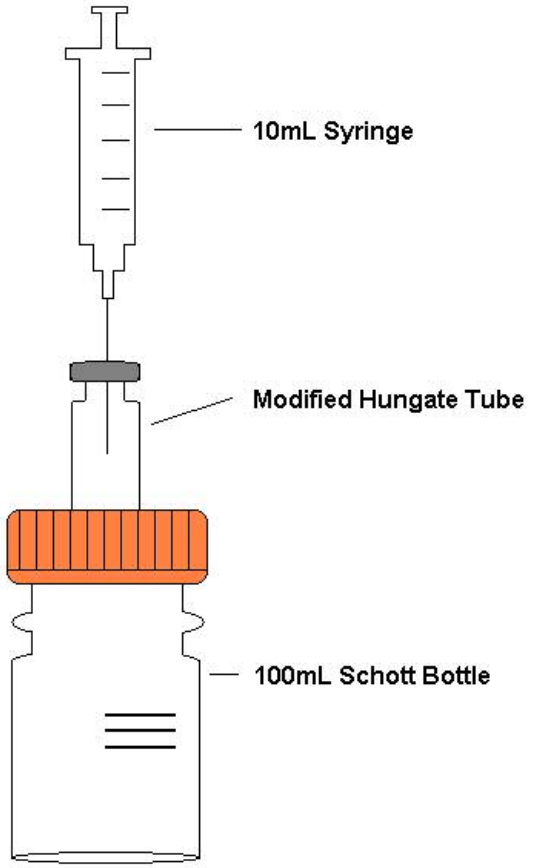


FIGURE 7: Syringe measurement of reactor biogas production

Biogas concentration was measured weekly using TCD analysis on an SRI 8610C Gas Chromatograph. Laboratory standards of CH_4 and CO_2 were used as control concentrations. For each sample, 100µL of gas was injected into the GC. The GC was operated at a constant 80ºC for approximately 5 minutes. Calibration data consisting of the following CH_4 to CO_2 ratios were collected: 100%:0%, 75%:25%, 50%:50%, 25%:75%, 0%:100%. Data from these ratios were exponentially plotted, yielding the following equation, which was used to determine the specific CH_4 to CO_2 ratio of each reactor.

$$y = 0.0057e^{0.084x} \quad (4)$$

Volatile fatty acid concentrations were analyzed using flame ionization detection (FID) on an SRI 8610C Gas Chromatograph. All VFAs were measured as acetate. Acetic acid standards of 100mg/L, 1000mg/L, and 10000mg/L were prepared in the laboratory. Short and long chain fatty acids cannot be examined with gas chromatography without preparation. VFAs must be protonized to an acidic state. For each analysis, 100µL of sample was withdrawn from each reactor and injected into a vial. One drop of formic acid was added, volatilizing the acid within the sample. Each vial was then subjected to approximately one minute of centrifugation in a Glas-Col Touch Vortexer. Then, 3µL of supernatant was withdrawn and injected into the GC. The GC initiated at 100ºC and terminated at 200ºC. Run time was approximately 11 minutes per sample.

Measurement of pH was conducted every 48 hours using a Fisher Scientific Accumet AR15 pH meter. A 5mL sample was withdrawn from each reactor, analyzed, and re-injected. The meter was standardized prior to each set of measurements using solutions of pH 4.00, 7.00, and 10.00.

Alkalinity was measured using Standard Methods procedure 2320 B (APHA, 1992). A 5mL sample was obtained from each reactor and subsequently placed on a magnetic stirring plate in order to provide a continually mixed sample. Additionally, pH was monitored with a Fisher Scientific Accumet AR15 pH meter. A 0.122N H_2SO_4 solution

was prepared as the titrant. Titrant was added until the swine waste sample reached the pH 4.3 endpoint. The following relationship between titrant volume and sample volume was used to calculate alkalinity as mg/L $CaCO_3$:

$$A = \frac{V_t * N * 50,000}{V_s} \tag{5}$$

In this expression, V_t is the titrant volume (mL), N is the normality of the titrant, and V_s is the sample volume (mL).

Chemical oxygen demand (COD) was determined at the beginning and end of each trial using Hach Method 8000 (Hach, 2003). Sample vials were prepared with Hach 0-1500 ppm COD Digestion Solution and incubated at 150ºC for 2 hours in a Hach COD Reactor. After cooling, each vial was analyzed using a Hach DR/2500 Spectrophotometer.

Nitrogen (NH_4^+-N) and phosphorus (PO_4^{3-}-P) were measured off site in the ATRC at Oklahoma State University with a HP Ion Chromatograph. Each was measured at the beginning and end of each trial.

The first kinetic trial was unseeded. Only prepared sample slurry was used. A sample volume of 75mL was added to each reactor. The trial ran for a total of 28 days. The second trial was seeded. An active methanogen population was obtained from Jim Tweet, Supervisor of the City of Tulsa Northside Wastewater Treatment Plant. During

this trial, 65mL of sample slurry and 10mL of seed sample were added to each reactor, maintaining a total sample volume of 75mL. The seeded trial, due to microbiological activity, was conducted over a span of 42 days. Final TS concentrations were examined upon completion of each trial.

V. RESULTS & DISCUSSION

Microbiological populations were dormant at the initiation of the 28-day unseeded kinetic trial. Prior to the trial, the swine slurry was maintained in a freezer and then at 4ºC for six months. Subsequently, little activity was noted over the course of the trial. Operating conditions of each reactor, noted in the experimental design matrix (Figure 6), is reviewed in Table 5.

TABLE 5: Reactor overview

Reactor	Unseeded %TS	Seeded %TS	Temperature (°C)
A	0.25	0.33	20
B	0.50	0.55	20
C	0.75	0.76	20
D	0.25	0.33	35
E	0.50	0.55	35
F	0.75	0.76	35

Total chemical oxygen demand (COD_T), presented in Table 6, was dependent upon the initial total solids (%TS) loading. Initial COD_T concentrations ranged from 505 mg/L for the 0.25% TS slurry samples to 1305 mg/L for the 0.75% TS slurry samples. Final COD_T concentrations increased for every sample except reactors A and D, the 0.25% TS samples subjected to 20ºC and 35ºC conditions, respectively.

TABLE 6: Average COD_T data, unseeded trial

Reactor	Initial COD_T (mg/L)	Final COD_T (mg/L)	% Change
A	505	450	- 10.9
B	662	909	+ 37.3
C	1305	1337	+ 2.5
D	505	474	- 06.1
E	662	906	+ 36.9
F	1305	1387	+ 06.3

Total solids more readily decreased during the course of the unseeded trial – an indication of the volatile nature of the various constituents of swine manure. Reduction in %TS generally ranged from 24% to 31% for the 20ºC reactors. All three 35ºC reactors showed TS reductions between about 30 and 32 percent. Reduction in %TS was independent of temperature regime. Total solids data for the unseeded trial are summarized in Table 7.

TABLE 7: Total solids data, unseeded trial

Reactor	Initial %TS	Final %TS	% Change
A	0.25	0.19	-24.0
B	0.50	0.37	-26.0
C	0.75	0.52	-31.0
D	0.25	0.17	-32.0
E	0.50	0.35	-30.0
F	0.75	0.52	-31.0

Alkalinity and pH remained constant through the duration of the unseeded trial. Initial pH was slightly lower for the 0.25% TS reactors, and remained so for the entire 28 days. Every reactor in the study ranged between pH 7 and pH 8 during the unseeded trial,

indicating the system contained enough bicarbonate alkalinity to prevent transition to an acidic state.

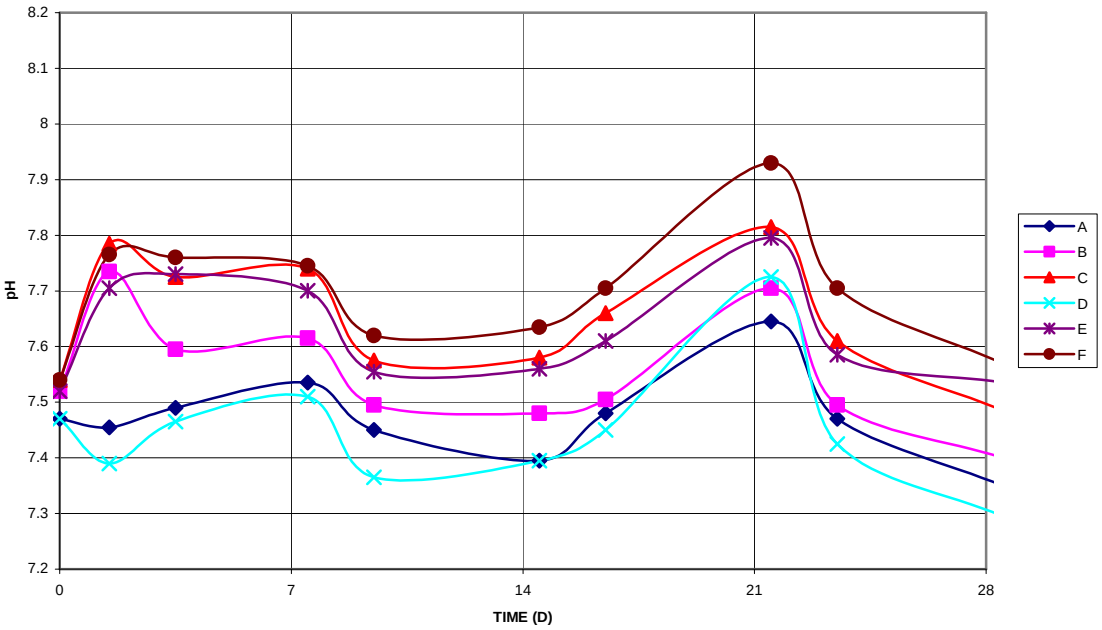


FIGURE 8: pH trends, unseeded trial

Accordingly, alkalinity remained fairly constant, exhibiting a slight decrease at the end of the trial period. It can be inferred that alkalinity was mostly unconsumed.

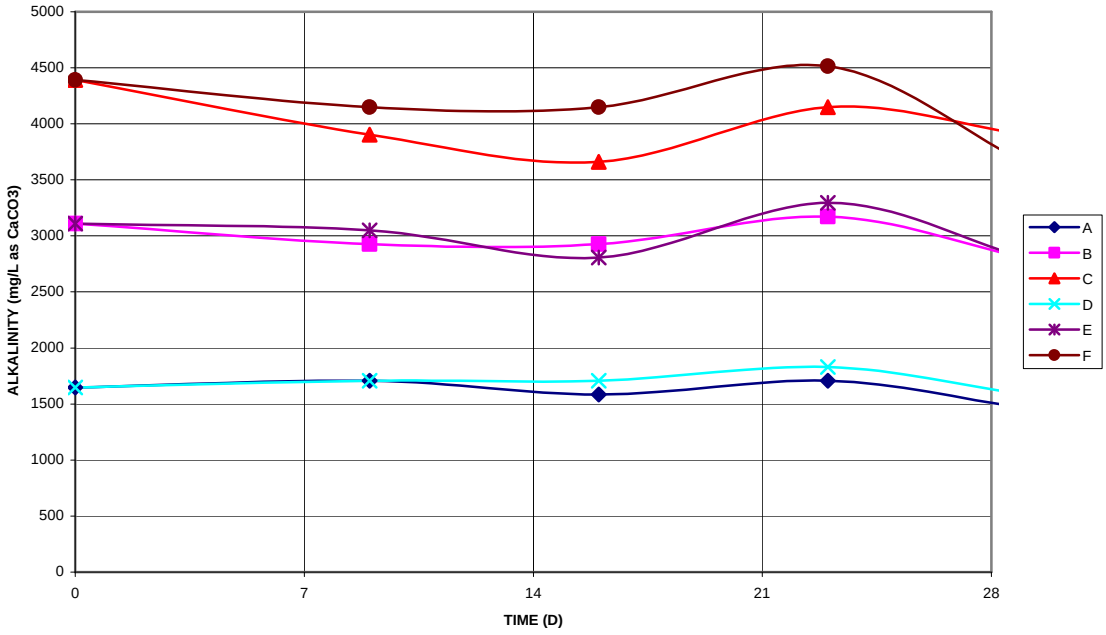


FIGURE 9: Alkalinity trends, unseeded trial

The two most explicit measures of microbiological activity, VFA concentration and biogas production, also showed little change during the unseeded experiment. VFA levels were initially high, indicating that acetogens were active while the swine slurry was in storage. In fact, initial VFA concentrations varied from 1500 mg/L for the 0.25% TS reactors to 4500 mg/L for the 0.75% TS reactors. Concentrations remained steady for every reactor except A, the 0.25% TS sample studied at 20ºC, which showed a marked increase in VFA concentrations by the 28th day. This result indicates activation of the acetogenic population.

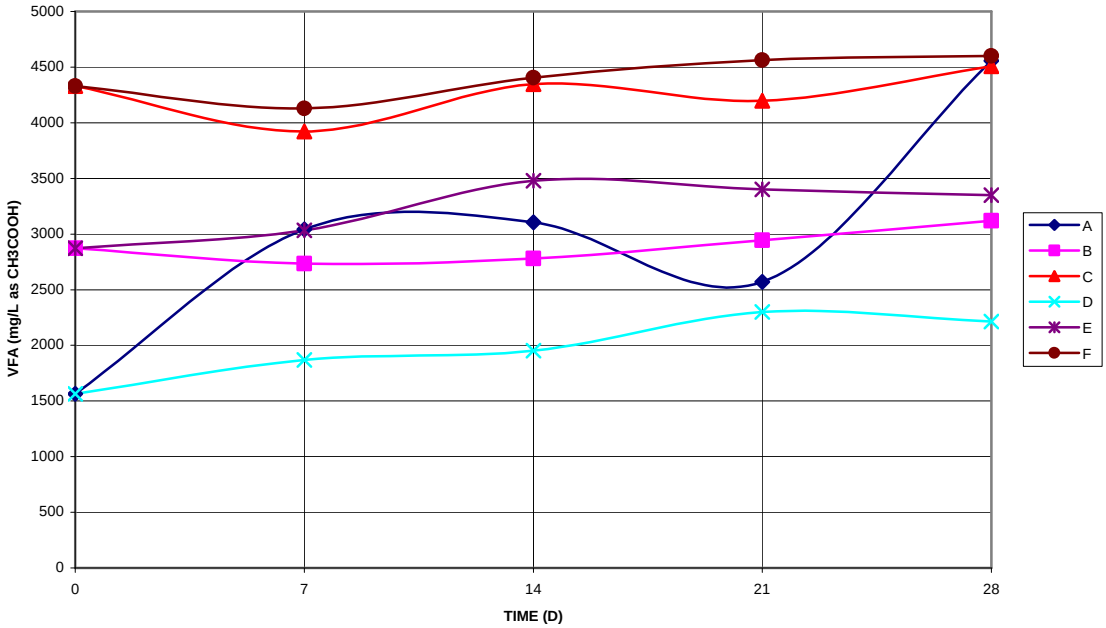


FIGURE 10: VFA trends, unseeded trial

Little or no biogas production was expected given the fact that VFA concentrations remained steady. The methanogens in the unseeded trial remained inactive for the entire period. The three reactors (D, E, F) operated at 35ºC produced minor volumes of gas. Otherwise, biogas production was nil. Gas chromatography indicated that the gas contained in the 35ºC reactors was substantially comprised of CO_2. CH_4 was undetectable.

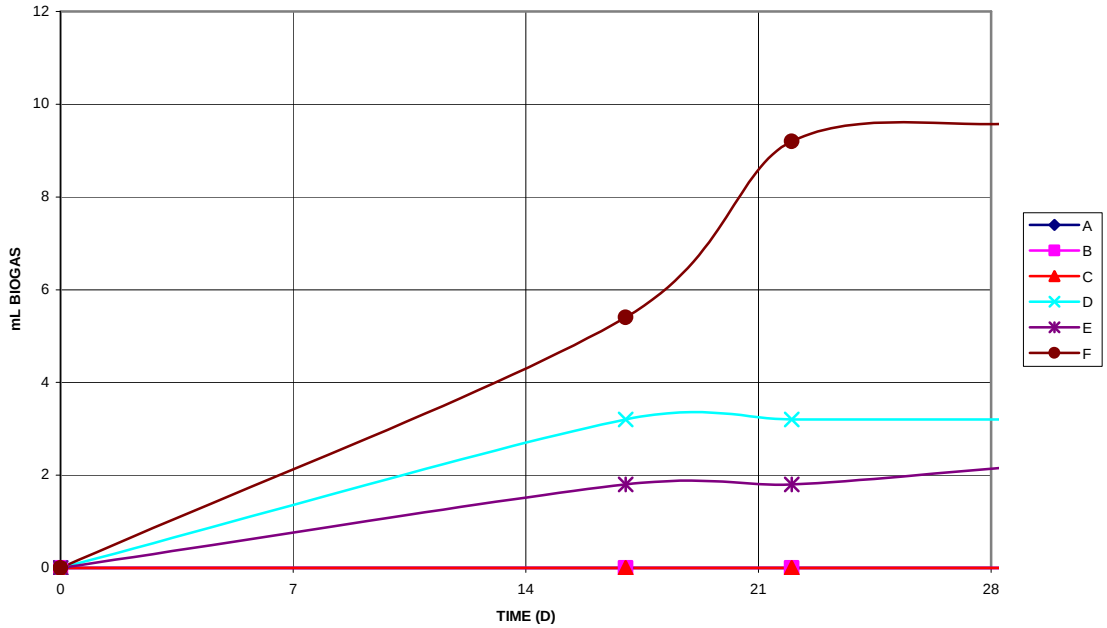


FIGURE 11 Cumulative biogas production, unseeded trial

Phosphorus, measured as PO_4^{3-}-P (phosphate), increased for all reactors except A and D, which contained the lowest percentage of total solids. No correlation is explicitly evident as the largest increases in PO_4^{3-}-P concentrations occurred in reactors B and F, with +89.9% and +90.3% change, respectively (Table 8).

TABLE 8: PO_4^{3-}-P concentrations, unseeded trial

Reactor	Initial (mg/L)	Final (mg/L)	% Change
A	45.9	28.5	- 37.9
B	91.8	174.3	+ 89.9
C	137.7	192.8	+ 40.0
D	45.9	38.9	- 15.2
E	91.8	125.4	+ 36.6
F	137.7	266.2	+ 93.3

Ammonium (NH_4^+-N) decreased in every reactor during the unseeded trial. Thus, at least one species of ammonium-consuming bacteria was active during Trial 1 even though the degradation process as a whole was slow and inefficient. The most significant consumption of NH_4^+-N occurred in reactor C, operated at 20ºC, indicating that the species present were not dependent on optimal mesophilic conditions (Table 9).

TABLE 9: NH_4^+-N concentrations, unseeded trial

Reactor	Initial (mg/L)	Final (mg/L)	% Change
A	347.1	306.2	- 11.8
B	694.1	536.7	- 22.7
C	1041.3	650.5	- 37.5
D	347.1	263.6	- 24.1
E	694.1	565.3	- 18.6
F	1041.3	794.3	- 23.7

Trial 2 was seeded with an active bacterial population in order to overcome the difficulties associated with the unseeded trial and thus record appreciable data. Each reactor contained approximately 87% raw slurry and 13% seed sludge. Adjusted concentrations of seeded COD_T, COD_S, %TS, and %VS are based on this ratio as well as the initial concentrations of both the initial slurry dilutions and the anaerobic seed sludge.

TABLE 10: Initial concentrations, swine slurry and anaerobic seed sludge

	Initial COD_T (mg/L)	Initial COD_S (mg/L)	Initial %TS	Initial %VS
0.25% Slurry	505	280	0.25	0.15
0.50% Slurry	662	532	0.50	0.29
0.75% Slurry	1305	875	0.75	0.43
Seed Sludge	2247	640	0.81	0.29

With an operational reactor, COD_T concentrations would be expected to decrease with consumption of waste substrate. Generally, this was the case. During the 42-day experiment, the most substantial decreases in COD_T occurred in the 35ºC reactors. COD_T in the 20ºC reactors remained fairly constant (Table 11). Reactor F, operated at 0.76% TS and 35ºC, showed a significant COD_T reduction of 56.1%.

TABLE 11: Average COD_T data, seeded trial

Reactor	Initial COD_T (mg/L)	Final COD_T (mg/L)	% Change
A	731	754	+ 03.2
B	868	792	- 08.8
C	1427	1452	+ 01.8
D	731	522	- 28.6
E	868	733	- 15.6
F	1427	627	- 56.1

A more accurate measure of biological activity is the soluble COD (COD_S). Unlike the determination of COD_T, COD_S samples are filtered before measurement. Filtering reduces the influence of particulates in COD measurement, thus accounting for the most readily biodegradable matter. All reactors experienced significant decreases in COD_S during the seeded trial (Table 12).

TABLE 12: Average COD_S data, seeded trial

Reactor	Initial COD_S (mg/L)	Final COD_S (mg/L)	% Change
A	327	94	- 71.3
B	546	125	- 77.1
C	844	330	- 60.9
D	327	96	- 70.6
E	546	180	- 67.0
F	844	125	- 85.2

Total solids decreased for every seeded reactor in Trial 2 (Table 13). The smallest percentage differences correspond to the lowest initial %TS reactors A and D. Both underwent a 6.1% TS reduction, respectively. Otherwise, every other seeded reactor exhibited considerable decline in %TS, ranging from –30.3% for reactor C to –39.5% for reactor F.

TABLE 13: Total solids data, seeded trial

Reactor	Initial %TS	Final %TS	% Change
A	0.33	0.31	- 06.1
B	0.55	0.36	- 34.5
C	0.76	0.53	- 30.3
D	0.33	0.31	- 06.1
E	0.55	0.35	- 36.4
F	0.76	0.46	- 39.5

Volatile solids also decreased for every reactor. Reduction was greatest in reactors with the highest initial volatile solids content. Similarly to %TS, COD_T, and COD_S of the seeded reactors in Trial 2, calculation of %VS was performed by considering the initial volatile solids content of the diluted swine samples coupled with the initial volatile solids

content of the anaerobic seed sludge (0.29%). Volatile solids reduction data are presented in Table 14.

TABLE 14: Volatile solids data, seeded trial

Reactor	Initial Slurry %VS	Initial Total %VS	Final Total %VS	% Change
A	0.15	0.17	0.16	-8.2
B	0.29	0.29	0.18	-38.6
C	0.43	0.42	0.28	-32.9
D	0.15	0.17	0.15	-14.1
E	0.29	0.29	0.16	-46.6
F	0.43	0.42	0.20	-51.7

pH initially surged in the seeded trial, followed by a slight decrease and stabilization. No reactor experienced pH values above 8.10 or below 7.20. By the end of the 42-day period, pH was trending slightly upward.

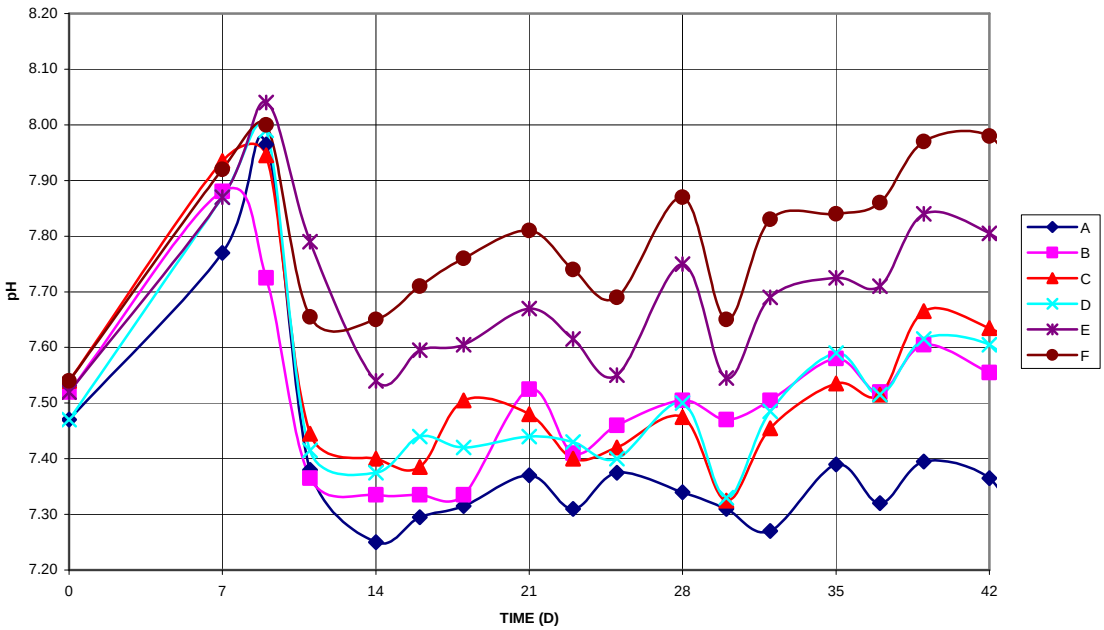


FIGURE 12: pH trends, seeded trial

As VFAs were consumed and methanogenesis maximized, the system became more basic. The reactors operating at the optimal mesophilic temperature and with the highest %TS loading increased most markedly as they were the highest rate reactors and VFAs were consumed quickly. Therefore, it is not surprising that the lowest rate reactor, A, had the lowest pH at the end of the seeded trial. Alkalinity remained constant for all reactors, with slight increases by the 42nd day, corresponding to pH increases. Bicarbonate alkalinity was present.

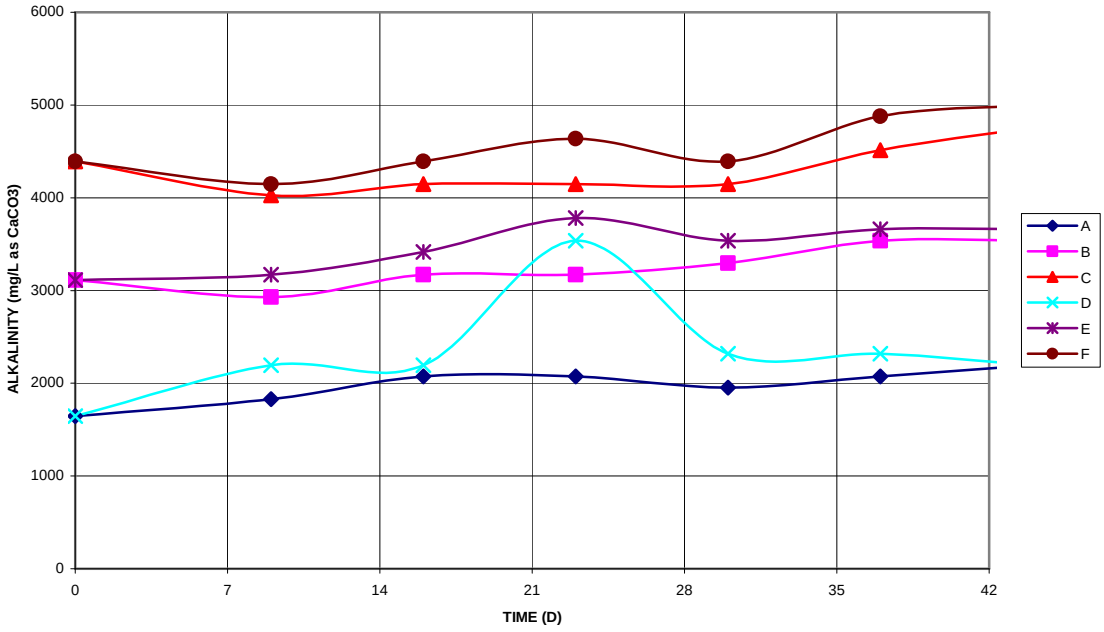


FIGURE 13: Alkalinity trends, seeded trial

Decreases in volatile fatty acid concentrations were pronounced for all reactors in the study. Reactors commissioned at 35ºC exhibited the most rapid transition of VFA levels, with each stabilizing at approximately 1000 mg/L. The net VFA change in reactor F was –86.3%. Reactors controlled at 20ºC experienced significant VFA consumption as well.

Bottles with the highest initial %TS underwent the greatest reduction. The initial VFA concentration average for all reactors was 2922 mg/L; final average VFA concentration was 905 mg/L.

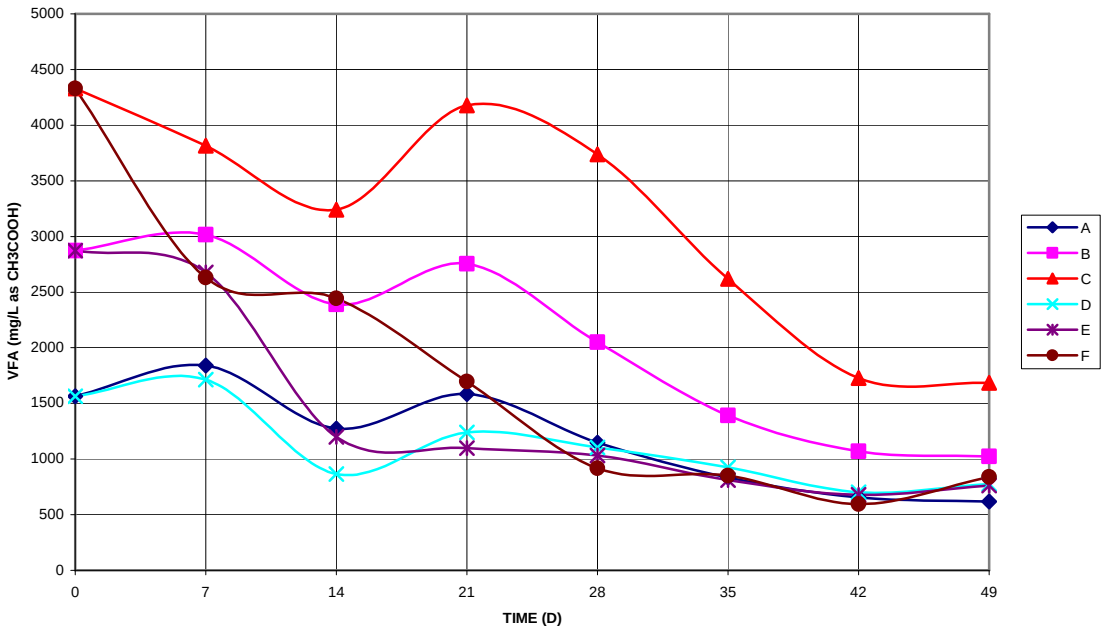


FIGURE 14: VFA trends, seeded trial

Biogas production quickly ensued. The greatest activity occurred in the 35ºC reactors, where cumulative volumes exceeded 100mL in bottles D, E, and F. Reactor F produced the largest volume of biogas – a total of 237.5mL.

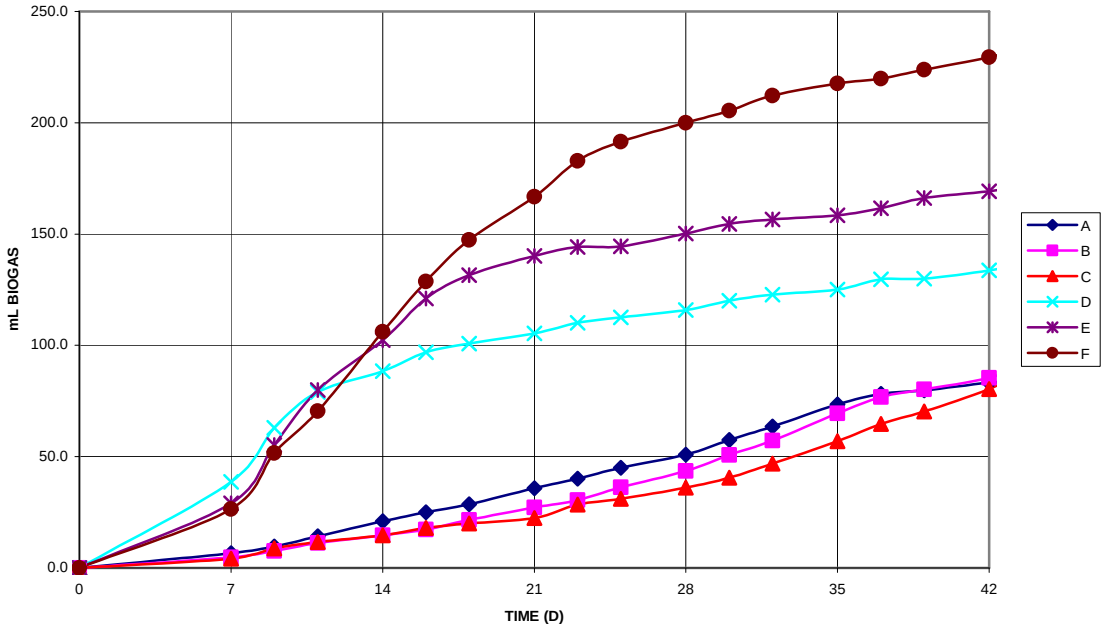


FIGURE 15: Cumulative biogas production, seeded trial

Maximum daily production of biogas in the high rate reactors peaked earlier in the study as compared to the 20ºC reactors. Generally, maximum biogas generation occurred between days 7 and 21 for reactors D, E, and F.

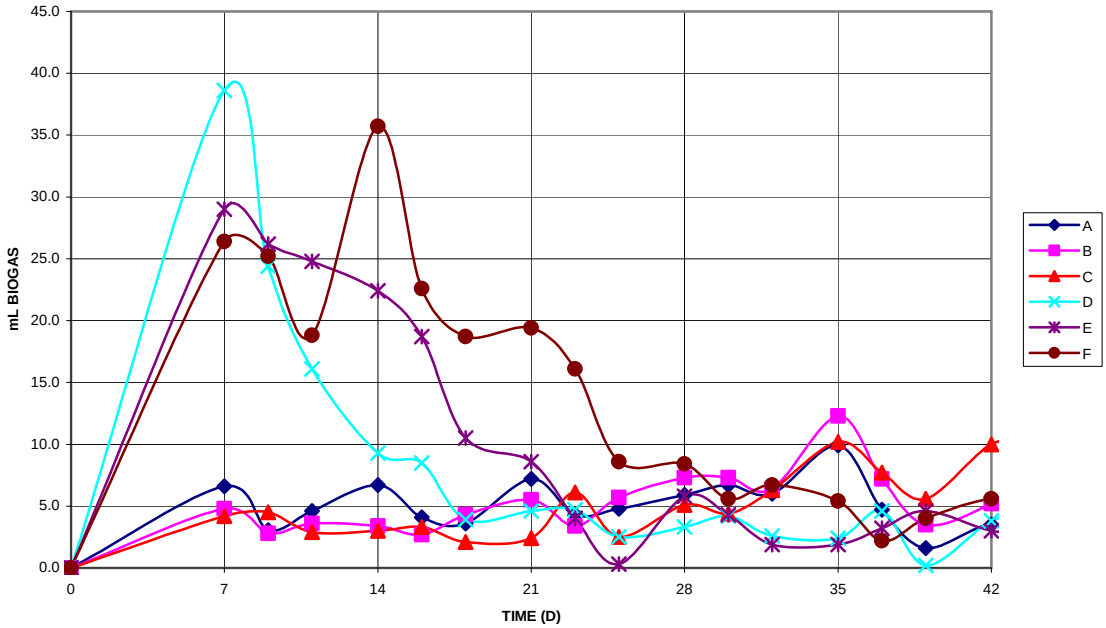


FIGURE 16: Daily biogas production, seeded trial

Biogas volume was quantitatively much lower and more gradual for reactors A, B, and C, which yielded 88.3mL, 98.4mL, and 95.8mL, respectively.

Biogas composition was measured twice during the seeded trial – the period of maximum biogas production in the 35°C reactors, which coincided with the midpoint of the trial, and the period of maximum biogas production in the 20°C reactors, which coincided with the end of the trial. During the middle of the experiment, measurements indicated that production in the high temperature reactors was dominated by CH_4. Methanogenic activity was slower in the 20ºC reactors as compared to those at 35ºC (Table 15). By the end of the trial, CH_4 had become the dominant gas in the low temperature reactors. Data were sparse for the high temperature reactors. For these bottles, the process had peaked several weeks prior, and gas production had slowed considerably. The lack of positive

pressure within the bottles coupled with injection holes in the rubber seal allowed bottles E and F to equalize. Reactor D registered very little gas, though it continued to be predominantly CH_4 (Table 16).

TABLE 15: Biogas composition, seeded trial, maximum 35°C production

Reactor	$\%CH_4$	$\%CO_2$
A	67	33
B	66	34
C	62	38
D	67	33
E	70	30
F	73	27

TABLE 16: Biogas composition, seeded trial, maximum 20°C production

Reactor	$\%CH_4$	$\%CO_2$
A	71	29
B	75	25
C	75	25
D	65	35
E	N/A	N/A
F	N/A	N/A

Phosphate increased for every reactor except E and F, the higher %TS bottles operated at 35ºC (Table 17). PO_4^{3-}-P increased significantly for all 20ºC reactors as well as the low %TS, 35ºC reactor D (+50.1%). Since reactor F experienced a 48.4% decrease in PO_4^{3-}-P, it appears that a species of phosphate-consuming bacteria was active in the higher %TS environment at optimal mesophilic conditions.

TABLE 17: PO_4^{3-}-P concentrations, seeded trial

Reactor	Initial (mg/L)	Final (mg/L)	% Change
A	45.9	65.7	+ 43.1
B	91.8	115.6	+ 25.9
C	137.7	182.3	+ 32.4
D	45.9	68.9	+ 50.1
E	91.8	87.6	- 04.6
F	137.7	71.1	- 48.4

The seeded trial resulted in nitrogen (NH_4^+-N) decreases for all reactors except those containing low %TS regardless of temperature (Table 18). As with the unseeded trial, reactor C experienced the greatest reduction in ammonium concentration, supporting the notion that the bacterial ammonium uptake was similar at the lower and higher temperatures. Demand for NH_4^+-N appears to be more closely related to the concentration of total solids.

TABLE 18: NH_4^+-N concentrations, seeded trial

Reactor	Initial (mg/L)	Final (mg/L)	% Change
A	347.1	382.4	+ 10.2
B	694.1	511.6	- 26.3
C	1041.3	659.1	- 36.7
D	347.1	386.5	+ 11.4
E	694.1	632.9	- 08.8
F	1041.3	830.0	- 20.3

VI. CONCLUSIONS AND RECOMMENDATIONS

Reaction rates are the most striking data from which conclusions can be established regarding the short-term kinetic study of swine slurry. The unseeded trial was unproductive, indicating dormancy of the various microbiological populations responsible for anaerobic degradation. During this 28-day study, COD_T increased for most reactors. Volatile fatty acid (VFA) concentrations remained constant, signaling the inactivity of acetogenic and methanogenic bacteria. This is further supported by the absence of CH_4 and CO_2 production. Methanogens are particularly responsible for CH_4 generation, and their failure to consume acetate (CH_3COOH) and produce CH_4 highlights the ineffectiveness of the unseeded anaerobic system. Differences between psychrophilic 20°C reactors and mesophilic 35°C reactors were minimal as neither was effective. The three 35°C reactors included in the study did produce a very small volume of biogas toward the end of the 28-day trial, indicating that the methanogenic populations within the mesophilic range may have been partially activating. Otherwise, activation of the unseeded reactors was not apparent. If the unseeded slurry had been fresh, it can be speculated that the trial would have been more productive. Long-term storage at low temperatures inactivated the various microbiological populations within the slurry, contributing to the slow initialization.

Addition of a fractional amount of anaerobic seed sludge to the raw slurry resulted in immediate and marked increases in reaction rates. Since the seed sludge was an active anaerobic population comprised of acidogens, acetogens, and methanogens, the raw slurry provided a substrate that was readily degradable. Production of CH_4 and CO_2 was rapid and dramatic within the mesophilic reactors, peaking by the 14^{th} day. Overall production exceeded 100mL for each of these reactors, with a maximum of 237.5mL generated by reactor F. Rates were slower for the psychrophilic 20°C reactors, but they were productive nonetheless. Peak biogas generation for these reactors occurred at the end of the 42-day seeded trial, and biogas volumes had been elevated since the 35^{th} day. VFA concentrations declined throughout the seeded study, and reduction was most evident for the initially high %TS reactors (0.76% reactors C and F). In fact, total VFA concentrations decreased by approximately 70% for reactor C and 80% for reactor F. Consumption of volatile fatty acids coupled with significant CH_4 production indicate a properly functioning anaerobic system in which intermediate VFAs are transformed into CH_3COOH by acetogens, and subsequently utilized by methanogens in the formation of CH_4 and CO_2. Performance during the kinetic study was optimized for seeded reactors, particularly those operating at the ideal mesophilic temperature of 35°C, where bioconversion was nearly complete.

Maximum optimization of the kinetic study clearly occurred for the highest initial %TS reactor operated at the ideal mesophilic reaction temperature – reactor F – verifying a hypothesis of the study. In fact, reactor F experienced the greatest reduction in COD_T, COD_S, %TS, %VS, and VFA concentration, and furthermore recorded the most

significant CH_4 and CO_2 production. It can be concluded that anaerobic degradation is most efficient at mesophilic (35°C) temperatures with high initial %TS and %VS loading. The initial loading of readily available bio-matter provides anaerobic bacterial populations with substrate necessary for the most efficient degradation reactions.

Another hypothesis of this study was that neither the concentration of NH_4^+-N nor PO_4^{3-}-P would change or significantly decrease as a result of the short term biological action. Results were mixed. For the unseeded trial, PO_4^{3-}-P decreased for the low initial %TS reactors and increased for all other temperature and %TS loading schemes. NH_4^+-N decreased for all reactors. Thus, the assumption can be made that since measurement of both is for soluble ammonium and phosphate, higher %TS loading increased the soluble PO_4^{3-}-P concentration, while no phosphate-reducing bacterial population was active. On the other hand, ammonium-consuming bacteria were active, thus accounting for NH_4^+-N reduction in every unseeded reactor.

Phosphate concentration increased in every seeded reactor except E and F. PO_4^{3-}-P decrease was pronounced for reactor F, with a net reduction of 48.4%. Thus, phosphate-consuming bacteria appear to have activated in the high initial %TS, mesophilic conditions. Unlike the unseeded trial, NH_4^+-N did not reduce for every reactor. The two reactors operating at the lowest initial %TS condition, A and D, experienced slight increases in soluble ammonium concentration. The most substantial decreases in NH_4^+-N occurred in reactors B and C, supporting the findings of the ammonium trends in the unseeded trial. Thus, it is reasonable to conclude that with the specific swine slurry

under consideration, ammonium-consuming species are most effective in the psychrophilic temperature range.

Although the most efficient anaerobic degradation occurred at the ideal 35°C mesophilic temperature, in many situations it may be more desirable to operate an anaerobic system at or near the 20°C psychrophilic temperature as it is a realistic ambient temperature, thus requiring little or no energy input for temperature adjustment. Maintenance of a 35°C system will conversely require a higher operating budget to keep temperatures within the mesophilic range. A goal of ASBR design is simplicity, further enhancing the desire for a reactor capable of efficient operation within the psychrophilic or psychrophilic-mesophilic temperature ranges. Review of the seeded trial data indicates appreciable 20°C anaerobic degradation, albeit at a slower rate than 35°C. Given a much longer trial time, cumulative biogas production at 20°C should approach 35°C production, as VFA consumption should result in similar final VFA concentrations. Acetogens and methanogens are active at 20°C as evidenced by the VFAs that were consumed and the CH_4 and CO_2 that was produced. If Figure 15 is considered, forecasting yields an estimated 20°C reaction time of 14 weeks, whereas the 35°C reaction was nearly complete at 7 weeks. Therefore, a doubled reaction time is a safe estimate for 20°C operating conditions as opposed to 35°C. Once anaerobic degradation at 20°C is complete, final concentrations of COD_T, COD_S, VFAs, as well as %TS, %VS and cumulative CH_4 and CO_2 production should be nearly identical to those achieved at 35°C. Slower reaction rates are an expected trade-off for lower energy input.

How do these results relate to ASBR development? A premise of sequencing batch reactor design is efficient operation within a broad temperature range. This study found that treatment can effectively be carried out over a range of process temperatures, but that efficiency is strongly correlated to the specific process temperature. Reactors set at 35ºC exhibited much faster degradation rates than those operated at 20ºC. Therefore, ASBRs operated at temperatures lower than 35ºC will accordingly require a longer SRT. If immediate treatment is required, 20°C operation is probably an undesirable option, as it has been established that short-term kinetic rates are significantly slower than at 35°C. Maximum biogas production occurred during the second week of the 35°C trial, much sooner than the maximum production of the 20°C trial, which occurred during the fifth week. If time is not a critical parameter, then initialization at 20°C is a viable preference. Another consideration is population dynamics. Different species of bacteria are responsible for degradation within the three reaction temperature ranges – psychrophilic, mesophilic, and thermophilic. If the faster reaction rate offered by mesophiles is desirable, then operation at a low mesophilic temperature, in the range of 25°C, offers rapid startup, a stable population, and minimal energy considerations.

A summary of concluding points:

- Seeding offers an instantaneously active anaerobic population
- Higher initial %TS provides a greater amount of consumable substrate
- Maximum reaction efficiency at ideal mesophilic temperature (35°C)

- Anaerobic degradation at 20°C requires an estimated doubled SRT when compared with 35°C
- NH_4^+-N consumed more readily at 20°C than at 35°C
- PO_4^{3-}-P generally unconsumed

Animal wastes are a growing threat to water resources in the United States and abroad. Water is an essential component of life. However, it is also a vector for disease. Treatment of animal wastes prior to discharge is therefore necessary to reduce the oxygen demand within streams and lakes, making them suitable for recreation and consumption. Anaerobic treatment of animal wastes is an effective technique that can achieve significant reductions in important environmental parameters, including COD_S. ASBR technology allows for temperature independent waste mitigation, with a burnable fuel, CH_4, as an end product. Swine waste can be treated in an ASBR, as it is a readily degradable substrate. Even though a functioning ASBR is not temperature dependent, initialization is, and that is an important consideration when choosing an operating temperature range. Furthermore, higher initial total solids waste streams are desirable in achieving maximum efficiency, provided that reactor design dictates an allowable %TS upper limit to prevent mechanical issues including but not limited to clogging. This research project has achieved its underlying goal by providing short term kinetic rate data for a range of temperature and solids loading schemes relevant to swine slurry specifically utilized in the ASBR research study being conducted at Oklahoma State University and is therefore provided as support for future reactor design.

BIBLIOGRAPHY

Ahring, B.K., Sandberg, M., and Angelidaki, I. (1995). Volatile fatty acids as indicators of process imbalance in anaerobic digestors. *Appl. Microbiol. Biotechnol.*, **43(3)**:559-565.

Alatiqi, I.M., Hamoda, M.F., and Dadkhah, A.A. (1998). Kinetic analysis of thermophilic anaerobic digestion of wastewater sludge. *Water, Air, Soil Pollut.*, **107(1-4)**:393-407.

Andreottola, G., Bortone, G. and Tilche, A. (1997). Experimental validation of a simulation and design model for nitrogen removal in sequencing batch reactors. *Wat. Sci. Tech.*, **35(1)**:113-120.

Angelidaki, I., and Ahring, B.K. (1993). Thermophilic anaerobic digestion of swine waste: the effect of ammonia. *Appl. Microbiol. Biotechnol.*, **38(4)**:560-564.

Angelidaki, I., Ellegaard, L., and Ahring, B.K. (1996). A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: focusing on codigestion of manure with lipid containing substrates. In: *Management of Urban Biodegradable Wastes*, J.A. Hansen (ed.). International Solid Waste Association, Copenhagen, Denmark., pp 142-152.

Angelidaki, I., Ellegaard, L., and Ahring, B.K. (1999). A comprehensive model of anaerobic bioconversion of complex substrates to biogas. *Biotechnol. Bioeng.*, **63(3)**:363-372.

Angenent, L.T., Sung, S., and Raskin, L. (2002). Methanogenic population dynamics during startup of a full-scale anaerobic sequencing batch reactor treating swine waste. *Wat. Res.*, **36(18)**:4648-4654.

American Society of Agricultural Engineers [ASAE] (1999). Manure production and characteristics. Final report Dec. 1999. ASAE D384.1 DEC99. 4p.

American Public Health Association [APHA] (1992). 18th Ed. *Standard Methods for the Examination of Water and Wastewater*. Washington, D.C.: Am. Public Health Assoc.

Bagley, D.M. and Brodkorb, T.S. (1999). Modeling microbial kinetics in an anaerobic sequencing batch reactor – model development and experimental validation. *Water Environ. Res.*, **71(7)**:1320-1332.

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H., and Vavilin, V.A. (2002). *Anaerobic Digestion Model No. 1*. International Water Association, 77p.

Brenner, A. (2000). Modelling of N and P transformations in an SBR treating municipal wastewater. *Wat. Sci. Tech.*, **42(1-2)**:55-63.

Cha, G.C., Chung, H.K., and Chung, J.C. (1997). Suppression of acidogenic activities due to rapid temperature drop in anaerobic digestion. *Biotechnol. Lett.*, **19(5)**:461-464.

Daffonchio, D., Thaveesri, J., and Verstraete, W. (1995). Contact angle measurement and cell hydrophobicity of granular sludge from upflow anaerobic sludge bed reactors. *Appl. Environ. Microbiol.*, **61(10)** 3676 -3680.

Dague, R.R., and Pidaparti, S.R. (1992). Anaerobic sequencing batch reactor treatment of swine wastes. *46th Purdue Industrial Waste Conference Proceedings;* 1992 May 15-16; West Lafayette, IN. Chelsea, MI: Lewis Publishers, Inc. p. 751-760.

Dague, R.R., Banik, G.C., and Ellis, T.G. (1998). Anaerobic sequencing batch reactor treatment of dilute wastewater at psychrophilic temperatures. *Water Environ. Res.*, **70(2)**:155-160.

Eismann, F., Glindemann., D., Bergmann, A., and Kuschk, P. (1997). Effect of free phosphine on anaerobic digestion. *Wat. Res.*, **31(11)**:2771-2774.

Griffin, M.E., McMahon, K.D., Mackie, R.I., and Raskin, L. (1998). Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids. *Biotechnol. Bioeng.*, **57(3)**:342-355.

Hach Co. (2003). Chemical Oxygen Demand – Reactor Digestion Method 8000. Loveland, Colorado. 8 p.

Hansen, K.H., Angelidaki, I., and Ahring, B.K. (1998). Anaerobic digestion of swine manure: inhibition by ammonia. *Wat. Res.*, **32(1)**:5-12.

Hansen, K.H., Angelidaki, I., and Ahring, B.K. (1999). Improving thermophilic anaerobic digestion of swine manure. *Wat. Res.*, **33(8)**:1805-1810.

Hill, D.T., and Baier, J.W. (2000). Physical and chemical properties of screened-flushed pig slurry waste. *J. Agric. Engng. Res.*, **77(4)**:441-448.

Hill, D.T., Taylor, S.E., and Grift, T.E. (2001). Simulation of low temperature anaerobic digestion of dairy and swine manure. *Bioresour. Technol*., **78(2)**:127-131.

Hobbs, P.J., Johnson, R., and Chadwick, D. (1999). A novel technique to determine organic processes in pig wastes. *J. Sci. Food Agric*., **79(2)**:199-205.

Hwang, S., Lee, Y., and Yang, K. (2001). Maximization of acetic acid production in partial acidogenesis of swine wastewater. *Biotechnol. Bioeng*., **75(5)**: 521-529.

Lahav, O., and Loewenthal, R.E. (2000). Measurement of VFA in anaerobic digestion: The five-point titration method revisited. *Water S.A.*, **26(3)**: 389-392.

Lawton, L.A., Robertson, P.K.J., Robertson, R.F., and Bruce, F.G. (2003). The destruction of 2-methylisoborneol and geosmin using titanium dioxide photocatalysis. *Appl. Catal. B. Environ.*, **44(1)**:9-13.

Lettinga, G., Rebac, S., and Zeeman, G. (2001). Challenge of psychrophilic anaerobic wastewater treatment. *Trends Biotechnol*., **19(9)**:363-370.

Le Van, T.D., Robinson, J.A., Ralph, J., Greening, R.C., Smolenski, W.J., Leedle, J.A., and Schaefer, D.M. (1998). Assessment of reductive acetogenesis with indigenous ruminal bacterium populations and *Acetitomaculum ruminis*. *Appl. Environ. Microbiol.*, **64(9)**: 3429-3436.

Levantesi, C., Serafim, L.S., Crocetti, G.R., Lemos, P.C., Rossetti, S., Blackall, L.L., Reis, M.A.M., and Tandoi, V. (2002). Analysis of the microbial community structure and function of a laboratory scale enhanced biological phosphorus removal reactor. *Environ. Microbiol.*, **4(10)**:559-569.

Mace, S., and Mata-Alvarez, J. (2002). Utilization of SBR technology for wastewater treatment: an overview. *Ind. Eng. Chem. Res*., **41(23)**:5539-5553.

Magbauna, B.S., Adams, T.T., and Johnston, P. (2000). Anaerobic codigestion of hog and poultry waste. *Bioresour. Technol*., **76(2)**:165-168.

Massé, D.I. and Droste, R.L. (2000). Comprehensive model of anaerobic digestion of swine manure slurry in a sequencing batch reactor. *Wat. Res*., **34(12)**:3087-3106.

Massé, D.I., Masse, L., Verville, A., and Bilodeau, S. (2001). The start-up of anaerobic sequencing batch reactors at 20ºC and 25ºC for the treatment of slaughterhouse wastewater. *J. Chem. Technol. Biotechnol*., **76(4)**:393-400.

Massé, D.I., Masse, L., and Croteau, F. (2003). The effect of temperature fluctuations on psychrophilic anaerobic sequencing batch reactors treating swine manure. *Bioresour. Technol*., **89(1)**:57-62.

Masters, G.M. (1998). Introduction to Environmental Engineering and Science. 2nd Ed. New Jersey: Prentice Hall. 651 p.

Ndegwa, P.M., Zhu, J., and Luo, A. (2001). Effects of solid levels and chemical additives on removal of solids and phosphorus in swine manure. *J. Envir. Engrg*., **127(12)**:1111-1115.

Obaja, D., Macé, S., Costa, J., Sans, C., and Mata-Alvarez, J. (2003). Nitrification, denitrification, and biological phosphorus removal in piggery wastewater using a sequencing batch reactor. *Bioresour. Technol*., **87(1)**:103-111.

Pagilla, K.R., Kim, H., and Cheunbarn, T. (2000). Aerobic thermophilic and anaerobic mesophilic treatment of swine waste. *Wat. Res*., **34(10)**:2747-2753.

Pavlostathis, S.G. and Giraldo-Gomez, E. (1991). Kinetics of anaerobic treatment. *Wat. Sci. Tech.*, **24(8)**:35-59.

Pavlostathis, S.G., and Gossett, J.M. (1986). A kinetic model for anaerobic digestion of biological sludge. *Biotechnol. Bioeng*., **28(3)**:1519-1530.

Pavlostathis, S.G., and Gossett, J.M. (1988). Preliminary conversion mechanisms in anaerobic digestion of biological sludges. *J. Envir. Engrg*., **114(3)**:575-592.

Pullammanappallil, P.C., Chynoweth, D.P., Lyberatos, G., and Svornos, S.A. (2001). Stable performance of anaerobic digestion in the presence of a high concentration of propionic acid. *Bioresour. Technol*., **78(2)**:165-169.

Rebac, S., Ruskova, J., Gerbens, S., vanLier, J.B., Stams, A.J.M., and Lettinga, G. (1995). High-rate anaerobic treatment of wastewater under psychrophilic conditions. *J. Ferment. Bioeng*., **80(5)**:499-506.

Rodríguez-Andara, A., and Lomas-Esteban, J.M. (1999).Kinetic study of the anaerobic digestion of the solid fraction of piggery slurries. *Biomass Bioenergy*, **17(5)**:435-443.

Rodríguez-Andara, A., and Lomas-Esteban, J.M. (2002). Transition of particle size fractions in anaerobic digestion of the solid fraction of piggery manure. *Biomass Bioenergy*, **23(3)**:229-235.

Smith, V.H., Tilman, G.D., and Nekola, J.C. (1999). Eutrophication: impact of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ. Pollut.*, **100(1-3)**:179-196.

Stumm, W., and Morgan, J.J. (1981). *Aquatic Chemistry*, 2nd Ed., Wiley, New York.

Sung., S. and Dague, R.R. (1995). Laboratory studies on the anaerobic sequencing batch reactor. *Water Environ. Res*., **67(3)**:294-301.

Tilche, A., Bortone, G., Malaspina, F., Piccinini, S., and Stante, L. (2001). Biological nutrient removal in a full-scale SBR treating piggery wastewater: results and modelling. *Wat. Sci. Tech*., **43(3)**:363-371.

Vezjak, M., Savsek, T., and Stuhler, E.A. (1998). System dynamics of euthrophication processes in lakes. *Eur. J. Oper. Res.*, **109(2)**:442-451.

Weiland, P. (2000). Anaerobic waste digestion in Germany—status and recent developments. *Biodegradation*, **11(6)**:415-421.

Wirtz, R.A. (1994). *Enhancement of granulation and start-up in the anaerobic sequencing batch reactor*. Ph.D. Dissertation, Iowa State University, Ames, Iowa.

Zhang, R.H., Yin, Y., Sung, S., and Dague, R.R. (1997). Anaerobic treatment of swine waste by the anaerobic sequencing batch reactor. *Transactions of the ASAE*, **40(3)**:761-767.

APPENDIX A: PRELIMINARY MEASUREMENTS

Seeded/Unseeded %TS

ALKALINITY	t	[Conc]
0.25/0.33	2.7mL	1647 mg/L as $CaCO_3$
0.50/0.55	5.1mL	3111 mg/L as $CaCO_3$
0.75/0.76	7.2mL	4392 mg/L as $CaCO_3$

PO_4^{3-}-P	[Conc]
0.25/0.33	45.9 mg/L
0.50/0.55	91.8 mg/L
0.75/0.76	137.7 mg/L

NH_4^+-N	[Conc]
0.25/0.33	347.1 mg/L
0.50/0.55	694.1 mg/L
0.75/0.76	1041.3 mg/L

pH	
0.25/0.33	7.47
0.50/0.55	7.52
0.75/0.76	7.54

VFA	[Conc]
0.25/0.33	1564 mg/L
0.50/0.55	2873 mg/L
0.75/0.76	4329 mg/L

APPENDIX B: UNSEEDED TRIAL MEASUREMENTS

ALKALINITY

- Titrant volume, mL

		2/18/2004	2/25/2004	3/3/2004	3/9/2004
A4		1.4	1.3	1.4	1.2
B4		2.4	2.4	2.6	2.3
C4		3.2	3.0	3.4	3.2
D4		1.4	1.4	1.5	1.3
E4		2.5	2.3	2.7	2.3
F4		3.4	3.4	3.7	3.0

- Concentration calculated mg/L as $CaCO_3$

		2/18/2004	2/25/2004	3/3/2004	3/9/2004
A4		1708	1586	1708	1464
B4		2928	2928	3172	2806
C4		3904	3660	4148	3904
D4		1708	1708	1830	1586
E4		3050	2806	3294	2806
F4		4148	4148	4514	3660

BIOGAS

- Volume

		2/26/2004	3/2/2004	3/9/2004
A1		0	0	0
B1		0	0	0
C1		0	0	0
D1		3.2	0	0
E1		1.8	0	0.4
F1		5.4	3.8	0.4

NUTRIENTS – PO_4^{3-}-P and NH_4^+-N

- All measurements

	PO_4^{3-} -P	NH_4^+-N
A1	6.4	333.7
A2	50.6	278.6
B1	200.9	550.4
B2	147.7	522.9
C1	184.6	650.4
C2	200.9	650.5
D1	36.7	266.3
D2	41.0	260.8
E1	99.3	554.8
E2	151.4	575.8
F1	262.6	765.8
F2	269.8	822.7

- Calculated averages

	PO_4^{3-}-P	NH_4^+-N
A	28.5	306.2
B	174.3	536.7
C	192.8	650.5
D	38.9	263.6
E	125.4	565.3
F	266.2	794.3

pH

- All measurements

		2/10/2004	2/11/2004	2/12/2004	2/13/2004	2/16/2004	2/17/2004	2/18/2004
A3		7.42		7.39		7.44		7.35
A4			7.49		7.59		7.63	
B3		7.78		7.52		7.57		7.40
B4			7.69		7.67		7.66	
C3		7.81		7.63		7.65		7.51
C4			7.76		7.82		7.83	
D3		7.37		7.41		7.45		7.23
D4			7.41		7.52		7.57	
E3		7.63		7.73		7.65		7.46
E4			7.78		7.73		7.75	
F3		7.69		7.81		7.72		7.53
F4			7.84		7.71		7.77	

		2/19/2004	2/20/2004	2/23/2004	2/24/2004	2/25/2004	2/26/2004	2/27/2004
A3			7.38		7.47		7.40	
A4		7.55		7.32		7.56		7.58
B3			7.52		7.55		7.45	
B4		7.59		7.41		7.56		7.58
C3			7.49		7.67		7.63	
C4		7.64		7.49		7.69		7.65
D3			7.16		7.41		7.39	
D4		7.50		7.33		7.51		7.55
E3			7.46		7.62		7.56	
E4		7.65		7.50		7.66		7.63
F3			7.55		7.72		7.66	
F4		7.71		7.55		7.75		7.71

		3/1/2004	3/2/2004	3/3/2004	3/4/2004	3/5/2004	3/8/2004	3/9/2004
A3		7.66		7.41		7.37		7.21
A4			7.63		7.53		7.49	
B3		7.67		7.52		7.44		7.28
B4			7.74		7.47		7.52	
C3		7.78		7.63		7.56		7.40
C4			7.85		7.59		7.57	
D3		7.53		7.37		7.30		7.13
D4			7.92		7.48		7.46	
E3		7.63		7.53		7.49		7.48
E4			7.96		7.64		7.59	
F3		7.77		7.68		7.63		7.48
F4			8.09		7.73		7.66	

- Calculated averages (days from initialization)

		1.5	3.5	7.5	9.5	14.5	16.5	21.5
A		7.46	7.49	7.54	7.45	7.40	7.48	7.65
B		7.74	7.60	7.62	7.50	7.48	7.51	7.71
C		7.79	7.73	7.74	7.58	7.58	7.66	7.82
D		7.39	7.47	7.51	7.37	7.40	7.45	7.73
E		7.71	7.73	7.70	7.56	7.56	7.61	7.80
F		7.77	7.76	7.75	7.62	7.64	7.71	7.93

		23.5	28.5
A		7.47	7.35
B		7.50	7.40
C		7.61	7.49
D		7.43	7.30
E		7.59	7.54
F		7.71	7.57

TOTAL SOLIDS

- Final Weights

	Tare Wt (g)	Final Wt (g)	ΔW
A1	49.9674	50.0133	0.0459
B1	45.6157	45.7064	0.0907
C1	47.8550	47.9850	0.1300
D1	50.5818	50.6257	0.0439
E1	48.2141	48.3036	0.0895
F1	48.3922	48.5208	0.1286
A2	48.7697	48.8167	0.0470
B2	49.3653	49.4586	0.0933
C2	48.5951	48.7239	0.1288
D2	49.2411	49.2823	0.0412
E2	48.8992	48.9876	0.0884
F2	45.5003	45.6293	0.1290

VOLATILE FATTY ACIDS

- All measurements (days from initialization; VFAs in mg/L CH_3COOH)

		7	14	21	28
A2		4480	4531	3092	7195
A3		1602	1681	2049	1919
B2		2845	2617	3112	3159
B3		2622	2940	2776	3079
C2		4139	4836	4243	4518
C3		3702	3860	4150	4500
D2		1826	1723	2223	2099
D3		1909	2179	2375	2331
E2		2891	3362	3115	3560
E3		3176	3594	3684	3137
F2		3960	4792	4705	4806
F3		4300	4019	4418	4391

- Calculated averages (days from initialization; VFAs in mg/L CH_3COOH)

		7	14	21	28
A		3041	3106	2571	4557
B		2734	2779	2944	3119
C		3921	4348	4197	4509
D		1868	1951	2299	2215
E		3034	3478	3400	3349
F		4130	4406	4562	4599

APPENDIX C: SEEDED TRIAL MEASUREMENTS

ALKALINITY

- Titrant volume, mL

		3/24/2004	3/31/2004	4/7/2004	4/14/2004	4/21/2004	4/28/2004
A4		1.5	1.7	1.7	1.6	1.7	1.8
B4		2.4	2.6	2.6	2.7	2.9	2.9
C4		3.3	3.4	3.4	3.4	3.7	3.9
D4		1.8	1.8	2.9	1.9	1.9	1.8
E4		2.6	2.8	3.1	2.9	3.0	3.0
F4		3.4	3.6	3.8	3.6	4.0	4.1

- Concentration calculated mg/L as $CaCO_3$

		3/24/2004	3/31/2004	4/7/2004	4/14/2004	4/21/2004	4/28/2004
A4		1830	2074	2074	1952	2074	2196
B4		2928	3172	3172	3294	3538	3538
C4		4026	4148	4148	4148	4514	4758
D4		2196	2196	3538	2318	2318	2196
E4		3172	3416	3782	3538	3660	3660
F4		4148	4392	4636	4392	4880	5002

BIOGAS

- All volume measurements, mL

		3/22/2004	3/24/2004	3/26/2004	3/29/2004	3/31/2004	4/2/2004	4/5/2004
A1		8.8	2.0	6.4	7.0	5.2	4.2	6.0
A2		4.4	4.1	2.8	6.4	3.0	3.0	8.4
B1		8.4	2.6	4.4	3.2	2.6	5.6	5.8
B2		1.2	3.0	2.8	3.6	2.8	3.0	5.2
C1		4.4	4.6	3.4	3.0	0.8	0.4	4.2
C2		4.0	4.4	2.4	0.0	5.8	3.8	0.6
D1		43.6	26.2	14.8	8.8	5.8	4.4	4.2
D2		33.6	22.6	17.4	9.8	11.2	3.4	5.0
E1		30.0	25.8	27.2	22.4	17.0	11.2	11.6
E2		28.0	26.6	22.4	22.4	20.4	9.8	5.6
F1		30.4	22.6	16.8	37.6	22.6	17.0	23.4
F2		22.4	27.8	20.8	33.8	22.6	20.4	15.4
		4/7/2004	4/9/2004	4/12/2004	4/14/2004	4/16/2004	4/19/2004	4/21/2004
A1		5.6	3.8	5.8	7.4	6.0	8.6	7.4
A2		3.0	5.8	6.0	6.0	6.0	11.2	2.0
B1		2.8	5.6	8.8	5.6	6.0	11.2	8.8
B2		4.0	5.8	5.8	9.0	6.8	13.4	5.6
C1		6.2	0.6	5.8	4.8	6.2	10.8	6.4
C2		6.0	4.4	4.4	4.0	6.4	9.6	9.0
D1		6.0	3.6	6.0	2.8	2.6	3.0	0.0
D2		3.4	1.4	0.6	5.6	2.6	1.8	4.6
E1		6.0	0.2	5.8	5.6	3.4	3.4	1.4
E2		2.0	0.4	5.8	3.0	0.4	0.4	5.0
F1		11.2	11.6	11.2	5.6	4.4	5.6	0.4
F2		21.0	5.6	5.6	5.6	9.0	5.2	4.0

		4/23/2004	4/26/2004	4/28/2004	4/30/2004
A1		0.2	3.2	2.8	2.6
A2		3.0	3.8	0.2	4.4
B1		4.8	4.8	5.6	6.2
B2		2.2	5.6	9.6	4.6
C1		5.5	14.4	8.4	5.6
C2		5.6	5.6	11.2	5.8
D1		0.2	3.8	4.2	0.0
D2		0.0	0.0	0.0	4.6
E1		4.6	0.0	2.8	0.0
E2		0.0	3.0	0.0	0.0
F1		4.0	5.6	4.2	3.8
F2		0.0	5.6	5.6	2.6

- Average volume calculations, mL

		3/22/2004	3/24/2004	3/26/2004	3/29/2004	3/31/2004	4/2/2004	4/5/2004
A		6.6	3.1	4.6	6.7	4.1	3.6	7.2
B		4.8	2.8	3.6	3.4	2.7	4.3	5.5
C		4.2	4.5	2.9	3.0	3.3	2.1	2.4
D		38.6	24.4	16.1	9.3	8.5	3.9	4.6
E		29.0	26.2	24.8	22.4	18.7	10.5	8.6
F		26.4	25.2	18.8	35.7	22.6	18.7	19.4

		4/7/2004	4/9/2004	4/12/2004	4/14/2004	4/16/2004	4/19/2004	4/21/2004
A		4.3	4.8	5.9	6.7	6.0	9.9	4.7
B		3.4	5.7	7.3	7.3	6.4	12.3	7.2
C		6.1	2.5	5.1	4.4	6.3	10.2	7.7
D		4.7	2.5	3.3	4.2	2.6	2.4	4.6
E		4.0	0.3	5.8	4.3	1.9	1.9	3.2
F		16.1	8.6	8.4	5.6	6.7	5.4	2.2

		4/23/2004	4/26/2004	4/28/2004	4/30/2004
A		1.6	3.5	1.5	3.5
B		3.5	5.2	7.6	5.4
C		5.6	10.0	9.8	5.7
D		0.2	3.8	4.2	4.6
E		4.6	3.0	2.8	0.0
F		4.0	5.6	4.9	3.2

NUTRIENTS – PO_4^{3-}-P and NH_4^+-N

- All measurements (final), mg/L

	PO_4^{3-}-P	NH_4^+-N
A1	61.0	387.5
A2	70.3	377.3
B1	113.0	522.1
B2	118.2	502.1
C1	185.2	646.6
C2	179.3	671.6
D1	78.0	390.8
D2	59.8	382.2
E1	74.9	623.9
E2	100.3	641.9
F1	78.2	828.5
F2	64.0	831.4

- Calculated averages (final), mg/L

	PO_4^{3-} -P	NH_4^+-N
A	65.7	382.4
B	115.6	511.6
C	182.3	659.1
D	68.9	386.5
E	87.6	632.9
F	71.1	830.0

pH

- All measurements

		3/22/2004	3/24/2004	3/26/2004	3/29/2004	3/31/2004	4/2/2004	4/5/2004
A3		7.81	7.66	7.55	7.27	7.32	7.32	7.39
A4		7.73	8.27	7.21	7.23	7.27	7.31	7.35
B3		7.88	7.46	7.37	7.31	7.33	7.33	7.55
B4		7.88	7.99	7.36	7.36	7.34	7.34	7.50
C3		7.91	7.94	7.45	7.37	7.37	7.62	7.50
C4		7.96	7.95	7.44	7.43	7.40	7.39	7.46
D3		7.88	7.96	7.41	7.40	7.43	7.46	7.43
D4		7.86	8.02	7.42	7.35	7.45	7.38	7.45
E3		7.91	7.99	7.71	7.57	7.58	7.61	7.69
E4		7.83	8.09	7.87	7.51	7.61	7.60	7.65
F3		7.96	7.89	7.70	7.65	7.71	7.76	7.81
F4		7.88	8.11	7.61	N/A	N/A	N/A	N/A

		4/7/2004	4/9/2004	4/12/2004	4/14/2004	4/16/2004	4/19/2004	4/21/2004
A3		7.34	7.48	7.37	7.32	7.29	7.41	7.31
A4		7.28	7.27	7.31	7.30	7.25	7.37	7.33
B3		7.41	7.46	7.52	7.51	7.50	7.60	7.53
B4		7.41	7.46	7.49	7.43	7.51	7.56	7.51
C3		7.41	7.45	7.48	7.35	7.45	7.57	7.54
C4		7.39	7.39	7.47	7.30	7.46	7.50	7.49
D3		7.44	7.38	7.48	7.35	7.47	7.52	7.52
D4		7.42	7.42	7.52	7.31	7.50	7.66	7.51
E3		7.62	7.54	7.75	7.55	7.66	7.71	7.71
E4		7.61	7.56	7.75	7.54	7.72	7.74	7.71
F3		7.74	7.69	7.87	7.65	7.83	7.84	7.86
F4		N/A	N/A	N/A	N/A	N/A	N/A	N/A

		4/23/2004	4/26/2004	4/28/2004	4/30/2004
A3		7.43	7.38	7.25	7.37
A4		7.36	7.35	7.25	7.37
B3		7.61	7.53	7.50	7.52
B4		7.60	7.58	7.54	7.55
C3		7.68	7.66	7.61	7.64
C4		7.65	7.61	7.58	7.63
D3		7.62	7.61	7.53	7.62
D4		7.61	7.60	7.53	7.60
E3		7.84	7.78	7.75	7.79
E4		7.84	7.83	7.74	7.83
F3		7.97	7.98	7.87	7.94
F4		N/A	N/A	N/A	N/A

- Calculated averages

		3/22/2004	3/24/2004	3/26/2004	3/29/2004	3/31/2004	4/2/2004	4/5/2004
A		7.77	7.97	7.38	7.25	7.30	7.32	7.37
B		7.88	7.73	7.37	7.34	7.34	7.34	7.53
C		7.94	7.95	7.45	7.40	7.39	7.51	7.48
D		7.87	7.99	7.42	7.38	7.44	7.42	7.44
E		7.87	8.04	7.79	7.54	7.60	7.61	7.67
F		7.92	8.00	7.66	7.65	7.71	7.76	7.81

		4/7/2004	4/9/2004	4/12/2004	4/14/2004	4/16/2004	4/19/2004	4/21/2004
A		7.31	7.38	7.34	7.31	7.27	7.39	7.32
B		7.41	7.46	7.51	7.47	7.51	7.58	7.52
C		7.40	7.42	7.48	7.33	7.46	7.54	7.52
D		7.43	7.40	7.50	7.33	7.49	7.59	7.52
E		7.62	7.55	7.75	7.55	7.69	7.73	7.71
F		7.74	7.69	7.87	7.65	7.83	7.84	7.86

		4/23/2004	4/26/2004	4/28/2004	4/30/2004
A		7.40	7.37	7.25	7.37
B		7.61	7.56	7.52	7.54
C		7.67	7.64	7.60	7.64
D		7.62	7.61	7.53	7.61
E		7.84	7.81	7.75	7.81
F		7.97	7.98	7.87	7.94

TOTAL SOLIDS

- Final weights

	Tare Wt. (g)	Final Wt. (g)	ΔW
A1	47.4659	47.5418	0.0759
A2	45.6665	45.7460	0.0795
B1	45.3698	45.4554	0.0856
B2	46.4650	46.5616	0.0966
C1	48.5779	48.7080	0.1301
C2	44.7190	44.8519	0.1329
D1	48.0244	48.1104	0.0860
D2	44.9382	45.0092	0.0710
E1	49.3326	49.4229	0.0903
E2	49.3344	49.4176	0.0832
F1	47.0278	47.1308	0.1030
F2	49.1318	49.2567	0.1249

VOLATILE FATTY ACIDS

- All measurements (days from initialization; VFAs in mg/L CH_3COOH)

	7	14	21	28	35	42
A2	1768	1225	1656	1014	838	689
A3	1912	1395	1513	1279	832	621
B2	3139	2542	2806	2009	1375	1040
B3	2892	2236	2701	2091	1411	1098
C2	3938	3173	4095	3668	2661	1796
C3	3690	3306	4259	3807	2577	1661
D2	1609	954	1414	1055	958	695
D3	1817	777	1063	1152	896	709
E2	2676	1054	1300	1034	820	640
E3	2676	1346	894	1030	800	715
F2	3830	2281	1408	1030	842	580
F3	1432	2609	1985	802	860	608

- Calculated averages (days from initialization; VFAs in mg/L CH_3COOH)

	7	14	21	28	35	42
A	1840	1275	1585	1147	835	655
B	3016	2389	2754	2050	1393	1069
C	3814	3240	4177	3738	2619	1729
D	1713	865	1239	1104	927	702
E	2676	1200	1097	1032	810	678
F	2631	2445	1697	916	851	594

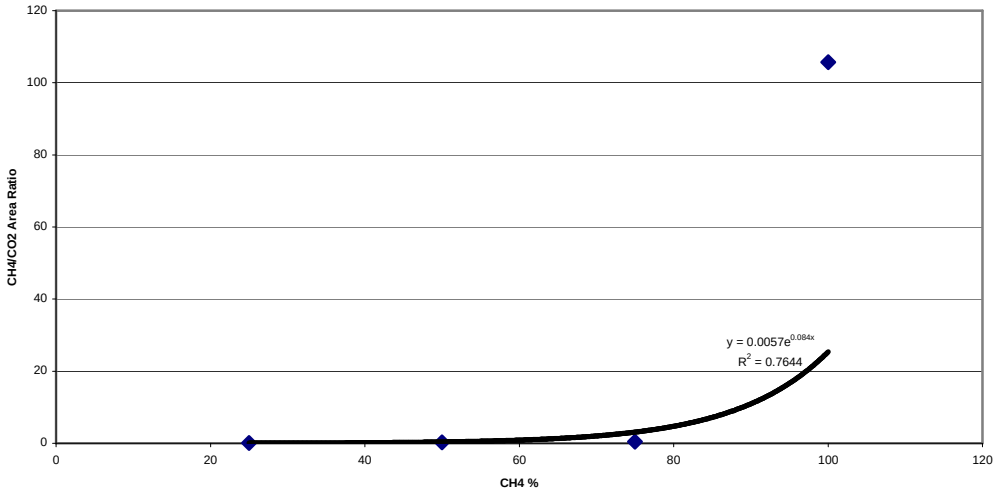
VOLATILE SOLIDS

- Final weights (g) - prepared samples, initial diluted slurry, and anaerobic seed sludge

	105°F	550°F	ΔW
A1	47.5418	47.5040	0.0378
A2	45.7460	45.7056	0.0404
B1	45.4554	45.4146	0.0408
B2	46.5616	46.5132	0.0484
C1	48.7080	48.6381	0.0699
C2	44.8519	44.7806	0.0713
D1	48.1104	48.0704	0.0400
D2	45.0092	44.9763	0.0329
E1	49.4229	49.3801	0.0428
E2	49.4176	49.3827	0.0349
F1	47.1308	47.0855	0.0453
F2	49.2567	49.2004	0.0563
0.25	48.7735	48.7355	0.0380
0.50	48.5684	48.4971	0.0713
0.75	45.7682	45.6596	0.1086
AS1	49.1482	49.0753	0.0729
AS2	46.1288	46.0545	0.0743

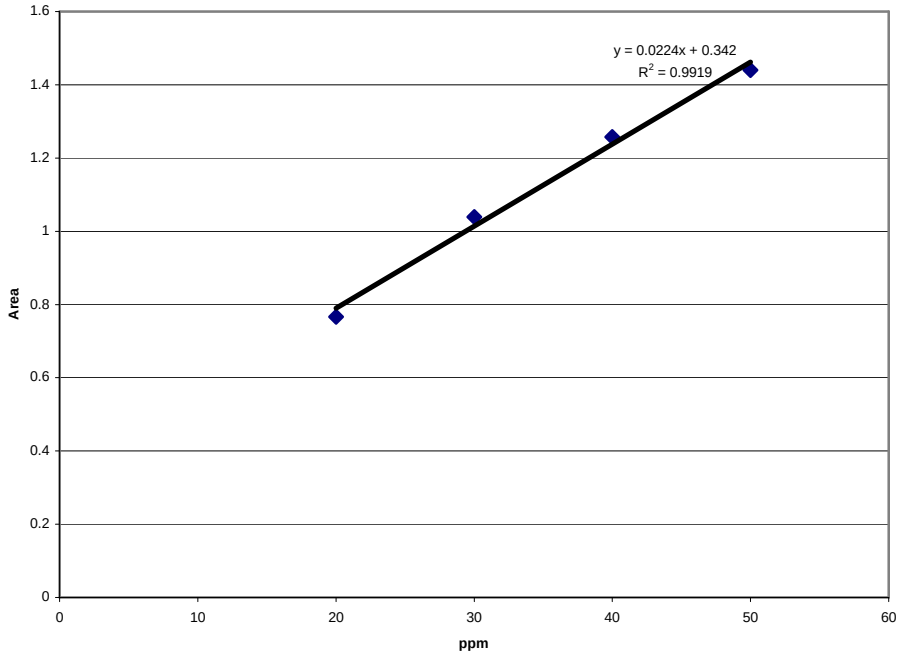
APPENDIX D: CALIBRATION DATA

BIOGAS (SRI GC – TCD ANALYSIS)

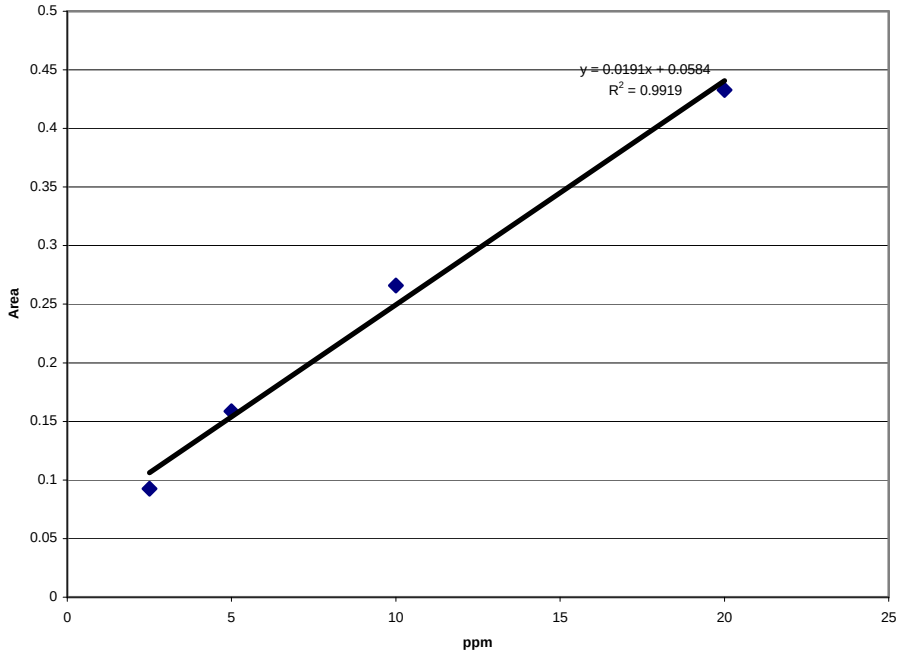


NITROGEN (NH_4^+ -N) (HP IC)

- Initial calibration

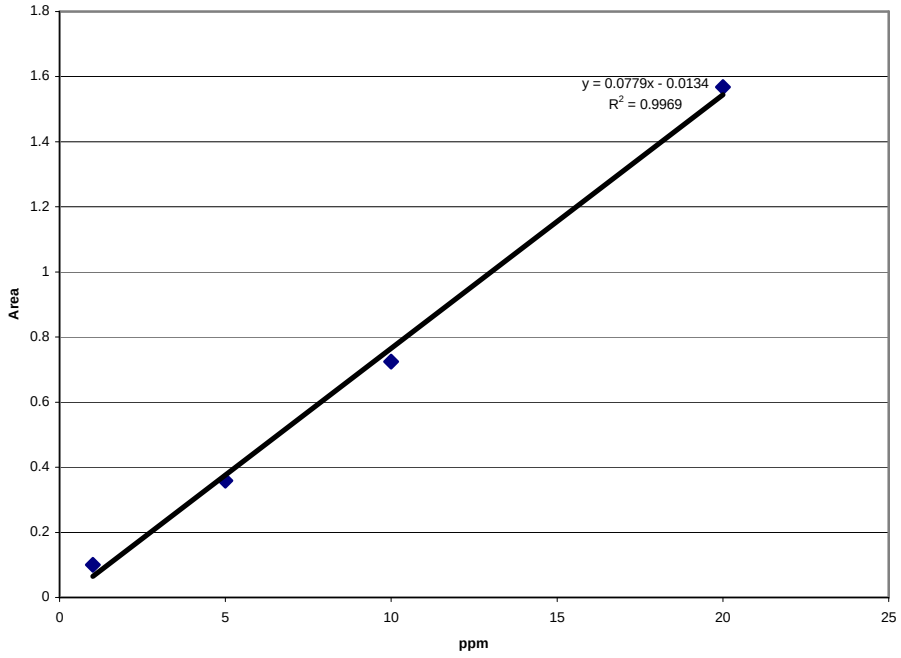


- Final calibration

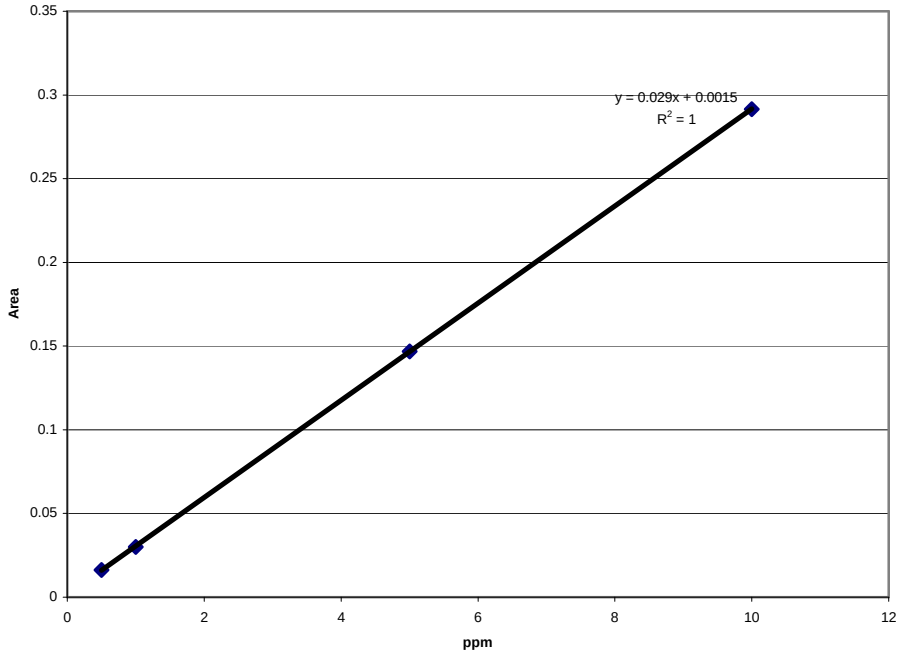


PHOSPHORUS (PO_4^{3-}-P) (HP IC)

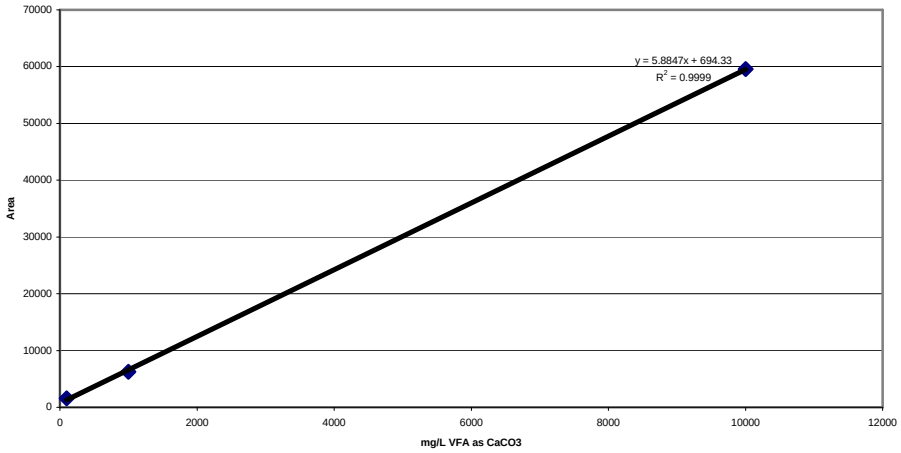
- Initial calibration



- Final calibration



VOLATILE FATTY ACIDS (SRI GC – FID ANALYSIS)



CURRICULUM VITAE

David Joe Williams

Candidate for the Degree of

Master of Science

Thesis: ANAEROBIC KINETIC STUDY OF SWINE SLURRY

Major Field: Environmental Engineering

Biographical:

Personal Data: Born in Claremore, Oklahoma on October 26, 1977

Education: Graduated from Catoosa High School, Catoosa, Oklahoma, in May 1996; received Bachelor of Arts degree in Geology from the University of Tulsa, Tulsa, Oklahoma, in December 1999 and Master of Environmental Science degree from the University of Oklahoma, Norman, Oklahoma, in December 2001. Completed the requirements for the Master of Science degree with a major in Environmental Engineering at Oklahoma State University in December 2004.

Academic Honors: Valedictorian, Catoosa High School, 1996; Oklahoma Academic All-State, 1996; American Radio Relay League Scholar Honoring Barry Goldwater, 1996; Oklahoma State Regents for Higher Education Academic Scholar, 1996-2001; American Meteorological Society Scholar, 1996-1997; American Indian Science and Engineering Society EPA Tribal Lands Scholar, 1999-2000, 2001-2002, 2002-2003; American Indian Science and Engineering Society AT Anderson Memorial Scholar, 2003-2004; Cherokee Nation Graduate Scholar, 2003-2004; Graduated Cum Laude, the University of Tulsa, 1999; Sigma Gamma Epsilon; Chi Epsilon; Tau Beta Pi; Sigma Xi; Order of the Engineer

Professional Memberships: American Society of Civil Engineers, International Water Association, American Meteorological Association, American Indian Science and Engineering Society, American Association of Petroleum Geologists

Name: David Joe Williams Date of Degree: December, 2004

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: ANAEROBIC KINETIC STUDY OF SWINE SLURRY

Pages in Study: 72 Candidate for Degree of Master of Science

Major Field: Environmental Engineering

Scope and Method of Study: The purpose of this study was to measure the anaerobic kinetic properties of diluted swine slurry. Anaerobic kinetic rates, particularly volatile fatty acid (VFA) consumption and biogas (CH_4 and CO_2) production were measured in a short-term study conducted at 20ºC and 35ºC. An initial study was conducted using swine slurry diluted to total solids loadings of 0.25%, 0.50%, and 0.75%, respectively. In order to ensure microbiological activity, a second study was conducted with the addition of anaerobic seed sludge. Adjusted total solids loadings were 0.33%, 0.55%, and 0.76%, respectively. The duration of the unseeded study was 28 days, and the duration of the seeded study was 42 days. Kinetic rates were measured as design considerations for an anaerobic sequencing batch reactor (ASBR).

Findings and Conclusions: Due to prolonged storage, microbiological populations were dormant in the unseeded slurry. With the addition of anaerobic seed sludge, appreciable kinetic results were recorded. Slurry at both 20ºC as well as 35ºC exhibited significant VFA consumption and biogas production. The largest biogas volumes were produced by the 35ºC, 0.76% TS slurry, with a cumulative biogas production of 237.5mL. All reactors operated at 35ºC produced biogas volumes in excess of 100mL. Reactors operated at 20ºC produced biogas more slowly. Maximum daily biogas production had peaked during the second week of the trial for the 35ºC reactors. Peak production was ongoing during the sixth week – the final week of the short-term study – with reactors operated at 20ºC. VFA consumption was most significant for the 35ºC slurry. VFA concentrations decreased by approximately 3,000mg/L for the 35ºC, 0.76% TS slurry. Based on the kinetic rates observed at 20ºC and 35ºC, it is estimated that slurry substrate in a reactor with an operational temperature of 20ºC will require a solids retention time (SRT) twice as long as the SRT at 35ºC. In terms of reactor design, a longer SRT will generate a longer treatment interval, thus providing less frequent sludge wasting.

Advisor's Approval: William Clarkson, Ph.D.