IMPROVED RAPID ANAEROBIC TREATABILITY-TOXICITY SCREENING TEST PROCEDURE

By

THOMAS RICE WYNN

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1993

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
July 1997

IMPROVED RAPID ANAEROBIC TREATABILITY-

TOXICITY SCREENING

TEST PROCEDURE

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

PREFACE

This study was performed to provide specific information and knowledge on an improved methodology for anaerobic treatability/toxicity screening test procedure. This methodology makes use of 125ml glass syringes as the reactor vessel. Healthy anaerobic bacteria produce methane, carbon dioxide and sometimes hydrogen sulfide. Measuring gas production accurately is critical in evaluating the performance of anaerobic bacteria. The goal of this study was to develop operating parameters for the new methodology so that the test procedure may be used properly, saving time and money. The new test procedure was reviewed in evaluating ammonia-nitrogen and nickel toxicity.

I wish to thank my committee, Drs. John N. Veenstra, Greg G. Wilber and William F. McTernan for their time and helpful comments. I would like to thank Enos Stover for providing the opportunity for research and for his efforts in the research. Thanks to Robert Rogers, Ron Helems and Tom Gorman for their assistance.

ACKNOWLEDGMENTS

I would like to express great thanks to my major advisor, Dr. John N. Veenstra, for his patience, advice and since of humor. My appreciation to my committee members Drs. Greg Wilber and William McTernan for taking the time to be on my committee. A special thanks to Dr. Enos Stover for providing me the opportunity to do research, providing the equipment, laboratory supplies and his time and patience throughout this research project.

Thanks to my fellow co-workers at The Stover Group for being extremely flexible and for checking the syringes: Scott Shannon, Thomas Gorman, Brian Diener and Mike Copenhaver. A big thanks to Ron Helems and Robert Rogers for their assistance in the project and Dr. Doug Fort for providing his statistical expertise. Last, but not least, a great thanks to Pat Lee for taking care of me.

Special appreciation to my parents Jack and Judy. Their encouragement, commitment, and understanding helped me achieve my goal. Thank you. Thanks to my family, Troy, Shelly, Vicky, Chris, Missy and Grandma Rice.

A very special thanks in memory of Dr. Bud Burks, who pointed me in the right direction.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

IMPROVED RAPID ANAEROBIC TREATABILITY-TOXICITY SCREENING TEST PROCEDURE

Anaerobic treatment of municipal and industrial waste has been used with great success in stabilizing organic waste. The popularity of anaerobic treatment has risen in recent years, mostly due to the high energy yielding by-product, methane. Other advantages include lower sludge yields and treatment of high strength organic waste streams more economically than aerobic systems. Anaerobic treatment involves the decomposition of waste in the absence of dissolved oxygen and consists of sequences of reactions in which one group of microorganisms produces substrate for another group of microorganisms. The synergistic relationship produces a delicately balanced environment which can diminish rapidly if environmental conditions are not satisfied. Anaerobic treatability/toxicity screening tests provide needed information to maintain favorable environmental conditions.

Anaerobic treatability/toxicity screening tests are bench scale tests that simulate full scale responses to waste streams. Information from these tests will determine the anaerobic treatability and waste characteristics of a particular waste stream and assist in developing large scale treatability studies. Types of treatability-screening tests include batch, continuous and semi-continuous feed assays. Each type of assay has advantages

and disadvantages, with the ultimate selection depending on the information desired. Batch assays are quick, inexpensive and reproducible and tend to produce a conservative value for a toxicity evaluation (Stucky *et al.*, 1980). Anaerobic toxicity assays are conducted with an active anaerobic inoculum, easily degradable carbon source and suspect chemicals or waste streams at various doses. Inhibition and toxicity can be evaluated from total volume and rate of gas produced. Gas production relates to the health of the inoculum and a decrease in the rate or total gas produced indicates a negative effect from the waste sample (Stover *et al.*, 1992). Accurate gas measurements are essential for this procedure.

A recent study by Brooks *et al.* (1994) evaluated the use of 125 ml glass luer-lock syringes for developing an anaerobic screening procedure. The glass luer-lock syringe was the reactor vessel and contained the biogas produced. Both liquid and gas samples can be removed without introducing oxygen. Gas production may be measured on a routine time sequence, to help in evaluating lag periods, kinetics and toxicity or inhibition. The procedure was simple and testing periods lasted from seven to twenty one days (Brooks *et al.*, 1994).

Goal of the Study

The goal of this study was to improve the methodology using 125 ml luer-lock glass syringes as the reactor vessel. The improvements focused on minimizing the

duration of the test and reducing variability through continuous mixing on a shaker table along with developing a protocol with standard operating conditions, such as organic loading and environmental conditions. Constant mixing should improve contact with the substrate, refining gas production rates, which is important when using the syringe in an anaerobic screening procedure. Improper test conditions can result in high gas production rates which may expel the plunger from the syringe, damaging the plunger and terminating the test. Therefore, operating parameters must be defined under mixing conditions to prevent excessive labor requirements for supervision of the syringes.

Objectives

There were four primary objectives of this study. The first objective was to determine specific operating conditions such as, solids loading and food-to-microorganism ratio (F/M ratio) in terms of mg COD/mg VSS (VSS is volatile suspended solids and COD is chemical oxygen demand). Defining these operating conditions was necessary to prevent the expulsion of the plunger from the syringe. A second objective was to evaluate the syringes for variability and reproducibility under mixing conditions. The third objective was to compare the response of continuous mixing versus static tests. The last experiments were examples of future practical administration of the syringe test. The fourth objective was to established toxicity levels for ammonia-nitrogen and nickel.

CHAPTER II

LITERATURE REVIEW

Information on existing methods for performing anaerobic toxicity assays was required, in order to evaluate the procedure developed in this study. Knowledge of the operating parameters, which indicated the overall health of the anaerobic bacteria, must be acquired to evaluate toxicity and inhibition. Methods, results and conclusions of previous studies will aid in developing a better procedure by identifying gaps in other procedures or evaluations of the toxicity of ammonia-nitrogen and nickel.

Anaerobic Treatment

Anaerobic digestion is a biological process used in stabilizing waste. This technology has been a mainstay in treating municipal waste sludges and has been successfully extende to treating high strength industrial wastewater. In the past ten years various anaerobic processes have been developed for the treatment of municipal sludges and industrial wastes (Metcalf and Eddy, 1991). Anaerobic treatment involves the decomposition of waste in the absence of dissolved oxygen and consists of sequences of reactions in which one group of microorganisms produces substrate for another group of microorganisms.

The biological conversion of organic matter to stabilized compounds may be classified into three or four sequencing reactions (Fox and Pohland, 1994). Figure 1 illustrates the sequences of reactions that occur during anaerobic metabolism of complex organic matter. The first reaction is the hydrolysis of complex organics such as lipids, polysaccharides and proteins by extracelluar enzymes into simpler soluble organics such as fatty acids, monosaccharides and amino acids. The second reaction is acidogenesis or the fermentation/acidification of soluble organics into volatile fatty acids, along with hydrogen and other fermentation products (alcohol's). These reactions are produced by facultative and obligate anaerobic bacteria. The third reaction may be separated into two reactions, acetogenesis and methanogenesis. The volatile fatty acids and hydrogen produced in acidogenesis reactions are converted to stable products, methane and carbon dioxide. This is accomplished by methanogenic bacteria (obligate anaerobes) and acetogenic bacteria (facultative and obligate anaerobes). Where the acetogenic bacteria convert the fermentation products into acetate and hydrogen which are substrates that methanogenic bacteria convert to methane and carbon dioxide. Acidification and acetogenic processes are not always distinguishable since both produce hydrogen and acetate (Fox and Pohland, 1994; Metcalf and Eddy, 1991).

Environmental parameters of concern are temperature, pH, sufficient supply of micronutrients and macronutrients and controlling materials producing toxic effects (Lawerence and McCarty, 1966). Indicators of operational performance are pH, volatile acids, alkalinity, methane production and gas composition. The pH is not a sensitive

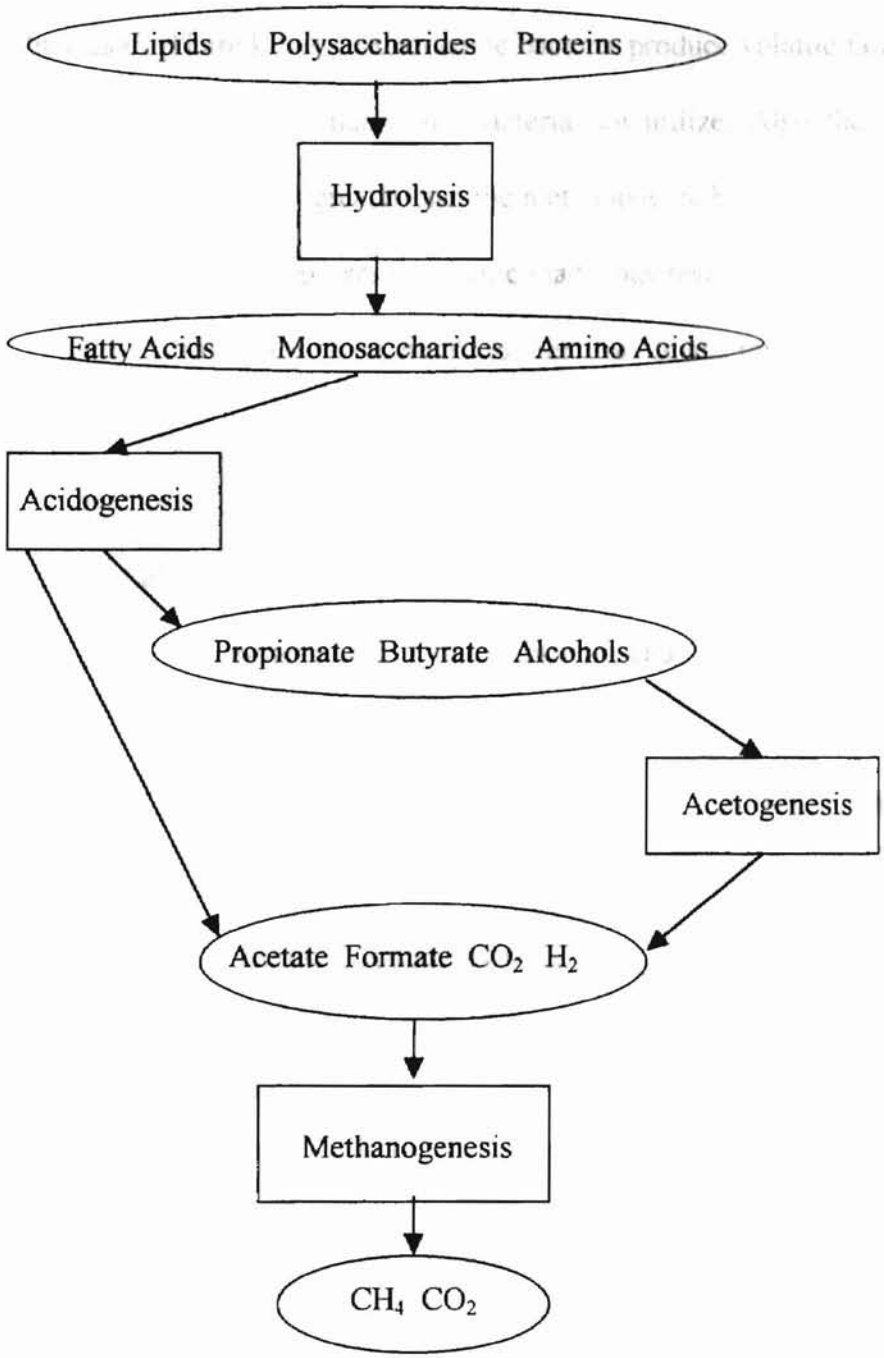

Figure 1. Sequences of Anaerobic Metabolism (Metcalf and Eddy, 1991).

indicator of reactor performance since significant environmental changes may have occurred before changes in pH are known. Acetogenic bacteria produce volatile fatty acids and hydrogen much faster than methanogenic bacteria may utilize. Also, the acetogenic bacteria population is much greater than the methanogenic bacteria population (Sawyer *et al.*, 1994). Sharp rises in organic loads may result in an increase in volatile fatty acids and hydrogen, lowering the pH to inhibitory levels for methanogenic bacteria. The pH should be between 6.5 - 7.7, however a neutral pH is generally more favorable (Grady and Lim, 1980). Sufficient alkalinity and buffering capacity must be supplied to maintain stability during normal and fluctuating organic loads (Grady and Lim, 1980). Chemicals used for alkalinity are caustic, sodium bicarbonate and lime.

Temperature has an effect on substrate uptake rates for anaerobic operations that is similar to other biochemical reactions. Higher temperatures, within a narrow band, result in greater removal rates and conversely for lower temperatures. Temperature influences the diffusion of substrate across cellular membranes. Emphasis should be on maintaining a uniform temperature, rather than maintaining a temperature for maximum removal rates, since small changes in temperature may have significant effects (Grady and Lim, 1980).

Bacteria may be classified by the temperature range in which they are able to survive. There are three ranges, psychrophilic, mesophilic and thermophilic.

Psychrophilic bacteria are efficient in the temperature range of 12 to18°C. Mesophilic bacteria function best in the temperature range of 25 to 40°C. Thermophilic bacteria prefer extremely warm environments with temperatures ranging from 55 to 65°C (Metcalf and Eddy, 1991).

Gas production and composition is a direct measure of the metabolic activity of methanogenic bacteria (Stover *et al.*, 1992). Stable conditions should produce gas with approximately 25 to 30% CO_2 and 65 to 70% CH_4 and small amounts of N_2, H_2 and H_2S. Gas production is the best measurement of progress of anaerobic metabolism (Metcalf and Eddy, 1991).

Successful anaerobic operations depend on maintaining an environment satisfactory to the symbiotic relationship between the methanogenic and acidogenic bacteria. Anaerobic treatability/toxicity screening tests provide needed information to maintain favorable environmental conditions.

Anaerobic Treatability/Toxicity Screening Test

Toxic materials are often the cause of anaerobic process failures. Biological assays have been developed using batch and semi-continuous flow systems (Owen *et al.*, 1979; Stucky et al., 1980). Each has advantages and disadvantages, with the ultimate

selection depending on the desired information, cost and equipment. Continuous procedures simulate full scale systems more accurately than batch systems. However, continuous systems are more expensive and labor intensive. Batch assays are relatively inexpensive and can provide the evaluation of a multitude of variables and scenarios and provide good toxicity information. Batch assays are useful in developing information for larger scale continuous feed assays (Owen *et al.*, 1979).

Common reactor vessels used in batch feed assays are serum bottles. Serum bottles are inexpensive and allow for measuring gas production and composition with the use of syringes. Syringes may also be used to extract liquid samples for subsequent analysis. The use of such devices in measuring the response of anaerobic bacteria to toxicants is called the "anaerobic toxicity assay" (ATA). A device commonly used in batch assays is the Warburg respirometer which has several limitations (Stucky *et al.*, 1980):

- costly and requires skill to operate,
- limited to the number of samples that can be analyzed at one time,
- sample size is limited, making subsequent analysis difficult,
- sampling the gas and liquid phase is difficult, and
- requires extended test periods which resluts in increased variability.

A study by Owen *et al.* (1979) developed a batch anaerobic bioassay technique for evaluating biological methane potential and ATA. The procedure made use of serum bottles which contained both the liquid and biogas. Syringes were used to extract gas and liquid samples. One of the objectives was to overcome some of the disadvantages to the

Warburg respirometer. Owen *et al.* (1979) concluded that the batch anaerobic bioassay technique was relatively quick and accurate. Several experimental conditions were evaluated and conditions may be screened for more detailed studies. The procedure was flexible which could allow for more rigorous studies.

Stuckey *et al.* (1980) compared batch and semi-continuous feed assays. In this study advantages were explored in each feed assay in the evaluation of methlyene chloride, vinyl acetate, ethlylene dichloride and vinyl chloride. The batch assay using serum bottles was found to be more practical, with testing periods lasting up to 10 days versus 60 days for the semi-continuous feed assay. A larger number of variables could be examined using the batch feed assay without additional labor or equipment. A characteristic noted by the authors was the ability of the inoculum to acclimate to toxic effects. This was observed in the batch assay by increased gas production rates after a period of time. The batch assay produced more conservative threshold estimates and provided a measure of the concentration of a given substance that would simulate a slug dose environment. The semi-continuous assay permitted evaluation of reduced toxicity due to volatilizing, acclimation or biodegradation.

Inhibition caused by a toxicant may be measured in terms of the concentration of the chemical that causes a 50% reduction in total gas production over a period of time compared to the feed control. This measurement is termed 50% inhibition (Stucky *et al.*, 1980). Owen *et al.* (1979) describes an alternative method for quantifying toxicity.

Total gas production of each sample is normalized by calculating ratios between the rates of the samples and the average of the controls. The ratio is termed the maximum rate ratio (MRR). Gas production rates are fairly accurate and ratios of 0.95 or lower indicate inhibition while a ratio less than 0.9 indicate significant inhibition. Complications of this method are sample decomposition and varying gas composition. Not all researchers agree that a 50% reduction in gas production over the control is needed to indicate indicate toxicity. This value may indicate excessive toxicity and 10% reduction over the control may indicate a toxic effect (Owen *et al.*, 1979). Reduced gas production alone does not indicate inhibition. Competitive inhibition could explain loss of gas production without accumulation of volatile fatty acids, as is the case with sulfides. Competitive inhibition occurs when sulfate reducing bacteria are present, which compete with the methanogenes for acetate and hydrogen. Several performance parameters should be investigated to develop a reasonable conclusion (Brooks *et al.*, 1994). Differentiating between toxicity and inhibition is not clear and often used interchangeably. In this study, toxicity referred to any negative effect.

Various devices and methods are available for measuring gas production from bench-scale anaerobic reactors. Gas production may be measured using volume displacement devices, wet-test meters, lubricated syringes, automatic anaerobic respirometers, manometer-assisted syringes, and calibrated pressure manometers or transducers. Each has advantages and disadvantages that should be considered, whether for batch, continuous, or semi-continuous procedures.

Young *et al.* (1991) evaluated the use of syringes as a gas measuring device, similar to the syringes used in this study. The method produced reasonably accurate gas measurements for cumulative gas production rates near 100 ml/d, but were inaccurate for hourly measurements. Disadvantages noted were the error due to resistance of the syringe movement and loss of gas through the plunger seal.

A recent study by Brooks *et a.* (1994) evaluated the use of 125 ml glass luer-lock syringes for developing an anaerobic screening procedure. This method is unique in that the syringe contains both the liquid sample and the biogas. The Young *et al.* (1991) study used serum bottles as the reactor vessel and the syringe was a separate device used to measure gas production. The syringes used in Brooks study allow for simple, quick and accurate gas measurement. The author noted that the syringes were accessible for both liquid and gas samples. However, results were fairly inconsistent, which may be due to inadequate contact between the anaerobic seed, carbon source and the toxicant.

Ammonia-nitrogen

Nitrogen is essential for the growth of bacteria and other forms of life. Nitrogen and phosphorous, in most cases, are the most important macronutrients in biological processes (Metcalf and Eddy, 1991). Nitrogen may exist in many forms and hasnumerous sinks and sources, as illustrated in the nitrogen cycle diagram in Figure 2. The two forms of nitrogen of most concern in anaerobic digestion are ammonium and ammonia.

Ammonia may be present during anaerobic digestion as either ammonium ion (NH_4^+) or as dissolved ammonia gas (NH_3). The two forms are in equilibrium with each other, as shown in the following equilibrium equation:

$$NH_3 + H_2O \leftrightarrow NH^+_4 + OH^- \quad (1)$$

The solubility constant or the equilibrium constant K, for this reaction is $5.4E10^{-10}$ at 35 °C and the pK_A is 9.27 (Kroeker *et al.*, 1979). The pK_A value represents the pH value at which the concentration of ammonia and ammonium are equal. This is illustrated by the pC-pH diagram in Figure 3. A pH of 7.0 is most favorable for anaerobic digestion and would contain mostly ammonium ion. Once the pH is above 7.0 equilibrium shifts to the left in equation 1. Another factor affecting the concentration of nitrogen species is temperature.

Temperature will affect the concentration of various chemical components. The temperature dependence on K is described in the following equation (Snoeyink and Jenkins, 1980). Both pH and temperature effect the distribution of ammonia and ammonium.

$$K = \exp(-\Delta G°/ RT) \quad (2)$$

where, $\Delta G°$ = Gibbs free energy at standard conditions
R = Gas constant
T = Absolute temperature
K = equilibrium constant

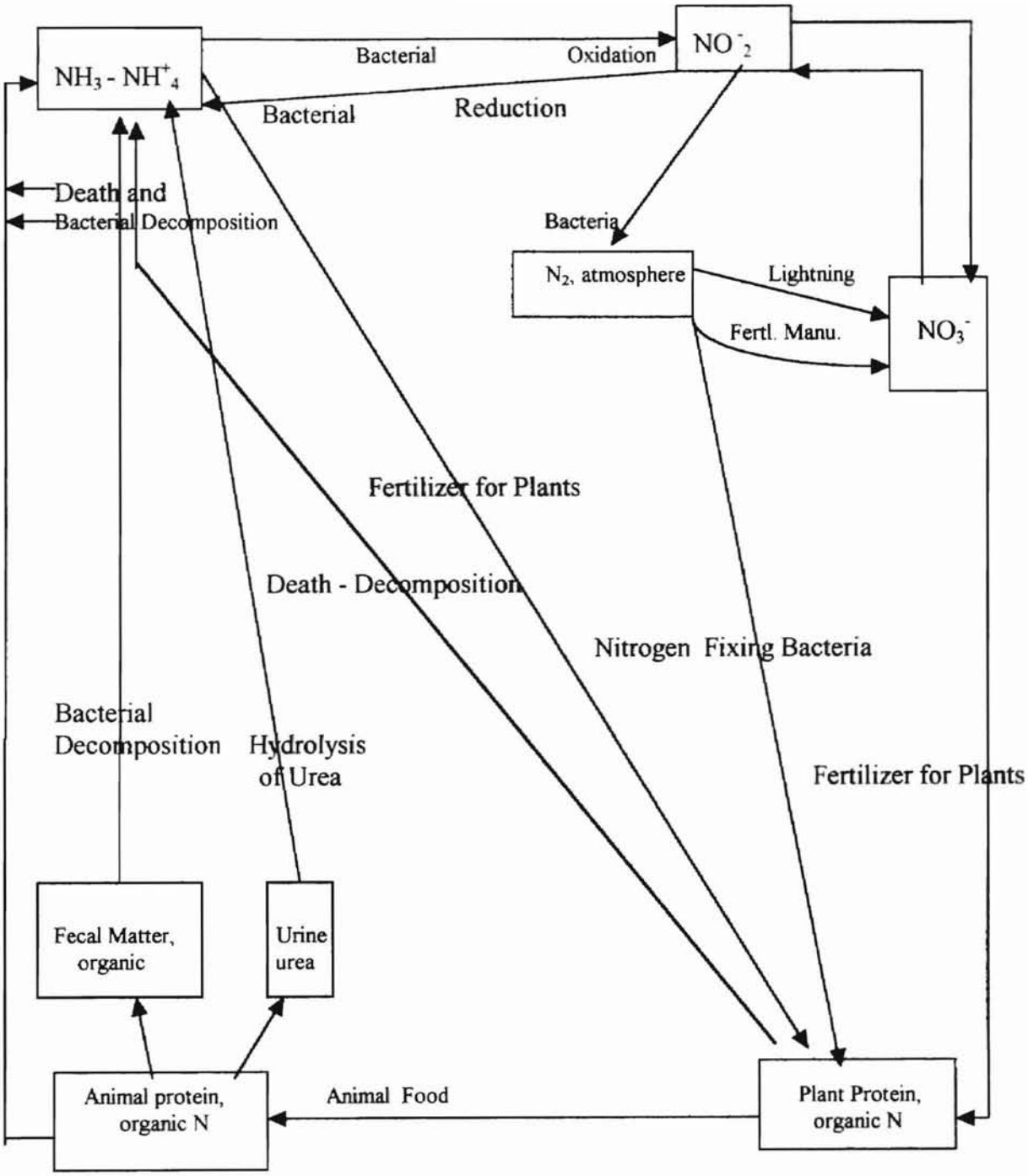

Figure 2. The Nitrogen Cycle (Metcalf and Eddy, 1991)

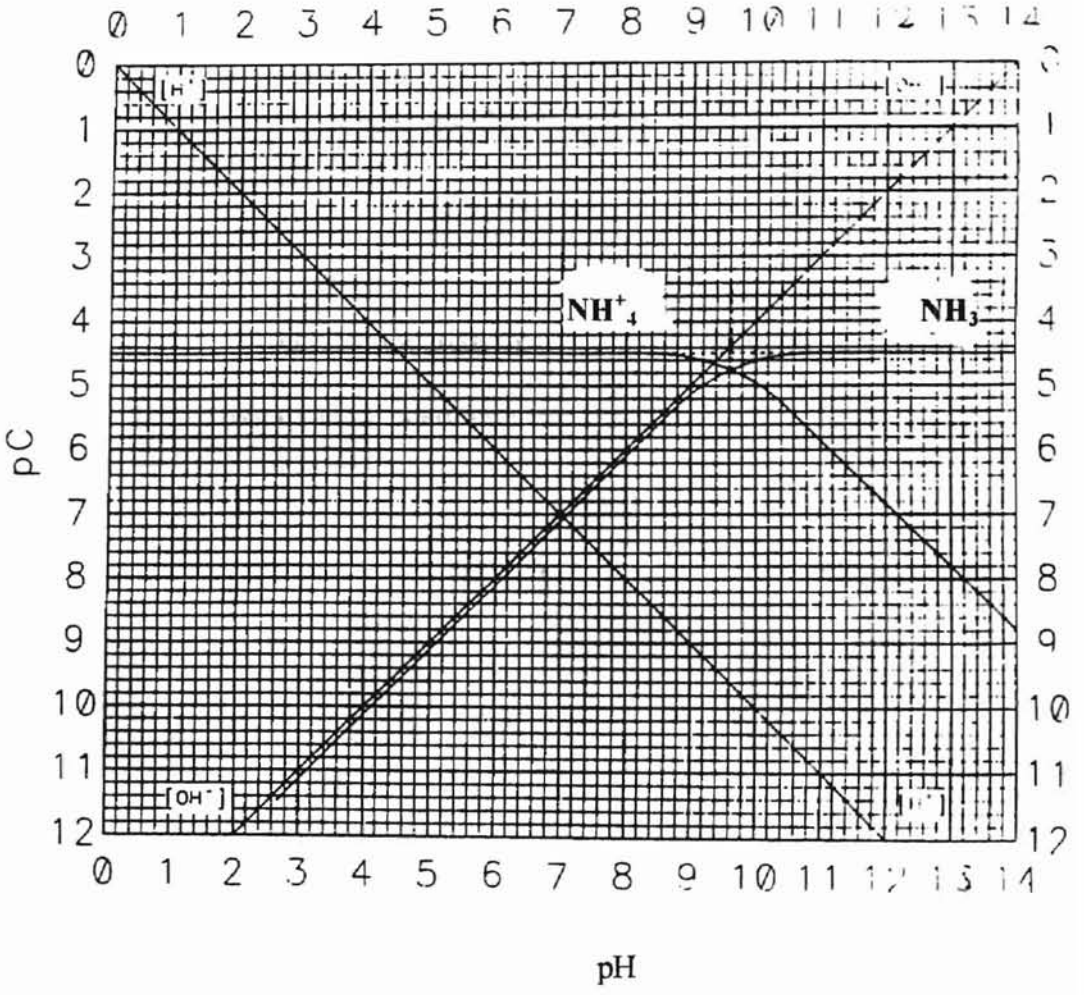

Figure 3. pC - pH Diagram for Ammonia-Nitrogen at $10E^{-4.5}$ M NH_4Cl

Ammonia in anaerobic digesters is produced from the digestion of organics (volatile suspended solids) containing protein. This is illustrated in the following equation for digestion of primary sludge at a solids residence time of 15 days at 35° C.

$$C_{10}H_9O_3N + 4.69H_2O \rightarrow 5.74CH_4 + 2.45CO_2 + 0.20C_5H_7O_2N + 0.80NH_4 + 0.8HCO_3 \quad (3)$$

Approximately 56 mg/L of ammonia-nitrogen is released for every g/l of volatile solid converted to methane, at the above conditions (Parkin and Owen, 1986).

There are conflicting reports on the toxicity of ammonia-nitrogen (ammonia-nitrogen = $NH^+_4 + NH_3$). Toxicity associated with free ammonia (NH_3-N) occurs near 100 mg/l and severe toxicity above 150 mg/l (Grady and Lim, 1980). McCarty (1964) reported that ammonia- nitrogen concentrations between 50 - 200 mg/l were beneficial and no adverse effect was observed up to 1000 mg/l ammonia-nitrogen. Inhibition occurred at 1,500 - 3,000 mg/l and toxicity occurred above 3,000 mg/l. Parkin *et al.* (1983) reported rate inhibition at 7,500 mg/l and extremely toxic responses at 10,000 and 12,000 mg/l ammonia-nitrogen. The experimental procedure was similar to the ATA proposed by Owen *et al.* (1979). The authors observed the capability of bacteria to produce gas after exposure to the toxicant (reversibility) at an ammonia-nitrogen concentration of 24,000 mg/l. Gas production was similar to the control after a four day exposure period at 24,000 mg/l ammonia-nitrogen.

Acclimation to ammonia-nitrogen has been observed. Kroeker *et al.* (1979) reported that with acclimation, 7,000 mg/l ammonia-nitrogen was not toxic. Studies by van Velsen (1979) suggest ammonia-nitrogen concentrations near 5,000 mg/l are tolerable (using batch feed assay) to methane forming bacteria, however long acclimation periods of up to 50 days were required. Other researches observed inhibition at ammonia-nitrogen concentrations near 2,000 mg/l (Dague et al., 1970; Kroeker et al., 1979; Melbinger and Donnellon, 1971). Parkin and Miller (1982) reported that with acclimation, ammonia-nitrogen concentrations in the range of 8,000 - 9,000 mg/l can be tolerated with little decrease in methane production. Researchers have observed ammonia-nitrogen concentration in excess of 1,500 mg/l with a pH range of 7.5-8.0 treating waste in anaerobic digesters with satisfactorily performances (Melbinger and Donnellon, 1971; Hobson and Shaw, 1976). Wide ranges of ammonia-nitrogen toxicity may be a function of solids retention time and acclimation.

Nickel

Heavy metals have been the primary cause of many anaerobic digester failures (Parkin and Owen, 1986). The heavy metals of most concern are nickel, zinc, copper and chromium (VI), since these are the most toxic of the heavy metals (McCarty, 1964; Parkin and Owen, 1986). The fabricated metal products industry is the greatest source of heavy metals. Nickel is used in electroplating and rinse waters from these industries are the main source of nickel (Sawyer *et al.*, 1994).

The toxicity of heavy metals in an anaerobic digester is dependent on the chemical species. Heavy metal toxicity is ultimately driven by the solubility of the cation which is linked to the uptake by bacteria. The solubility is governed by the anaerobic conditions and pH (Ashley *et al.*, 1982). Bound metals or complexes exhibit lower bioavailability compared to ionic species. Metals may be highly toxic at low levels, but less toxic at high concentrations provided sulfide, carbonate and in some cases phosphate are present to complex the metals to lower the cations in solution (Mosey, 1971; Mosey *et al.*, 1971). Chelating agents may be added or present, rendering the cation less available (Callender and Barford, 1983). Gould and Genetelli (1978) reported nickel complexation was pH dependent and was the weakest complexed metal.

The primary anions capable of precipitating metals in an anaerobic digester are sulfide (S^{-2}), carbonate (CO_3^{-2}) and less importantly, phosphate (PO_4^{-3}). The concentrations of these species are dependent on pH. An anaerobic digester is commonly operated near a pH of 7.0 and at a pH of 7.3 the dominant species present are HS^-, HCO_3^-, with equal portions of HPO_4^{-2} and H_2PO_4. The typical sulfide, carbonate and phosphate ion distribution as a percent of all the related species is extremely small, with carbonate the largest at 0.089 and sulfide the smallest at 0.00017 %. Thus the ion species with the greatest affinity to complex with metal is relatively small (Callender and Barford, 1983).

Researchers have examined the distribution of heavy metals in anaerobic digester sludges (MacNicol and Beckett, 1989; Hays and Thies, 1978; Gould and Gennetelli, 1975). MacNicol and Beckett (1989) investigated the distribution of heavy metals based on particle size. The researchers used elutriation and filtration to separate the sludge into four fractions; particulate, supracolloidal, colloidal and soluble. Generally more than 90% of the heavy metals were found in the particulate fractions that were greater than 100 μ. The majority of the remaining metals were found in the supracolloidal fraction. Hayes and Theis (1978) investigated the soluble, precipitated, the extracellular and the intracellular fractions. The majority of the metals examined, including nickel, were found to be in the insoluble fraction. Heavy metal removal from the digester effluent was greater than 95%.

Several researchers have investigated nickel toxicity in batch and semi-continuous feed assays. Most researchers have examined the effect of nickel on methanogenic bacteria (Parkin *et al.*, 1983) or different physiological groupings (Ashley *et al.*, 1982). Parkin *et al.* (1983) examined the reversibility of four toxicants including nickel.

Nickel toxicity has been evaluated by batch and semi-continuous feed assays (Parkin et al., 1983). Rate inhibition was observed at 50 to 200 mg/L as $NiCl_2$. Greater than 50% reduction in total gas produced was observed at concentrations of 300 and 500

mg/l $NiCl_2$. Greater tolerance was observed with the semi-continuous feed assay. No decrease in gas production was observed under 70 mg/l $NiCl_2$. Methane production was inhibited at 80, 90, and 100 mg/l $NiCl_2$. Inhibition was not revealed until after four days of exposure to nickel. Reversibility experiments suggest unacclimated methanogens can recover from high nickel concentrations, but are limited in concentration and duration of exposures. In that study, concentrations of 400, 800, 2,400 mg/l Ni^{2+} were exposed at 1 hour, 1 day and 4 days. The serum bottles were centrifuged and the supernatant was removed and replaced with supernatant from uncontaminated serum bottle with sludge.

Parkin and Miller (1982) investigated nickel toxicity towards methanogenic bacteria using semi-continuous feed assays at different solids retention times (15, 25, and 50 days) and temperatures (25, 35 and 42.5°C). The maximum tolerable concentration was in the range of 100 to 200 mg/l among the various conditions.

Ashley *et al.* (1982) examined the response of different physiological groupings of microorganisms such as, starch, lipid, and protein hydrolyzing bacteria. In addition, the relationship of added and dissolved nickel ion concentrations in the anaerobic digester was evaluated. The highest dose of 250 mg/L Ni^{2+} resulted in a dissolved nickel concentration of 15 mg/l. This was possibly due to complexing with sulfides and organic components. Results of this study showed decreases in the populations of each type after each incremental dose and recovery followed by an increase in most populations.

The following is brief summary of what has been accomplished in reference to ATA's and nickel and ammonia-nitrogen toxicity (batch) assays. Most ATA's are performed with serum bottles and few have been performed under continuous mixing conditions. Syringes are generally used to measure gas production, and not used as the reactor vessel. The study by Brooks *et al.*, (1994) was the first to use the 125 ml glass luer-lock syringes. However, this study was performed under static conditions between intermittent mixing twice per day. Also, the syringes were not evaluated for variability and reproducibility to ensure the syringes used under these conditions would measure gas production accurately.

Researchers have evaluated nickel and ammonia-nitrogen toxicity on methanogens and other physiological groupings (Parkin and Miller, 1982; Ashley *et al.* 1982; Parkin et al., 1983). This has been accomplished by using acetate as the primary substrate. The literature reviewed in this study did not find any studies that evaluated toxicity on the entire sequence of metabolic reactions. This is important in application of this procedure, since most applications of anaerobic reactors will involve all sequences of anaerobic metabolism and encounter a variety of complex substrates.

CHAPTER III

METHODS AND MATERIALS

Experimental Procedure

The scope of this study was to improve an anaerobic treatability/toxicity screening procedure using 125 ml glass syringes as the reactor vessel. There were four objectives to this study. First, determine the specific operating conditions such as, the range of volatile suspended solids and the F/M ratio. Second, examine the variability and reproducibility of the syringes. Third, determine the effects of mixing versus static condition. Fourth, apply the method in evaluation of ammonia-nitrogen and nickel toxicity.

Six 125 ml glass leur-lock syringes were used in this study. The syringes were fastened to a shaker table throughout the study, except for one experiment. The syringe was comprised of three parts, the barrel, plunger, and the leur-lock valve. The leur-lock valve is secured at one end of the barrel, and pressure from the production of biogas moved the inserted plunger. Biogas was evacuated through the leur-lock valve. The barrel of the syringe contained a measurable volume of 100 ml with the smallest defined unit of two milliliters.

The anaerobic seed source used in the study was from a hybrid anaerobic reactor treating high strength carbohydrate wastewater. The wastewater is from a candy manufacturing plant. The anaerobic seed was shipped directly from the industry to The Stover Group in Stillwater, OK. The sludge was stored in an air tight container and kept at a temperature of approximately 35 degrees Celsius. Gas was released once a day until the gas pressure was minimal, which occurred after approximately seven days. At that time, pH, alkalinity, volatile fatty acids (VFA), and soluble COD analysis were conducted every two to three days until endogenous conditions were established. Experimental procedures were initiated when the seed source reached endogenous conditions, represented by insignificant changes in COD and VFA analysis. Endogenous conditions represented the removal of the biodegradable organic matter. This condition was preferred so that, the influent COD represented the desired soluble COD at the beginning of the test.

The set up procedure was similar for all test runs. In the first experiment, a 120 ml stock solution of sucrose diluted with BOD dilution water and anaerobic seed was mixed thoroughly, and then a 60 ml subsample was withdrawn and placed into each syringe. The pH was adjusted with sodium hydroxide or sulfuric acid to 7.0 to 7.3 s.u. before the a 60 ml subsample was withdrawn from the stock solution. The remaining 60 ml of solution was used for chemical analysis except for volatile suspended solids, which were analyzed at the end of the tests. This parameter was measured at the end so that the subsamples were withdrawn from the solution contained in the syringes.

Volatile suspended solids measurements were exceedingly high when the subsample was withdrawn from the remaining 60 ml of the stock solutions. In experiments 2 and 3, one stock solution was made and all initial chemical analyses were prepared from that stock solution.

In experiments three, four and five, one stock solution containing a mixture of sucrose, anaerobic seed, phosphate buffer and BOD dilution water was mixed thoroughly. Subsamples from the stock solution were transferred to 200 ml beakers and were spiked with the desired concentration of ammonia-nitrogen or nickel (total volume was 120 ml). All 60 ml syringe samples were then withdrawn from the beakers containing the 120 ml stock solutions. The remaining 60 ml was used for chemical analyses (except VSS which was measured at the end of the test). Micronutrients were not added in experiments one through three since micronutrient additions occurred in the process from which the seed source was collected. However, micronutrients were added in experiments four and five to ensure that reactor failure was not caused by low micronutrients. Micronutrients added were $(NH_4)_6 \bullet Mo_7O_{24}$, $NiCl_2 \bullet 6H_2O$, $CuSO_4 \bullet 5H_2O$, $CoCl_2 \bullet 6H_2O$ and $ZnSO_4 \bullet 7H_2O$. The micronutrient stock solution concentrations were 0.1 mg/l as the micronutrient and 4 ml of solution was added to the make up the 120 ml stock solution (Brooks *et al.*, 1994).

The first experiment was designed to determine the optimum range of volatile suspended solids and F/M ratio, for practical administration of the test. Syringes loaded

with high VSS concentrations or high organic loads may produce gas too quickly, possibly separating the plunger from the syringe, and terminating the test. In addition, high VSS and F/M ratios may require intense supervision and multiple evacuations of the biogas, increasing test variability. Conversely, a low volatile suspended solids concentration or low F/M ratio may produce a low, inconclusive volume of biogas. An F/M ratio range must be determined to prevent reactor failure.

The objective of Experiment one; Test A, was to determine the optimum range of volatile suspended solids. Syringe A was loaded with 1,000 mg/l VSS, syringe B with 2,000 mg/l, syringe C with 3,000 mg/l, syringe D with 4,000 mg/l, syringe E with 5,000 mg/l and syringe F with 6,000 mg/l VSS. These values represent nominal VSS concentrations. All syringes contained an F/M ratio around 0.25. This value was selected to ensure that gas production rates were low enough to monitor the syringes over time and that a high organic load would not impair reactor performance. The objective of test B was to determine an acceptable range of F/M ratios. Two VSS concentrations were selected based on the results of test A. The criteria for VSS selection were the volume of gas produced, number of times gas was evacuated from a syringe and reactor performance. F/M ratios were set at 0.5, 1.0 and 1.5 for each VSS concentration selected. These values are representative of full scale anaerobic reactors (Droste, 1997). Criteria for selecting the F/M ratios were based on practical consideration of administering the test. The test procedure was developed so that gas production was

examined twice daily without supervision between inspections. Initial operating conditions for Experiment 1; Tests A and B are listed in Table 1.

Table 1. Initial Operating Conditions for Experiment 1; Test A,VSS Determination and Test B, F/M Determination

Parameter	Syringe A	B	C	D	E	F
Test A						
Initial Conditions						
VSS (mg/l)	900	1600	2700	3850	4650	5800
Sucrose (mg/l)	250	500	750	1,000	1,250	1500
COD (mg/l)	350	460	750	900	1250	1450
Alkalinity (mg/l)	500	500	800	1,150	1,450	2,400
pH (s.u.)	7.2	7.2	7.2	7.2	7.2	7.2
Test B						
Initial Conditions						
VSS (mg/l)	2,700	3,100	-	3,650	4,200	-
F/M Ratio	0.38	0.77	1.5*	0.56	0.71	1.5*
Sucrose (mg/l)	1,500	3,000	4,500	2,000	4,000	6,000
COD (mg/l)	1,025	2,415	3,000	1,305	3,000	5,000
Alkalinity (mg/l)	705	720	670	906	916	950
pH (s.u.)	7.1	7.2	7.2	7.2	7.2	7.3

- Syringes expelled plunger, therefore VSS was not analyzed (VSS was measured at the end of the test so that VSS)

* Nominal values

Biogas production relates to performance of the anaerobic bacteria, thus syringe variability must be distinguished from inhibition produced from the test media. The objective of the second experiment was to examine the variability and reproducibility of the syringes. Six syringes were prepared with a VSS concentration of 4,000 mg/l and an F/M ratio of 0.5. An F/M ratio of 0.5 was selected due to favorable gas production rates

and COD removal. The test media for each syringe was withdrawn from a stock solution of anaerobic seed (308 ml), sucrose and phosphate buffer to ensure uniformity. Initial operating conditions for Experiment 2 are listed in Table 2. Two test runs were used to assess the variability of the method. Since comparisons were made between tests, the accuracy of each set up was important for maintaining uniformity.

Table 2. Initial Operating Conditions for Experiment 2; Test A and B Variability/Reproducibility and Experiment 3; Comparison of Mixed Versus Static Test Conditions

	Parameter					
	VSS (mg/l)	**Sucrose (mg/l)**	**sCOD (mg/l)**	**Alkalinity (mg/l)**	**pH s.u.**	**KH_2PO_4 (mg/l)**
Experiment 2						
Test A	4,100	2,000	2,125	1,600	7.2	20
Test B	3,650	2,000	1,900	2,250	7.2	20
Experiment 3						
Test A	3,575	2,000	2,000	2,250	7.2	20

Improvements in the test procedure focused on reducing the duration of the test and improving contact between the anaerobic seed and the substrate or toxicants. This was accomplished by utilizing a mixing device (shaker table). The objective of the third experiment was to evaluate the effects of mixing versus static conditions. Three syringes were placed on a shaker table and three syringes were mixed twice per day remaining under static conditions between mixing. Initial operating conditions for Experiment 3 are listed above in Table 2.

The improved syringe method was evaluated as an anaerobic toxicity assays (ATA). Two compounds, ammonia and nickel, were evaluated for toxicity. Operating conditions were based on the results of experiments devoted to developing the methodology. The operating conditions were set at a VSS concentration of 3,000 to 4,000 mg/l and an F/M ratio of 0.5. A stock solution containing sucrose, anaerobic seed (NH_3-N was added in the nickel toxicity assay as a macronutrient) and phosphate buffer was mixed thoroughly and pH was adjusted to 7.0 to 7.3 before subsamples were withdrawn. Ammonium was the dominant species present at that pH. Subsamples were then spiked with toxicants. In each ATA, one syringe was designated as a control. The objective was to determine what concentrations of NH_3-N and nickel caused inhibitory conditions.

The first ATA evaluated NH_3-N toxicity. The objective of test A was to assess the beginning of inhibition or toxicity. Test B was developed based on the results of test A, to further define the concentration range of toxicity or inhibition. The NH_3-N source was reagent grade NH_4Cl, which is commonly used as a nitrogen source. The NH_3-N (nominal) concentrations in test A ranged from 250 mg/l in the control and 400, 800, 1,200, 1,600 and 2,000 mg/l NH_3-N in the remaining syringes. In test B, NH_3-N concentrations ranged from 2,500 to 5,000 mg/l NH_3-N. Ammonium chloride was added (50 mg/l as NH_4Cl) to the controls to ensure that macronutrients were not the cause of reactor failure. Initial operating conditions for Experiment 4 Tests A and B are listed in Table 3.

Nickel was also evaluated for inhibition and toxicity. The setup conditions were similar to the NH_3-N study and are listed in Table 4. Ammonium chloride was added as a macronutrient. Two test were performed using reagent grade $NiCl_2 \bullet 6H_2O$. Test A was set up with a control receiving nickel only from the micronutrient stock solution. The remaining syringes were set up with nickel concentrations of 11, 45, 90, 136 and 181 mg/l. Test B was setup with a control and nickel concentrations of 90, 136, 181, 226 and 272 mg/l. The syringe containing 136 mg/l nickel was spiked to 226 mg/l during the test. This was done to determine the cause of lag periods exhibited by the anaerobic seed source. This will be discussed further in Chapter 5.

Wet Chemistry Analysis

All samples for wet chemistry analysis were obtained from the 60 ml of excess solutions made for each syringe. The contents were mixed well before a subsample was withdrawn. Samples which required filtration were filtered through 4.25 micron glass filter (Whatman AH934). All analyses were performed at The Stover Group's analytical laboratory.

Soluble Chemical Oxygen Demand (sCOD)

The chemical oxygen demand (COD) was determined colorimetrically using the reactor digestion method and HACH chemical reagents. The detection range used for all

Table 3. Initial Operating Conditions for Experiment 4; Test A and B NH_3-N Toxicity Assay

Parameter	Syringe A	B	C	D	E	F
Test A						
Initial Conditions						
VSS (mg/l)	3,500	3,800	4,050	3,700	4,250	5,200
Sucrose (mg/l)	1,750	1,750	1,750	1,750	1,750	1,750
COD (mg/l)	1,900	1,900	1,900	1,900	1,900	1,900
Alkalinity (mg/l)^	2,250	2,250	2,250	2,250	2,250	2,250
pH (s.u.)	7.2	7.2	7.2	7.2	7.2	7.2
NH_3-N (mg/l)	-	-	-	1,310	1,560	1,800
KH_2PO_4 (mg/l)	20	20	20	20	20	20
Sludge VFA (mg/l)	140	140	140	140	140	140
Test B						
Initial Conditions						
VSS (mg/l)	3,500	3,800	4,050	3,700	4,250	5,250
Sucrose (mg/l)	1,750	1,750	1,750	1,750	1,750	1,750
TOC (mg/l)	824	887	777	695	813	986
Alkalinity (mg/l)^	1,666	1,600	1,650	1,650	1,600	1,750
pH (s.u.)	7.2	7.2	7.2	7.2	7.2	7.2
NH_3-N (mg/l)	204	2,760	2,860	3,150	3,630	3,768
KH_2PO_4 (mg/l)	20	20	20	20	20	20
Sludge VFA (mg/l)	140	140	140	140	140	140

- Was not able to measure NH_3-N

^ Expressed as $CaCO_3$

Table 4. Initial Operating Conditions for Experiment 5; Tests A and B
Nickel Toxicity Assay

Parameter	Syringe A	B	C	D	E	F
Test A						
Initial Conditions						
VSS (mg/l)	4,150	3,850	3,900	3,800	4,100	3,950
Sucrose (mg/l)	2,000	2,000	2,000	2,000	2,000	2,000
COD (mg/l)	2,175	2,000	2,000	2,000	2,000	2,000
Alkalinity (mg/l)^	1,800	1,900	1,850	1,900	2,000	1,800
pH (s.u.)	7.2	7.2	7.2	7.2	7.2	7.2
NH_4Cl (mg/l)	50	50	50	50	50	50
KH_2PO_4 (mg/l)	20	20	20	20	20	20
Sludge VFA (mg/l)	140	140	140	140	140	140
Nickel (mg/l)	-	11	45	90	136	181
Test B						
Initial Conditions						
VSS (mg/l)	4,000	3,000	3,700	3,600	3,900	4,000
Sucrose (mg/l)	2,000	2,000	2,000	2,000	2,000	2,000
TOC (mg/l)	2,400	2,000	2,200	2,000	2,000	2,000
Alkalinity (mg/l)^	1,950	2,150	1,950	1,950	1,950	2,000
pH (s.u.)	7.1	7.1	7.1	7.1	7.1	7.1
NH_4Cl (mg/l)	50	50	50	50	50	50
KH_2PO_4 (mg/l)	20	20	20	20	20	20
Sludge VFA (mg/l)	100	100	100	100	100	100
Nickel (mg/l)	-	90	136	181	226	271

^ Expressed as $CaCO_3$

sCOD analysis was 0-1,500 mg/l. In this method, 5 ml were filtered. The total volume of sample used for each COD vial was 2 ml. Appropriate dilution factors were incorporated when COD concentration were expected to be above the range of the test method. The chemical reagents in the COD vial consist of concentrated sulfuric acid (H_2SO_4),

mercuric sulfate ($HgSO_4$), and silver sulfate ($AgSO_4$) and dichromate with a total volume of 1.5 ml. All samples were digested for two hours at 150°C, cooled and analyzed colorimetrically by measuring the absorbance at 620nm by a HACH DR/3 spectrophotometer. In each batch of tests a HACH standard of 300 mg/l and a blank were run to ensure accuracy. All samples were run in duplicate.

Total Organic Carbon (TOC)

The Total Organic Carbon (TOC) was measured by injecting a 20 ml sample into an Astro 2001 TOC/TIC/TC analyzer. A 5 ml sample from each syringe was filtered and diluted with deionized water to 20 ml. A calibration standard of potassium acid phthalate at a concentration of 100 mg/l as carbon, a blank (deionized water) and one duplicate was run with each series of test.

Ammonia-Nitrogen (NH_3-N)

Ammonia-nitrogen was determined using a Fisher Accumet 25 pH-ion meter. The meter was calibrated with HACH NH_3-N standards of 1.0, 10, and 100 mg/l NH_3-N. All samples were diluted with deionized water to produce a concentration within the standard range of 100 mg/l -N. The total diluted volume of each syringe sample was 50ml and a 1.0 ml solution of 10N NaOH was added prior to analysis to increase the pH so that all of the NH^+_4 was converted to NH_3.

Nickel

Total and soluble nickel was analyzed on a Perkin-Elmer atomic absorption spectrophotometer using the flame method. Samples were filtered with a 4.25 micron filter to separate the soluble fraction. Samples were digested using EPA method 3005 (EPA, 1986). Nickel analysis was performed by Ron Helems of The Stover Group.

Total and Volatile Suspended Solids (TSS/VSS)

Total and volatile suspended solids determinations were made according to Standard Methods (2540 D. and 2540 E., respectively) 18th Edition (1992). Total suspended solids (TSS) were dried in a Fisher Isotemp 500 oven overnight. Volatile suspended solids (VSS) were ignited in a Linberg furnace at 550°C ± 50°C for fifteen minutes. A syringe sample was selected for duplication to check reproducibility. The weight of each sample was determined on a Ohaus GA200D balance.

Alkalinity

Total alkalinity was determined titrametrically in accordance with Standard Methods (2320 B.) 18th Edition (1992). Alkalinity measurements were conducted with a limited number of samples under the recommended volume of 50 ml.

Volatile Fatty Acid

Volatile fatty acid analysis was determined by suppressing the pH of a 50 ml sample to 3.5 s.u., boiling for three minutes and cooling. The pH was raised with 0.05 or 0.1 N NaOH to 4.5 s.u., then raised to 7.0 s.u. The volume required to raise the pH from 4.5 to 7.0 s.u. was used for VFA determination. The formula used to determine the VFA is presented below.

VFA (mg/l) = (NaOH Normality) * (NaOH Vol. (ml)) * 1000 (4)

if VFA > 150 mg/l, multiply by 1.5 for final VFA (mg/l)

pH

The pH of all samples were determined using a Fisher Accument 900. The pH meter was calibrated at pH of 4.0 and 7.0 s.u. before analysis and intermittently rechecked at a pH of 7.0 s.u. to ensure precision. The final pH was measured by removing the syringe valve and pushing the liquid sample through the end of syringe, directing the contents into a beaker with a pH probe in position This procedure produced an accurate representation of the final pH. The pH increased when exposed to ambient conditions due to CO_2 release; therefore, the pH was determined first.

Gas Analysis; Volume Production Rate

The gas production rate was measured twice a day by subtracting the previous volume of gas produced from the total volume gas measured at that time. The volume of gas was measured from the tip of the plunger, or if the plunger extended past the measurable volume of the barrel, the syringe was placed vertically and measured from the meniscus of the sample. Gas was evacuated if it was believed gas production rates would cause the expulsion of the plunger between inspections.

Carbon Dioxide (%CO_2)

Carbon dioxide was determined by injecting a 0.7 ml sample of biogas into an Astro 2001 TOC/TIC/TC analyzer. A silicon tube was connected to the end of the luer-lock valve, the valve was then opened, flushing the silicon tube with biogas and then clamped to capture the biogas. This procedure required approximately 10 to 15 ml of gas. A sample was extracted from the tube using a 5 ml syringe. A 30 % CO_2 and 70 % helium standard was made on site using the same type of syringe used in this study. Three to four standards were analyzed and averaged for calibration during each test.

CHAPTER IV

RESULTS

The results of this study are reported in two sections. The first section includes the experiments which were designed to develop the operating conditions of the syringes and the effects of mixing. The second section evaluated the method with the ammonia-nitrogen and nickel toxicity assays. Results of this study are summarized in Tables 6 through 10 . Cumulative gas curves for the syringes are presented in Figures 4 through 12.

The scope of this project was to improve an existing anaerobic treatability/toxicity screening procedure. The objectives of this study, as outlined in Table 5, were to define operational conditions such as volatile suspended solids concentrations and F/M ratios, while evaluating the variability of the test procedure and reproducibility. Another objective was to minimize the length of the test period, while maintaining accurate, reproducible results. The final objective of the study was to assess the method in evaluating ammonia-nitrogen and nickel toxicity. The procedure employed 125 ml glass leur-lock syringes as the reactor vessel under mixing conditions on a shaker table. The syringes are unique, in that both the test media and biogas are contained in the same vessel, allowing real time measurement of biogas production.

Table 5. Objectives of Each Experiment

EXPERIMENT NUMBER	OBJECTIVE
1	Determine Optimum Range of VSS Determine Optimum F/M Ratio
2	Examine the Variability and Reproducibility of the Syringes
3	Determine the Effects of Mixing Versus Static Conditions
4	Review Method in Evaluating NH_3-N Toxicity
5	Review Method in Evaluating Nickel Toxicity

Experiment 1; Tests A and B

Test A

The syringe performance summaries for Experiment 1; Test A are presented in Table 6. Cumulative gas production curves are presented in Figure 4. The VSS concentration in syringe A was 900 mg/l and the F/M ratio was 0.38 mg COD/mg VSS. The total gas production was 22 ml with a 9.2 % CO_2 content. The COD removal (CODr) for syringe A was 74 %. The VSS concentration in syringe B was 1,950 mg/l and the F/M ratio was 0.23 mg COD/mg VSS. The total gas production was 46 ml with a 15.5 % CO_2 content. The COD removal for syringe B was 39 %. The VSS concentration in

syringe C was 2,800 mg/l and the F/M ratio was 0.26 mg COD/mg VSS. The total gas production was 86 ml of which 15.4 % was CO_2. The COD removal for syringe C was 86 %. The VSS concentration in syringe D was 3,600 mg/l and the F/M ratio was 0.25 mg COD/mg VSS. The total gas production was 106 ml and was comprised of 15.0 % CO_2. The COD removal for syringe D was 90 %. The VSS concentration in syringe E was 4,600 mg/l and the F/M ratio was 0.27 mg COD/mg VSS. The total gas production was 141 ml which consisted of 14.3 % CO_2. The COD removal for syringe E was 92 %. The VSS concentration in syringe F was 5,750 mg/l and the F/M ratio was 0.25 mg COD/mg VSS. The total gas production was 160 ml, consisting 13.8 % CO_2. The COD removal for syringe F was 92 %.

Test B

The syringe performance summaries of Experiment 1; Test B are presented Table 6. Cumulative gas production curves are presented in Figure 5. The objective of this test was to determine the optimum F/M ratio. Syringes C and F were set up at 3,000 mg/l and 4,000 mg/l VSS, respectively, and an F/M ratio near 1.5 mg COD/mg VSS. These syringes produced gas too rapidly and were terminated. Gas pressure expelled the plunger on syringe C on the first day and syringe F was removed from the test due to rapid gas production. The four remaining syringes completed the test. The F/M ratio in syringe A was 0.38 mg COD/mg VSS and the VSS concentration was 2,700 mg/l. Total gas production was 54 ml, with a 32 % CO_2 content. The COD removal in syringe A was

38%. The F/M ratio in syringe B was 0.78 mg COD/mg VSS and the VSS concentration was 3,100 mg/l. Total gas production was 31 ml, of which 48% was CO_2. The COD removal in syringe B was 0%. The F/M ratio in syringe D was 0.35 mg COD/mg VSS and the VSS concentration was 3,650 mg/l. Total gas production was 121 ml which was comprised of 33 % CO_2. The COD removal in syringe D was 92%. The F/M ratio in syringe E was 0.74 mg COD/mg VSS and the VSS concentration was 4,200 mg/l. Total gas production was 55 ml, with a 59 % CO_2 content. The COD removal in syringe E was 0%.

Experiment 2; Tests A and B

Test A

The syringe performance summaries of Experiment 2; Test A are presented in Table 7. Cumulative gas production curves are given Figure 6. The objective of the next two tests was to examine variability and reproducibility among the six syringes. All syringes had a VSS concentration of 4,100 mg/l and an F/M ratio of 0.52 mg COD/mg VSS. Syringe A produced a total of 108 ml of gas, which was comprised of 23.6 % CO_2. The COD removal for syringe A was 94.8 %. Syringe B produced a total of 110 ml of gas, which was comprised of 25.3 % CO_2. The COD removal for syringe B was 95.2 %. Syringe C produced a total of 104 ml of gas, which comprised of 22.5 % CO_2. The COD

Table 6. Final Conditions of Experiment 1; Test A, VSS Determination and Test B, F/M Determination

Parameter	Syringe A	B	C	D	E	F
Test A						
Final Conditions						
	900	1,600	2,700	3,850	4,650	5,800
Total Gas (ml)	22	46	86	106	141	160
CODr (%)	74	39	46	90	92	92
Alkalinity (mg/l)^	250	850	1,200	1,650	2,050	2,400
pH (s.u.)	6.5	6.5	6.8	7.0	7.0	7.1
CO_2 (%)	9.2	15	15	15	14	14
Test B						
Final Conditions						
	0.38	0.77	1.5*	0.56	0.71	1.5*
Total Gas (ml)	54	31	-	121	55	-
CODr (%)	38	0	-	90	0	-
Alkalinity (mg/l)^	990	760	-	1322	980	-
pH (s.u.)	6.5	5.4	-	6.9	5.5	-
VFA (mg/l)	300	1,125	-	100	1,275	-
CO_2 (%)	32	48	-	33	59	-

* Nominal F/M ratio

^ Expressed as $CaCO_3$

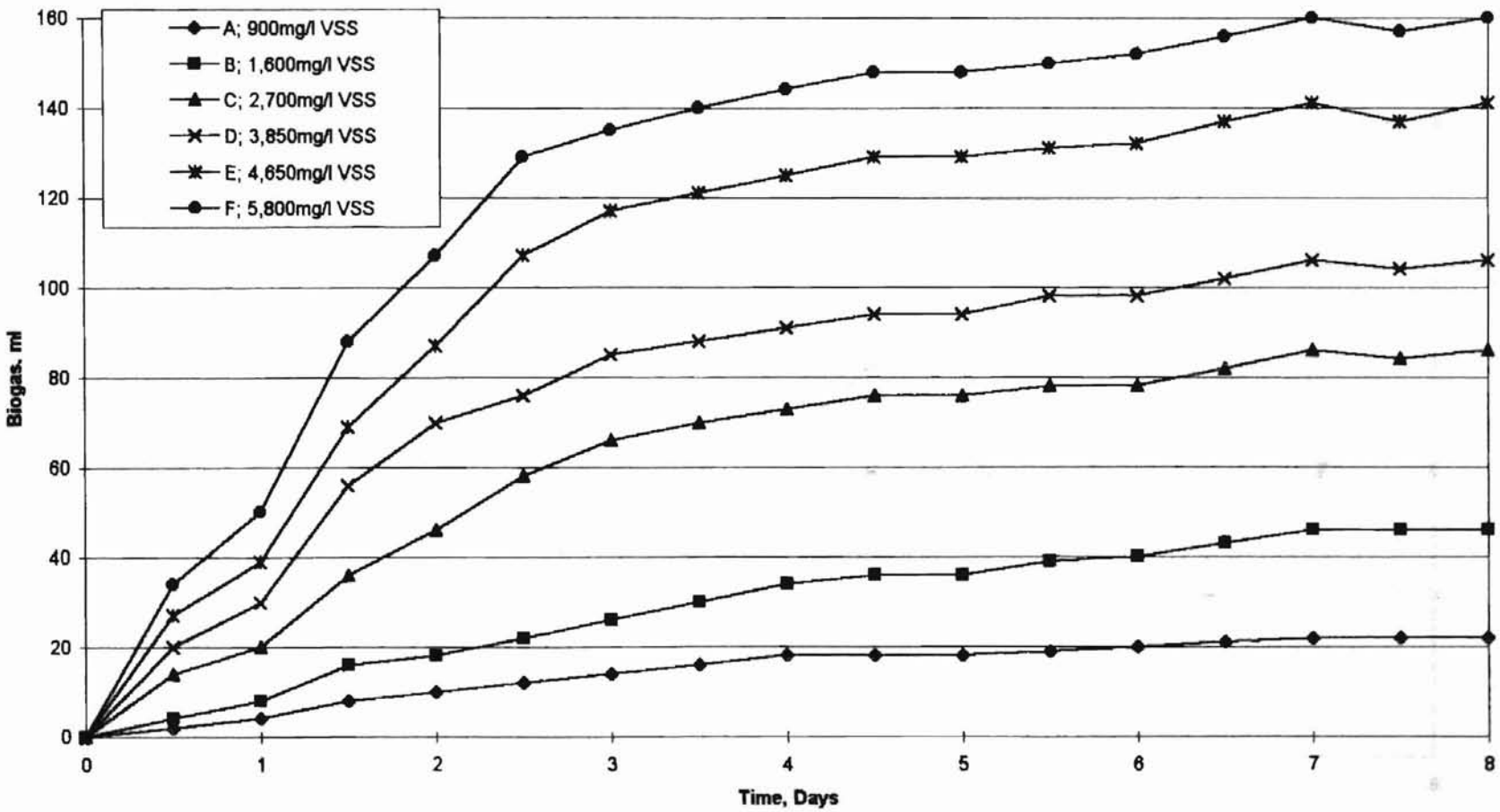

Figure 4. Cumulative Gas Curves For Experiment 1; Test A, VSS Determination at a F/M of 0.25

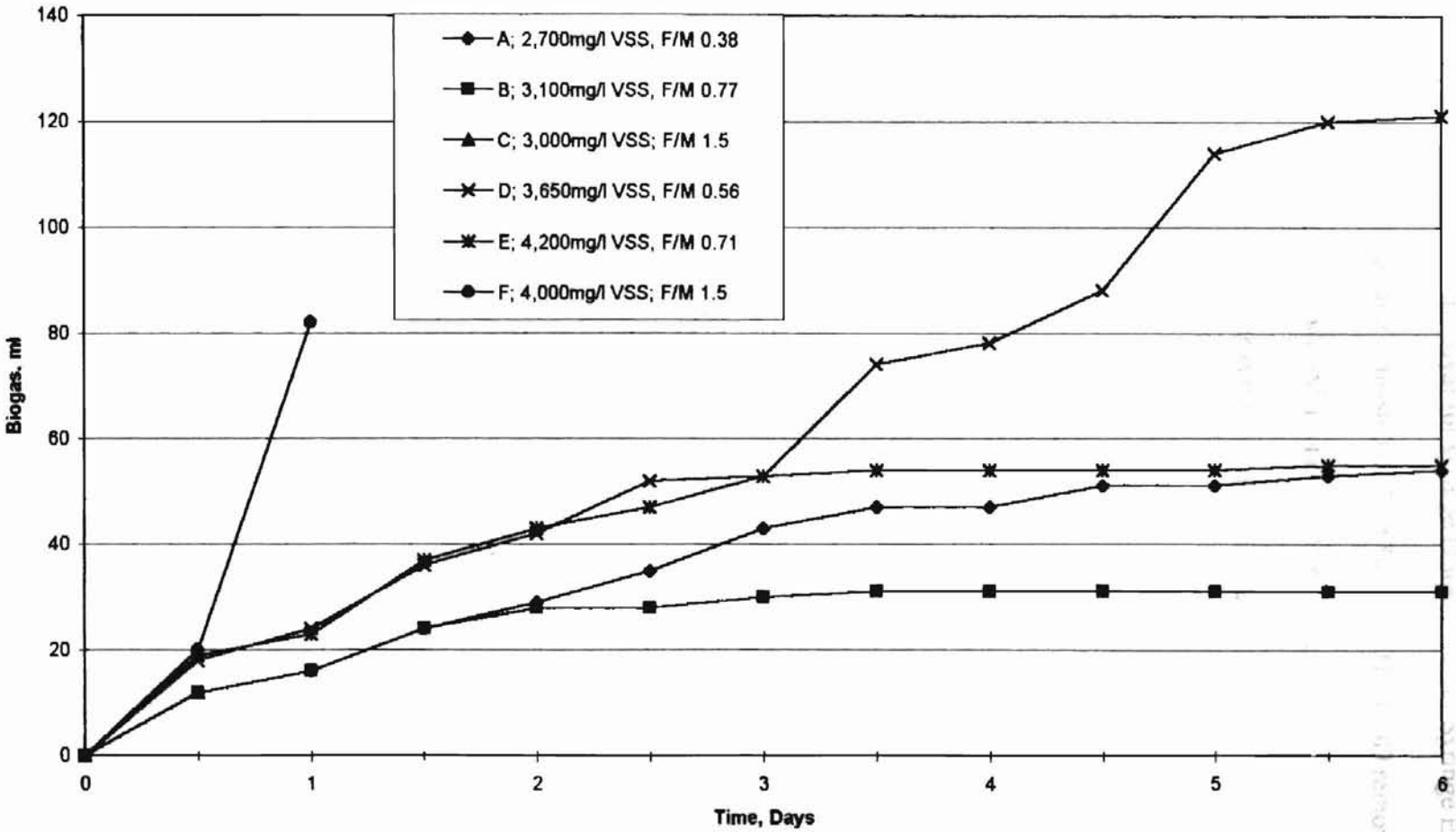

Figure 5. Cumulative Gas Curves For Experiment 1; Test B, F/M ratio Determination

removal for syringe C was 95.2 %. Syringe D produced a total of 108 ml of gas, which contained 24.2 % CO_2. The COD removal for syringe D was 95.7 %. Syringe E produced a total of 105 ml of gas and consisted of 22.8 % CO_2. The COD removal for syringe E was 85.7 %. Syringe F produced a total of 105 ml of gas, which was comprised of 22.3 % CO_2. The COD removal for syringe F was 95.7 %.

Test B

Syringe performance summaries for Experiment 2; Test B are listed in Table 7. Cumulative gas production curves are presented in Figure 7. All syringes had a VSS concentration of 3,650 mg/l and an F/M ratio of 0.52 mg COD/mg VSS. Syringe A produced a total of 103 ml of gas, which was comprised of 30.3 % CO_2. The COD removal for syringe A was 94 %. Syringe B produced a total of 98 ml of gas, of which 30.3 % was CO_2. The COD removal for syringe B was 95 %. Syringe C produced a total of 105 ml of gas, which was comprised of 31.5 % CO_2. The COD removal for syringe C was 95 %. Syringe D produced a total of 100 ml of gas, with a 28.5 % CO_2 content. The COD removal for syringe D was 95 %. Syringe E produced a total of 104 ml of gas, which was comprised of 28 % CO_2. The COD removal for syringe E was 95 %. Syringe F produced a total of 103 ml of gas, which was comprised of 28.4 % CO_2. The COD removal for syringe F was 95.7 %.

Table 7. Final Conditions for Experiment 2; Test A and B Variability/Reproducibility

Parameter	Syringe A	B	C	D	E	F
Test A						
Final Conditions						
Total Gas (ml)	108	110	104	108	105	105
CODr (%)	95	95	95	96	86	96
Alkalinity (mg/l)^	1,850	1,700	1,611	1,700	1,750	1,750
pH (s.u.)	6.9	7.0	7.0	7.0	7.0	7.0
CO_2 (%)	24	25	23	24	23	22
Test B						
Final Conditions						
Total Gas (ml)	103	98	105	100	104	103
CODr (%)	94	95	95	95	95	96
Alkalinity (mg/l)^	1,800	1,850	1,700	1,750	1,750	1,850
pH (s.u.)	7.0	6.9	6.9	7.0	7.0	7.0
CO_2 (%)	30	30	32	29	28	28

^ Expressed as $CaCO_3$

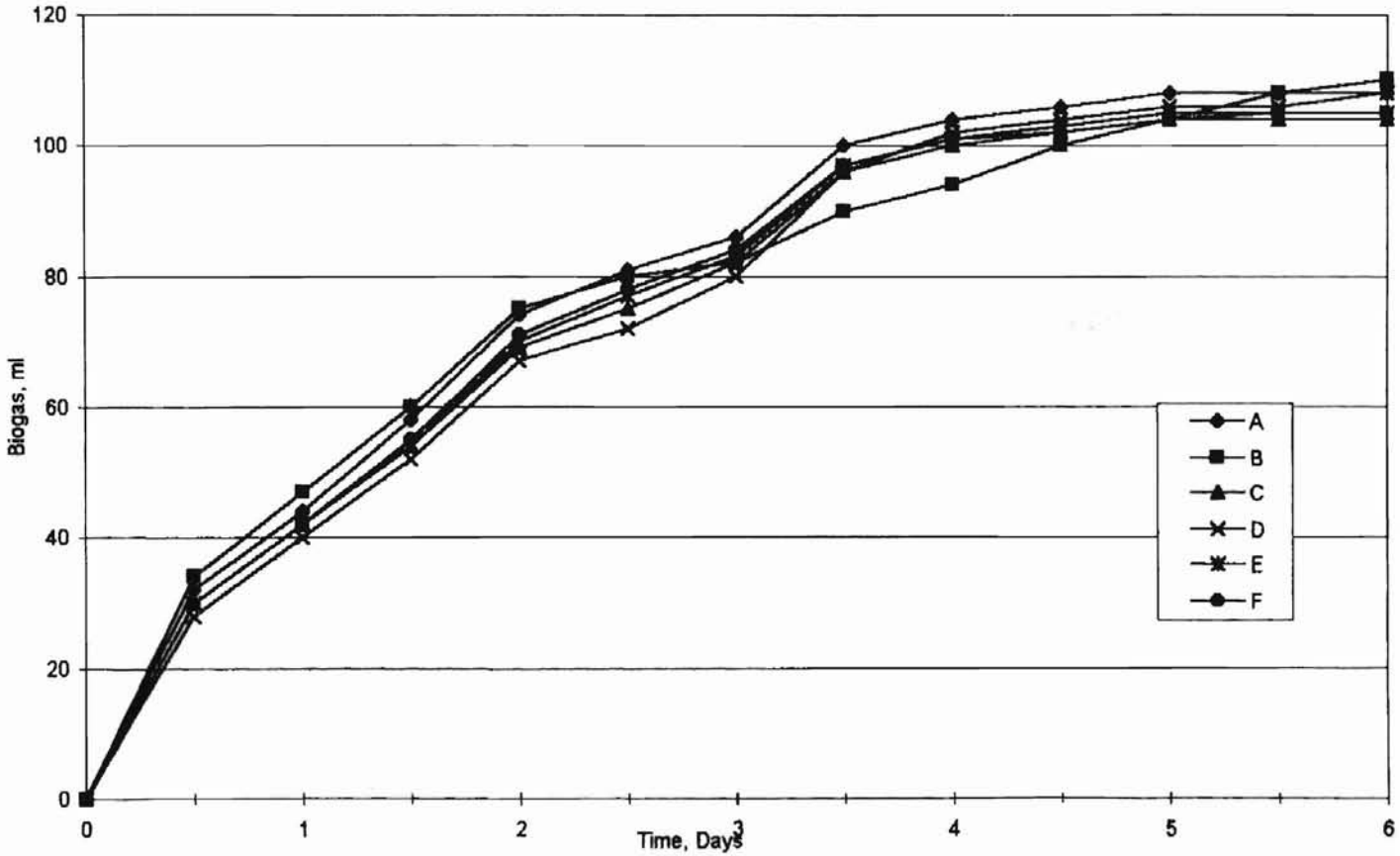

Figure 6. Cumulative Gas Curves For Experiment 2; Test A, Variability/Reproducibility at 4,100 mg/l VSS, F/M ratio of 0.52

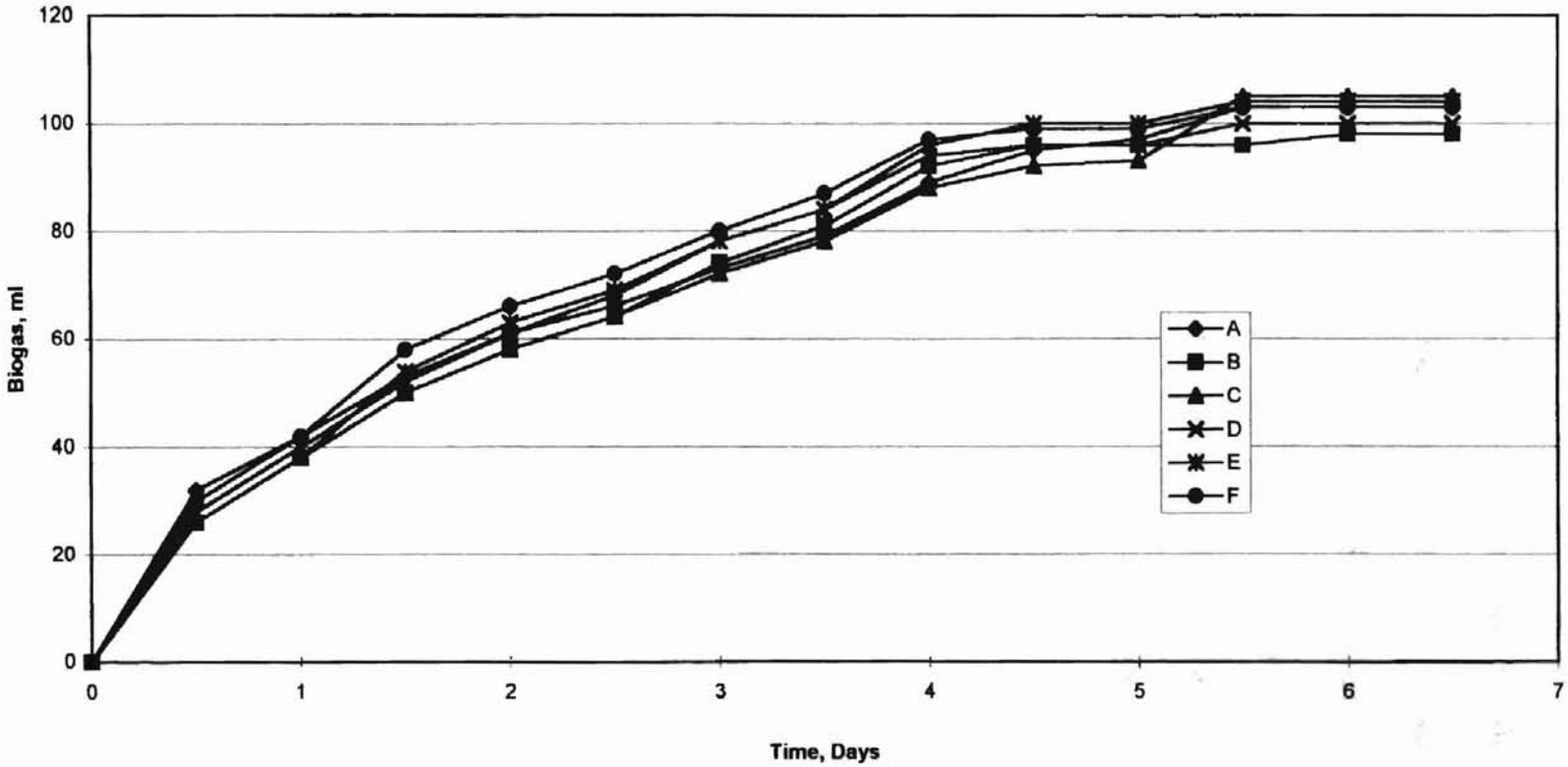

Figure 7. Cumulative Gas Curves for Experiment 2; Test B, Variability/Reproducibility at 3,650 mg/l VSS, and F/M Ratio of 0.56

Experiment 3

The objective for the third experiment was to evaluate the effect of mixing conditions versus static conditions. Operating conditions were the same for all syringes, with an F/M ratio of 0.56 mg COD/mg VSS and a VSS concentration of 3,575 mg/l. Results of the mixed syringes, A, C, and E, will be presented first, static syringes B, D, and F, will follow. Syringe performance summaries of Experiment 3 are presented in Table 8. Cumulative gas production curves are presented in Figure 8.

Mixed Syringes

Syringe A produced a total of 99 ml of gas, which was comprised of 23 % CO_2. The COD removal for syringe A was 96 % and the gas production rate was 0.86 L/g COD removed. Syringe C produced a total of 99 ml of gas, consisting of 22.6 % CO_2. The COD removal for syringe B was 92.5 % and the gas production rate was 0.89 L/g COD removed. Syringe E produced a total of 92 ml of gas, with the biogas containing 22.6 % CO_2. The COD removal for syringe E was 95 % and the gas production rate was 0.81 L/g COD removed.

Static Syringes

Syringe B produced a total of 94 ml of gas, which was comprised of 26 % CO_2. The COD removal for syringe B was 95 % and the gas production rate was 0.82 L/g COD

removed. Syringe D produced a total of 95 ml of gas, with a content of 24.5 % CO_2. The COD removal for syringe D was 94 % and the gas production rate was 0.84 L/g COD removed. Syringe F produced a total of 92 ml of gas, and consisted 26 % CO_2. The COD removal for syringe F was 94.5 % and the gas production rate was 0.81 L/g COD removed.

Table 8. Final Conditions of the Experiment 3; Static and Mixed Conditions.

	Mixed Syringes			Static Syringes		
Parameter	**A**	**C**	**E**	**B**	**D**	**F**
Test A						
Total Gas (ml)	99	99	92	94	96	92
CODr (%)	96	93	95	95	94	95
Alkalinity (mg/l)^	2,000	1,800	1,800	1,887	1,850	1,850
pH (s.u.)	7.0	7.0	7.0	6.8	6.9	7.0
CO_2 (%)	23	23	23	25	25	26

^ Expressed as $CaCO_3$

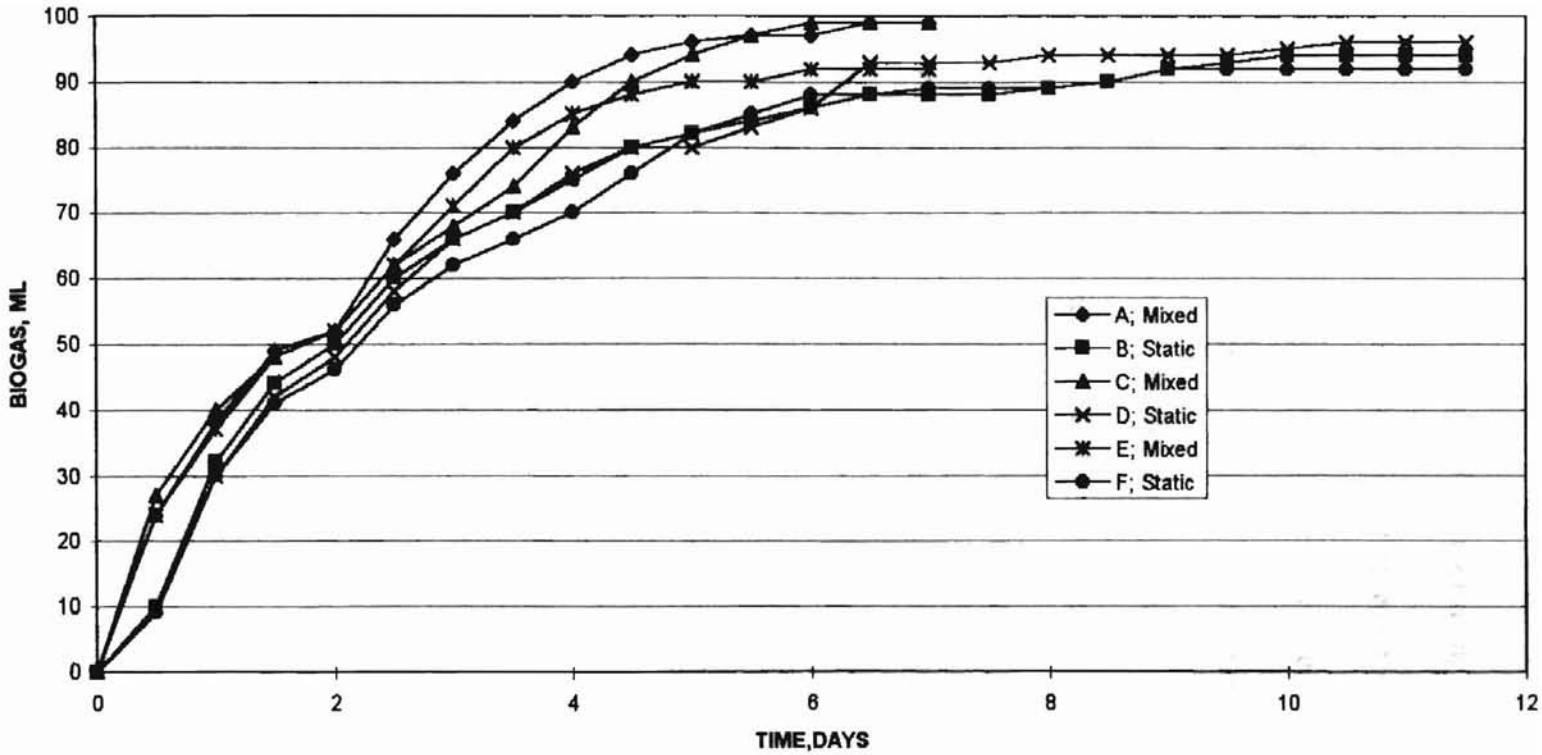

Figure 8. Cumulative Gas Curves for Experiment 3; Static and Mixed at 3,750 mg/l VSS and F/M Ratio of 0.56

Experiment 4; Tests A and B

Test A

Syringe performance summaries for Experiment 4; Test A are presented in Table 9. Cumulative gas production curves are presented in Figure 9. The objective of Experiment 4 was to apply the method in an evaluation of ammonia-nitrogen toxicity. Test A showed no reduction in total gas produced compared to the control syringe, however the gas production rate was inhibited. Syringe A was operated as a control and was fed 250 mg/l NH_3-N to ensure reactor failure was not caused by insufficient nitrogen. The COD removed from the control syringe was 97% with an average 28% CO_2 content in the biogas and with a prodcution of 74 ml of biogas. Syringe B was fed approximately 400 mg/l NH_3-N. The COD removal was 87% with an average 27% CO_2 content in the biogas. Gas production was 105% of the control. Syringe C was fed approximately 800 mg/l NH_3-N. The biogas was 27% CO_2 and the gas production rate was 97% of the control. Syringe D was fed approximately 1,310 mg/l NH_3-N. The biogas was 29% CO_2 and the gas production rate was 99% of the control. Syringe E was fed approximately 1,560 mg/l NH_3-N. The biogas was 30% CO_2 and the gas production rate was 99% of the control. Syringe F was fed approximately 1,800 mg/l NH_3-N. The biogas was 29% CO_2 and the gas production rate was 97% of the control.

Test B

Syringe performance summaries for Experiment 4; Test B are presented in Table 9. Cumulative gas production curves are presented in Figure 10. Test B was set up to extend the range of concentration of NH_3-N. TOC analysis was substituted for the COD analysis for Test B due to interference's from chloride. A significant reduction in total gas produced over the control was observed. Toxicity was initiated in syringe B and toxic responses were observed with syringes C through F. Syringe A was operated as a control and was fed 238 mg/l NH_3-N to ensure reactor failure was not caused by insufficient nitrogen. The TOC removed for the control syringe was 94% with an average 20% CO_2 content in the74 ml of biogas produced. The average biogas production rate was 0.29 L/g TOC removed • day. Syringe B was fed approximately 2,550 mg/l NH_3-N. The TOC removal was 42% with an average 40% CO_2 content in the biogas. Gas production was 66% of the control and the biogas production rate was 0.25 L/g TOC removed • day. Syringe C was fed approximately 3,036 mg/l NH_3-N. The TOC removal was 22% with an average 43% CO_2 content in the biogas. Gas production was 51% of the control and the biogas production rate was0.29 L/g TOC removed • day. Syringe D was fed approximately 3,566 mg/l NH_3-N. The TOC removal was 0% with an average 42% CO_2 content in the biogas. Gas production was 33% of the control and the biogas production rate was 0 L/g TOC removed since final TOC was higher than initial. Syringe E was fed

approximately 3,825 mg/l NH_3-N. The TOC removal was 2.4% with an unknown CO_2 content in the biogas (gas volume was too low to measure). Gas production was 38% of the control and the biogas production rate was 0.33 L/g TOC removed • day. Syringe F was fed approximately 4,350 mg/l NH_3-N. The TOC removal was 16% with an unknown CO_2 content in the biogas (gas volume was too low to measure). Gas production was 32% of the control and the biogas production rate was 0.32 L/g TOC removed • day.

Table 9. Final Conditions for Experiment 4; Tests A and B NH_3-N Toxicity Assay

	Syringe					
Parameter	**A**	**B**	**C**	**D**	**E**	**F**
Test A						
Final Conditions						
Total Gas (ml)	74	78	72	73	73	72
CODr (%)	-	-	-	-	-	-
Alkalinity (mg/l)^	1,820	1,920	1,900	1,940	1,920	1,880
pH (s.u.)	7.0	6.9	6.9	7.0	7.0	7.0
CO_2 (%)	28	27	27	29	30	29
NH_4Cl (mg/l)	195	262	825	1,125	1,438	1,750
VFA (mg/l)	40	50	50	50	50	50
Test B						
Final Conditions						
Total Gas (ml)	74	49	38	25	28	24
TOCr (%)	94	42	23	0	2.4	15.5
Alkalinity (mg/l)^	1,666	1,600	1,650	1,650	1,600	1,750
pH (s.u.)	6.8	6.5	6.4	6.4	6.2	6.2
CO_2 (%)	20	40	43	42	*	*
NH_3-N (mg/l)	238	2,550	3,036	3,566	3,825	4,350
VFA (mg/l)	110	532	660	735	735	750

- COD was not measurable

* Gas volume too low to measure

^ Expressed as $CaCO_3$

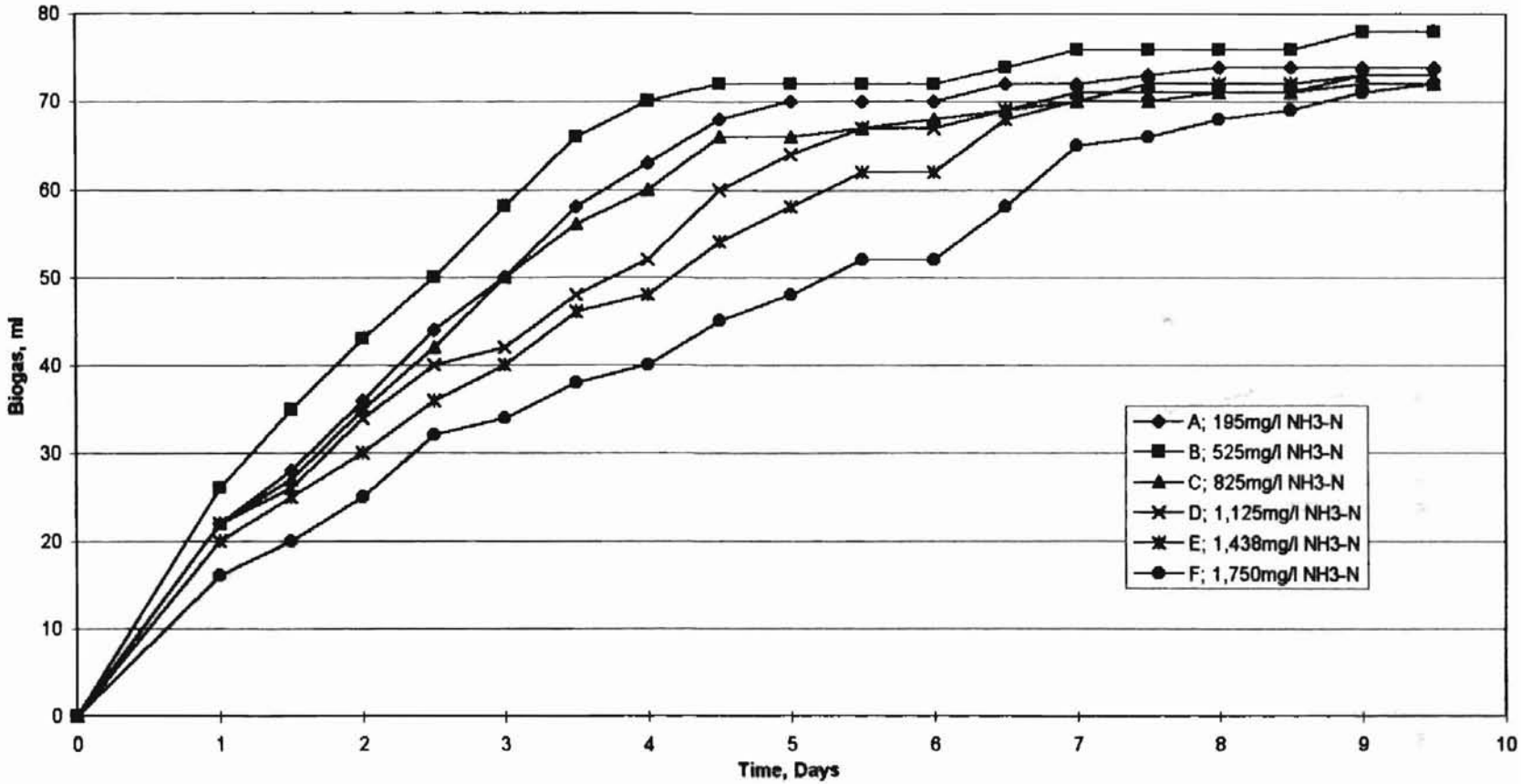

Figure 9. Cumulative Gas Curves for Experiment 4; Test A, NH_3-N Toxicity Assay

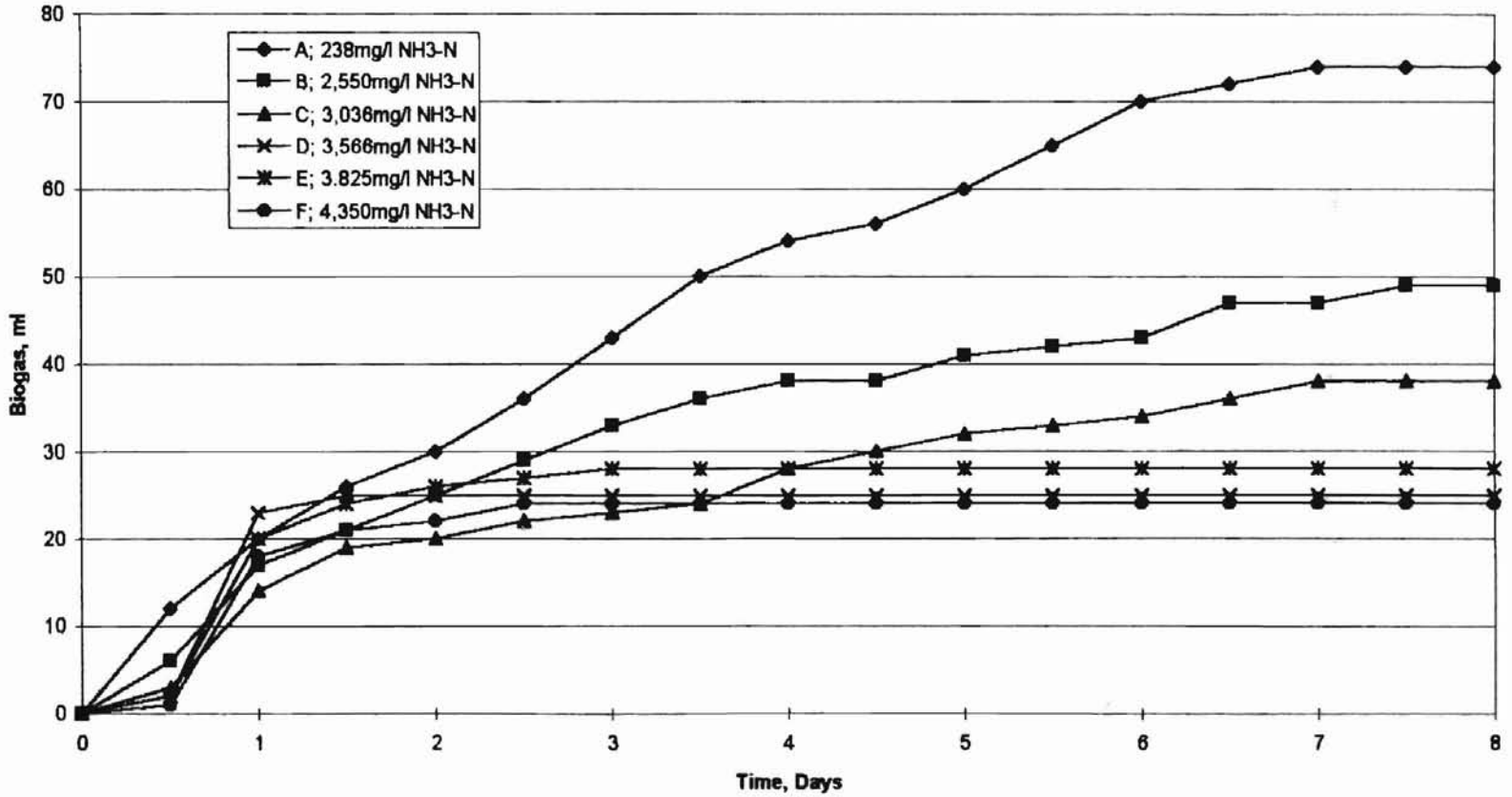

Figure 10. Cumulative Gas Curves For Experiment 4; Test B, NH_3-N Toxicity Assay

Experiment 5; Tests A and B

Test A

The syringe performance summaries for Experiment 5; Test A are presented in Table 10. Cumulative gas production curves are presented in Figure 11. The objective of Experiment 5 was to apply the method in an evaluation of nickel toxicity. A significant observation in Test A was lag period associated with the four largest concentrations of nickel. The lag period increased with increasing concentrations of nickel. Syringe A was operated as a control and was fed 3.3 μg/l nickel to ensure reactor failure was not caused by insufficient nickel, which is less than the micronutrient concentrations fed by Parkin *et al.* (1982). However, micronutrients (including nickel) were fed in the process from which the seed was collected. The COD removed for the control syringe was 93% with an average 33% CO_2 content in the biogas. The average biogas production rate was 0.88 L/g COD removed • day. Syringe B was fed approximately 11 mg/l nickel. The COD removal was 95% with an average 35% CO_2 content in the biogas. Gas production was 106% of the control and the biogas production rate was 0.096 L/g COD removed • day. Syringe C was fed approximately 45 mg/l nickel. The COD removal was 81% with an average 42% CO_2 content in the biogas. Gas production was 91% of the control and the biogas production rate was 0.095 L/g COD removed • day. Syringe D was fed approximately 90 mg/l nickel.

The COD removal was 25% with an average 54% CO_2 content in the biogas. Gas production was 61% of the control and the biogas production rate was 0.21 L/g COD removed • day. Syringe E was fed approximately 136 mg/l nickel. The COD removal was 25% with an average 56% CO_2 content in the biogas. Gas production was 52% of the control and the biogas production rate was 0.19 L/g COD removed • day. Syringe F was fed approximately 181 mg/l nickel. The COD removal was 25% with an average 52% CO_2 content in the biogas. Gas production was 63% of the control and the biogas production rate was 0.26 L/g COD removed • day.

Test B

The syringe performance summaries for Experiment 5; Test A are presented in Table 10. Cumulative gas production curves are presented in Figure 12. The range of nickel was extended in Test B. Syringe C was spiked with Nickel to 226 mg/l 3.5 days into the test to determine if the lag period was created by either precipitation reactions or by acclimation of the inoculum to nickel. Syringe A was operated as a control and was fed 3.3 μg/l nickel to ensure reactor failure was not caused by insufficient nickel. The COD removed for the control syringe was 92% with an average 31% CO_2 content in the biogas. The average biogas production rate was 0.93 L/g COD removed • day. Syringe B was fed approximately 90 mg/l nickel. The COD removal was 22% with an average 42%

CO_2 content in the biogas. Gas production was 64% of the control and the biogas production rate was 0.32 L/g COD removed • day. Syringe C was fed approximately 136 mg/l nickel. The COD removal was 20% with an average 46% CO_2 content in the biogas. Gas production was 37% of the control and the biogas production rate was 0.2 L/g COD removed • day. Syringe D was fed approximately 181 mg/l nickel. The COD removal was 20% with an average 59% CO_2 content in the biogas. Gas production was 31% of the control and the biogas production rate was 0.16 L/g COD removed • day. Syringe E was fed approximately 226 mg/l nickel. The COD removal was 20% with an average 46% CO_2 content in the biogas. Gas production was 46% of the control and the biogas production rate was 0.23 L/g COD removed • day. Syringe F was fed approximately 271 mg/l nickel. The COD removal was 30% with an average 53% CO_2 content in the biogas. Gas production was 32% of the control and the biogas production rate was 0.13 L/g COD removed • day.

Table 10. Final Operating Conditions for Experiment 5; Tests A and B Nickel Toxicity Assay

Parameter	Syringe A	B	C	D	E	F
Test A						
Final Conditions						
Nickel (mg/l)*	-	11	45	90	136	181
Total Gas (ml)	87	93	79	53	45	55
CODr (%)	93	95	81	25	25	25
Alkalinity (mg/l)^	2,150	2,200	2,150	1,650	1,450	1,600
pH (s.u.)	7.0	7.0	7.0	6.5	6.3	6.4
CO_2 (%)	33	35	42	54	56	52
VFA (mg/l)	120	100	130	540	735	615
Test B						
Final Conditions						
Nickel (mg/l)*	-	90	136	181	226	271
Total Gas (ml)	98	63	36	30	45	31
CODr (mg/l)	92	22	20	20	20	30
Alkalinity (mg/l)^	2,114	1,583	1,600	1,621	1,621	1,568
pH (s.u.)	6.9	6.2	6.34	6.3	6.5	6.4
CO_2 (%)	31	42	46	59	46	53
VFA (mg/l)	80	540	435	465	420	375

* Represents the concentration of nickel fed at the beginning of the test

^ Expressed as $CaCO_3$

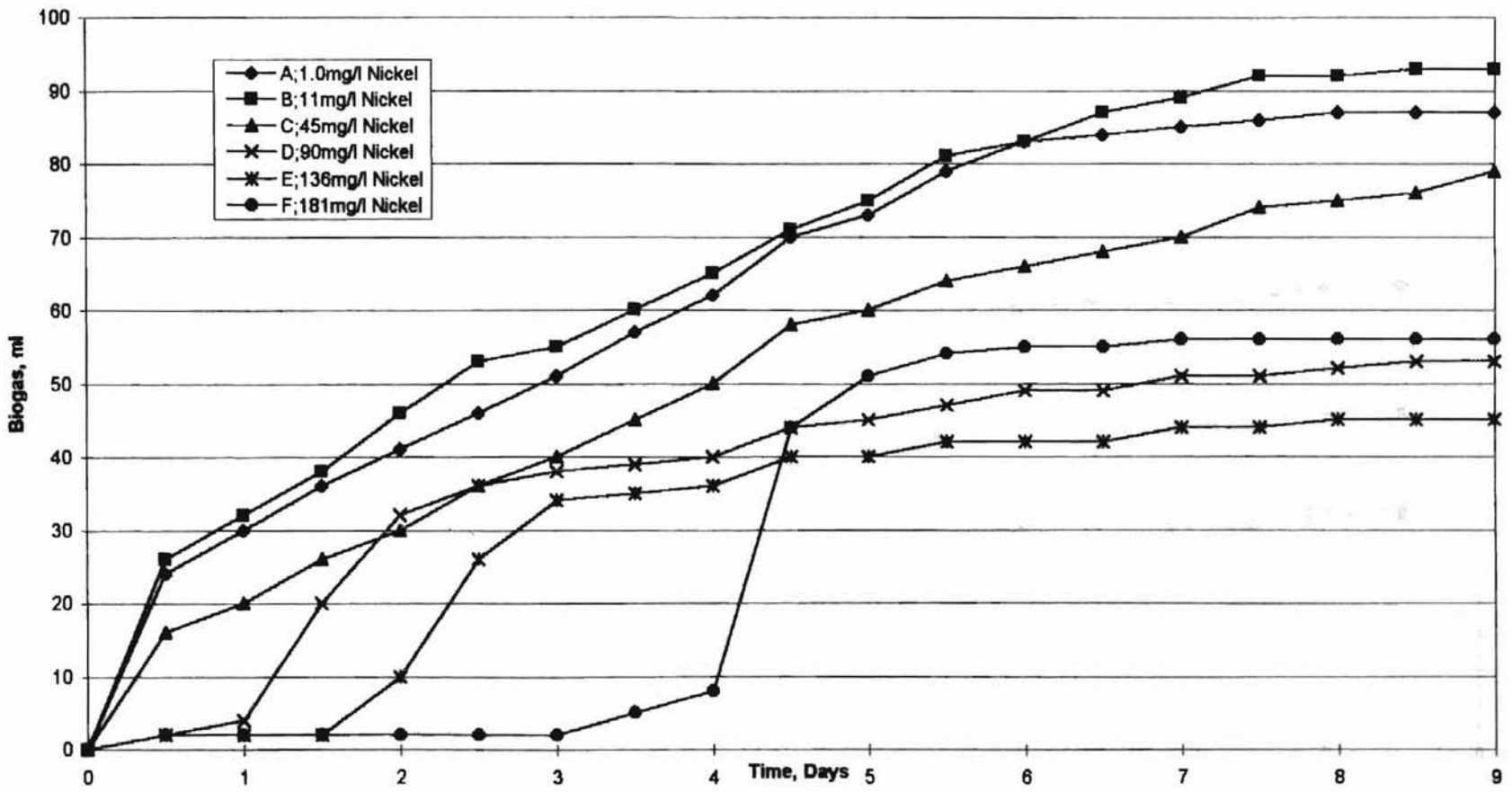

Figure 11. Cumulative Gas Curves for Experiment 5; Test A, Nickel Toxicity Assay

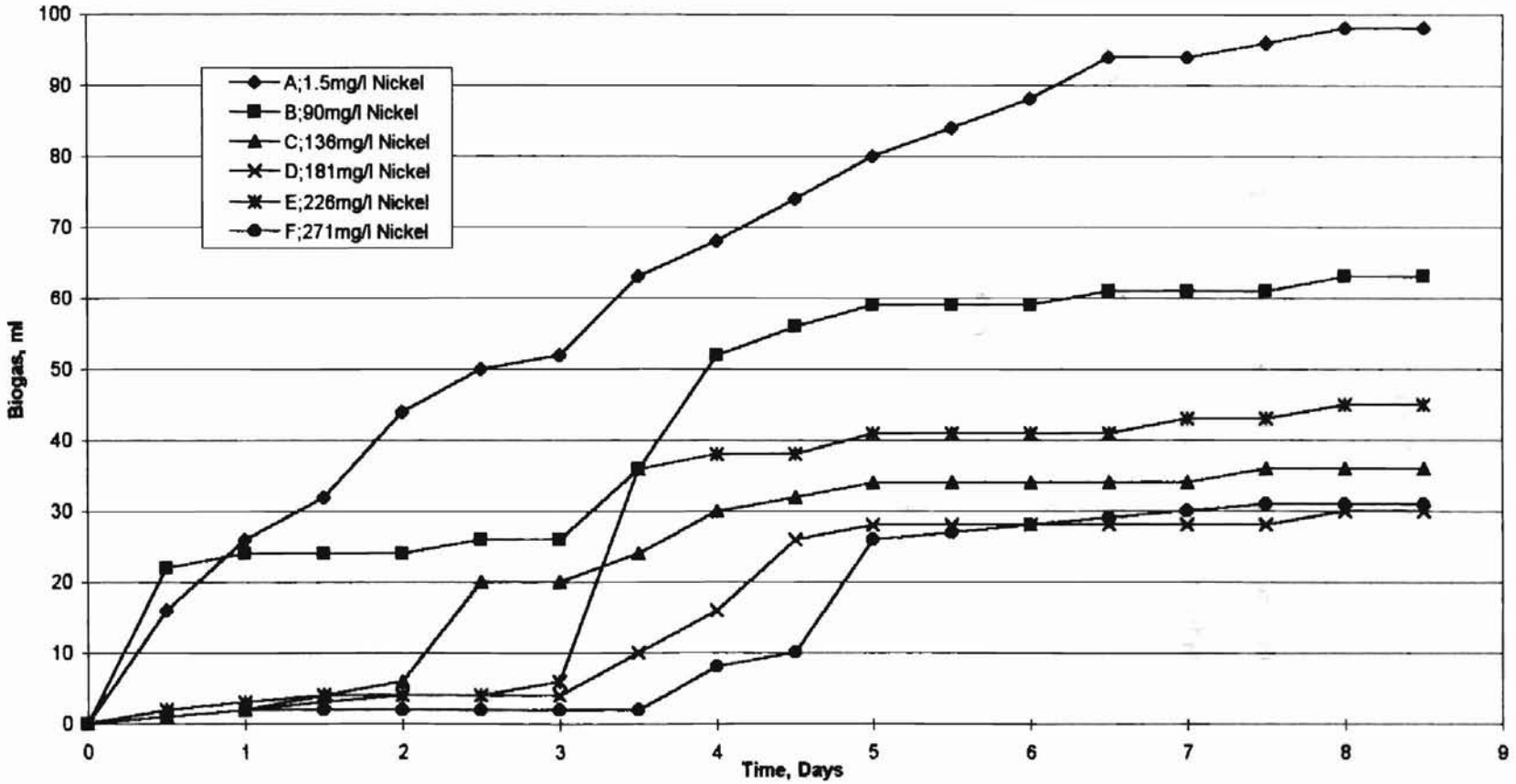

Figure 12. Cumulative Gas Curves for Experiment 5; Test B, Nickel Toxicity Assay

CHAPTER V

DISCUSSION

Discussions on the results of this study are presented in three sections. The first section discusses the syringe methodology. The objectives in this section were to determine optimum range for VSS loading, determine the optimum range of F/M ratios, evaluate variability and reproducibility and evaluate the effect of mixing versus static conditions. The objective of the second section was to apply the syringe method in evaluating ammonia-nitrogen toxicity. The third section applied the syringe methodology in evaluating nickel toxicity.

Syringe Methodology

The results of Test A experiment one are presented in Table 6 and Figure 4. In test A, syringes were loaded with varied VSS concentrations and an F/M ratio near 0.25 mg COD/mg VSS. Gas production increased with the increase in VSS concentrations and organic load. The syringes loaded with 5,750 and 4,600 mg/l VSS required numerous gas evacuations the first five days. Gas was evacuated three times for the syringe with 3,850 mg/l VSS, twice for the syringe with 2,800 mg/l VSS and gas was not evacuated from the two lowest concentrations. The two highest concentrations required frequent inspections to

prevent expulsion of the plunger. The time required for inspecting the two syringes made the VSS loads impractical. The gas production rate was slow for syringes with 900 and 1,600 mg/l VSS, requiring three days to produce enough gas for analysis, and subsequent gas production was not adequate for additional gas analysis. A larger total volume of gas would be more beneficial for distinguishing between responses to toxicity or treatability and variability among the syringes. The VSS loads of 2,800 and 3,600 mg/l VSS required a maximum of three evacuations and produced volumes of gas adequate for gas analysis. The optimum range of VSS for this test sludge was determined to be 3,000 to 4,000 mg/l. Gas production rates above 4,000 mg/l VSS were impractical to monitor and below 2,000 mg/l VSS gas production was too low.

The results of Test B are presented in Table 6 and Figure 5. Three F/M ratios near 0.5, 1.0 and 1.5 were evaluated with VSS concentrations around 3,000 to 4,000 mg/l. Gas production rates were too high for syringes with F/M ratios near 1.5, as seen in Figure 5. These syringes required constant monitoring and were terminated the first day. Results of the chemical analyses presented in Table 6 indicated that the syringes with F/M ratios near 1.0 failed. The effluent pH was below 6.0, VFA's were over 1,200 mg/l, CO_2 comprised nearly 50 percent of the gas and no COD was removed. Reactor failure may be due to inadequate buffering. Initial alkalinity's for syringes B and E were 760 and 980 mg/l as $CaCO_3$, respectively. Brooks *et al.* (1994) did not encounter reactor

failure at an F/M near 1.0 with an initial alkalinity near 1,400 mg/l as $CaCO_3$. However, percent COD removal was lower under these conditions. Syringes with an F/M ratio near 0.5 were easily monitored and produced adequate volumes of gas for analysis. The final VFA's were 300 mg/l in syringe A, with an initial alkalinity of 990 mg/l, where syringe D had a final VFA of 100 mg/l and an initial alkalinity of 1,322 mg/l as $CaCO_3$. Percent COD removed (CODr) was much higher in syringe D than syringe A. The difference in CODr may be explained by the difference in the F/M ratio. The F/M ratio was higher in syringe D providing the bacteria with a greater amount of substrate. Buffers were not initially added since the anaerobic seed source had an alkalinity of 2,570 mg/l. Phosphate buffer (KH_2PO_4) was however added in subsequent experiments. The optimum range for the F/M ratio was determined to be 0.5 to 1.0.

Gas production is an indication of the health of anaerobic bacteria and was monitored to determine the effects of a chemical component or waste stream. One advantage of syringes was the variable time scale over which the gas production may be monitored, daily or hourly. However, differentiating between the levels in the severity of toxic responses and the variability of the syringes must be understood. If variability of the syringes is minimal, an accurate threshold inhibition concentration may be determined. This information can be beneficial for trouble shooting tests, process control and developing data for large scale treatability studies or full scale operation assistance. The objective of the

third experiment was to evaluate the variability and reproducibility of the syringes used in this study. Two tests were duplicated to evaluate variability and reproducibility. Variability among the syringes was low in terms of total gas produced and gas production rates, as seen in Figures 6 and 7. Also, the results of the % CO_2 analysis in Table 7 showed a minor amount of variability. Total gas production from the two tests were combined to calculate the mean, standard deviation, standard error and 95 % confidence interval. These results are summarized in Table 11. The standard deviation was 3.3 ml, which was 1.3 ml greater than the smallest measurable unit of 2.0 ml. The data indicate that the variability among the syringes was low. Syringe B exhibited the greatest variability with gas measurement of 6 ml above and below the mean of 104 ml. Additional tests should be conducted to further investigate and refine estimates of variability and reproducibility. An easy method for evaluating variability is to use a control chart as presented in Figure 13. This figure was developed from the statistical results presented in Table 11. The control chart illustrated the trends of duplicate tests by plotting the mean and one standard deviation of total gas production of the duplicate tests. The objective of the control chart was to monitor the performance for the syringes. If the gas production of a syringe was outside the acceptable range of variability, (i. e. one or two standard deviations) the syringe or the plunger may need to be replaced, other potential causes in variation may be human error. Duplicate tests should be run periodically or before a treatability test is initiated to develop an accurate control chart. Results

of duplicate tests in this study indicate minimal variability, with a standard deviation of 3.3 ml. In addition to monitoring syringes, the control chart may serve as a quality control chart and illustrate the treatability of a waste water or the toxicity of a chemical. For example, once several syringe tests have been completed at a defined VSS and F/M ratio with a biodegradable substrate, such as sucrose, a well defined standard deviation can be defined. Gas production in the test reactors may be compared to the standard deviation to determine the degree of toxicity or the biodegradability of a waste water relative to sucrose. Also, gas production in liters of gas produced per gram of COD removed can be calculated for total gas volume to assist in evaluating the treatability or toxicity of a waste water or chemical compound. A quality control assessment may be administered using the control chart. For example, if the control syringe is ubiquitously outside the desired standard deviation from the mean, an error in the set up procedure may have occurred.

A previous study with this type of syringe utilizing intermittent mixing had individual test runs which lasted up to two to three weeks (Brooks et al., 1994). An objective of this study was to minimize the test period and reduce variability by continuously mixing the syringes. A comparison study of three static syringes and three continuously mixed syringes showed a reduction in the duration of the test. The test duration for the static syringes exceeded the continuously mixed syringes by five days. The total volume of gas produced was

Table 11. Statistical Evaluation of the Comparison of Static and Mixing Conditions

	Total Gas Production (ml)
First Test	108
	110
	104
	108
	105
	105
Second Test	103
	98
	105
	100
	104
	103
Combined Test One and Two	
Sum	1253
Count	12
Statistical Results	
Standard Div.	3.3
Standard Error	0.96
Mean	104.4

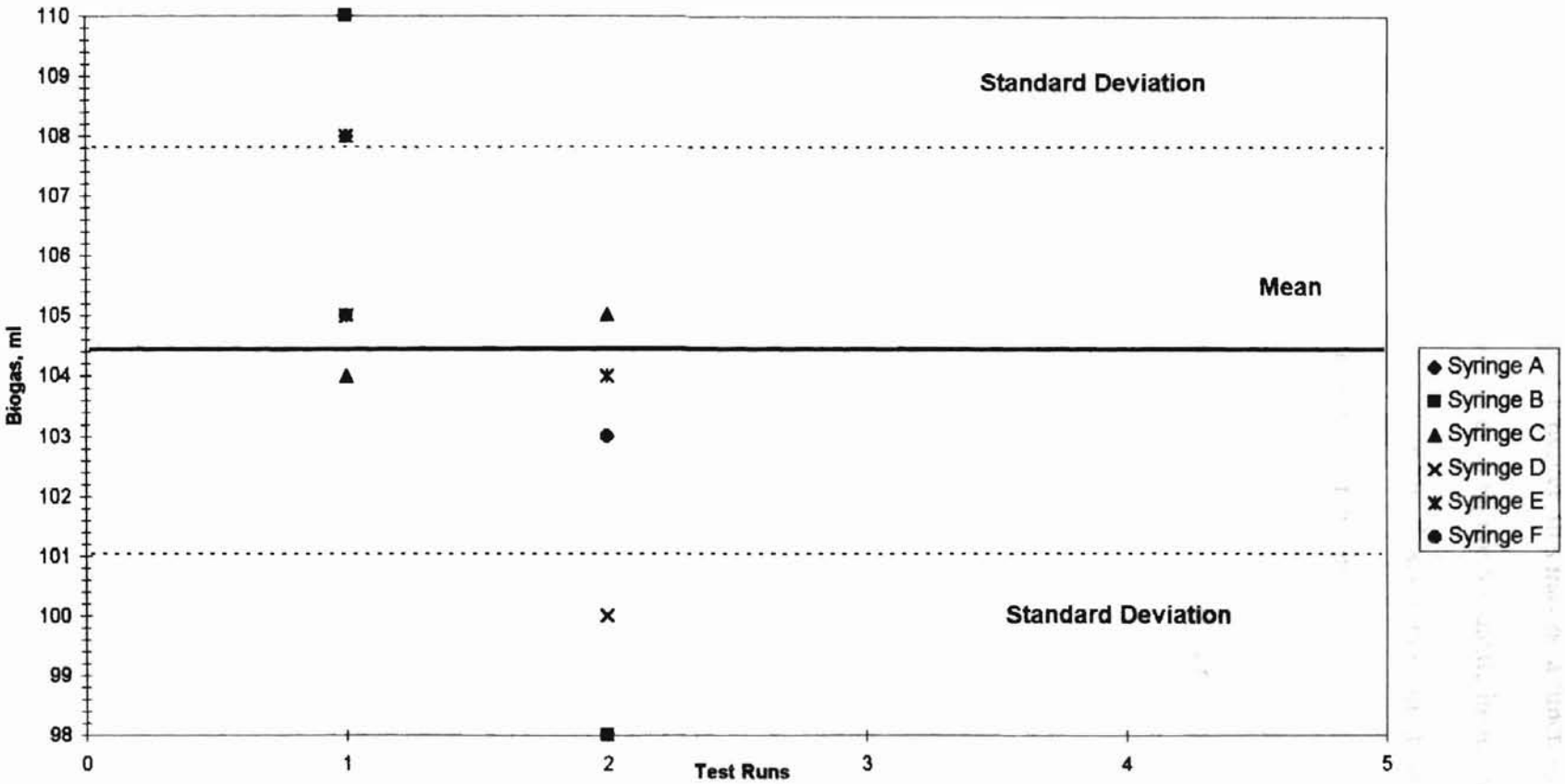

Figure 13. Control Chart For Monitoring Syringe Variability And Reproducibility

equal or less in the static syringes; however, this may be a function of greater variability. The cumulative gas curves in Figure 8 indicate lower gas production rates up to two days with the static syringes. The mixing conditions may have increased contact between the anaerobic organisms and the sucrose. The mixing conditions improved (increased) gas production rates. A similar study should be performed with toxicants to further evaluate the effect of mixing.

Ammonia-Nitrogen Toxicity Tests

Test A

The objective of this phase of the study was to review the syringe method in evaluating ammonia-nitrogen toxicity. Ammonia-nitrogen concentrations up to 200 mg/l have been shown to have a beneficial effect and no detrimental effect up to 1000 mg/l (McCarty, 1964). McCarty's observation was not observed in this study. Cumulative gas curves in Figure 9 suggest that ammonia-nitrogen concentrations in the range of 800 to 2,000 mg/l slowed the rate of gas produced. Final gas produced after 9.5 days for all syringe conditions were within the one standard deviation of syringe variability (developed from the control chart) and the control syringe. The gas production rate and final volume of gas produced in syringe B (262 mg/l NH_3-N) was greater than the control by 4 ml. However, syringe B exhibited the widest range in variability and reproducibility in

Experiment 2. Thus, 4 ml difference in gas produced may be inconclusive. Syringe C (825 mg/l NH_3-N) gas production rate was the same as the control, suggesting that ammonia-nitrogen concentrations above 200 mg/l may be beneficial with no observed effect up to 800 mg/l. However, this study indicates gas production rates were slowed between 1,000 mg/l and 2,000 mg/l NH_3-N, as seen in Figure 5. Ammonia-nitrogen concentrations of approximately 1,000 to 2,000 mg/l reduced gas production rates, requiring five additional days to reach the total volume of the control syringe. Parkin *et al.* (1983) did not observe rate inhibition until 7,500 mg/l NH_3-N under batch conditions. Gas production rates decreased slowly with increasing ammonia-nitrogen concentration from 300 to 600 mg/l under batch conditions in a study performed by van Velsen (1979).

Gas production rates were affected but the final volume of gas produced was similar in all syringe conditions for test A. Observations from other researchers suggest that bacteria are capable of becoming acclimated to the ammonia-nitrogen (Parkin *et al.*, 1983; Parkin and Miller, 1982). Under semi-continuous feed assays Parkin and Miller (1983) observed the acclimation of methanogens to ammonia-nitrogen concentrations of 8,000 to 9,000 mg/l at a solids residence times of 50 days.

Test B

Ammonia-nitrogen concentrations were extended from approximately 2,500 to 5,000 mg/l to further investigate the affects of ammonia-nitrogen toxicity. Cumulative gas curves in Figure 10 illustrate the reduction of the gas production rate and total volume produced. Syringe B at 2,550 mg/l NH_3-N produced 66% of the control, exhibiting toxicity. Syringe C at 3,036 mg/l NH_3-N produced 51% of the control's total gas volume, which illustrates the 50% inhibition level described by Stucky *et al.* (1980). The remaining syringes produced approximately 30% of the control's gas volume, signifying severe toxicity. In addition to gas production, all other parameters indicated a negative effect above 2,500 mg/l NH_3-N. The results of this study are in agreement with McCarty (1964) where inhibitory responses were observed at 1,500 to 3,000 mg/l NH_3-N and toxic responses were seen at 3,000 mg/l NH_3-N and above, regardless of pH. Parkin and Miller (1982) concluded that concentrations of 8,000 to 15,000 mg/l NH_3-N are lethal at various temperatures and solids residence times. Under optimal conditions of 25°C and 50 day solids residence time, 8,000 mg/L NH_3-N were tolerable, without a decrease in gas production.

Final VFA's were 5 to 7 times greater than the control syringe in syringes B through F, as seen in Table 9. This observation suggest that the methanogens experienced toxicity. Methanogens convert acetate and H_2 to methane and carbon

dioxide. The majority of the high concentration of VFA's was assumed to be acetate. Gas analysis results indicated toxicity with approximately 40% CO_2 in all syringes, which was twice that of the control. Increase VFA's and above normal (20-30%) CO_2 suggest acidogenesis and acetogenesis reactions were the dominating reactions which overwhelmed the methanogens. Final pH for all syringes below 7.0, and as low as 6.2, indicating an increase in H_2 and VFA concentration from acidogenesis and acetogenesis and that NH^+_4 was the dominant species.

Nickel Toxicity Test

Test A

The objective of this phase of the study was to review the syringe methodology in evaluating nickel toxicity. In Test A, 11, 45, 90, 136 and 181 mg/l nickel was evaluated for toxicity. Syringe B, at 11 mg/l nickel, produced gas at a rate similar to the control, (refer to Figure 11), and showed no evidence of toxicity . Syringe C, at 45 mg/l nickel produced less gas and a greater percentage of CO_2 than the control. However, the next three syringes D, E and F corresponding to 90, 136 and 181 mg/l nickel demonstrated lag periods, (Figure 11). The 90 mg/l nickel concentration had a lag period of 1.0 day, the 136 mg/l had a lag period of 1.5 days and the 181 mg/l had a lag period of 3.5 to 4.0 days.

All three of these syringes produced a smaller volume of gas than the control. Other parameters such as, VFA's, percent CO_2 production and COD removal, showed signs of toxicity.

In the sequence of reactions that occur in anaerobic metabolism the first two reactions are hydrolysis and acidogenesis. A study by Ashley *et al.* (1982) analyzed the effect of nickel on the hydrolysis of certain substrates including starch, which is comparable to sucrose. They observed fluctuations in the populations of amylolytic (starch hydrolyzing) bacteria. Populations would decrease after each increasing inoculation of nickel. The same response was observed in acid producing bacteria. These observations may help explain the lag periods in this study. However, similar batch studies were performed by Parkin *et al.* (1983) and no lag periods were observed. However, the methodology was different in the Parkin study. Acetate was fed as the primary substrate, eliminating the hydrolysis step. Ashley *et al.* (1982) observed decreases in methane production after each initial inoculation of increasing nickel concentrations, but increasing methane production occurred after a long period of time. The acclimation period was much greater than the lag period observed in this study.

Similar observations were made in this study. Syringe F with the highest concentration of nickel (181mg/l), produced more gas than the proceeding two

concentrations of 90 and 136 mg/l Nickel and had lower final VFA's, suggesting than methanogens exhibit a greater toxic response at lower concentrations of nickel. This test was followed with Test B. Test B was set up similar to Test A, with duplicates and larger doses of nickel.

Test B

Test B was set up with a control syringe and 90, 136, 181, 226, 271 mg/l Nickel. Duplicates of Test A (90, 136 and 181 mg/l nickel) were set up to determine if the lag periods were reproducible. Cumulative gas curves (Figure 12) showed similar lag periods, except for syringe B at 90 mg/l nickel, which exhibited no lag period this time. Syringe B produced a light brown color after the first day and slowly became black, identical in color to the remaining syringes. Lag periods did not exceed 3.5 days in both tests. The total volume of gas produced in the duplicate syringes were different, with syringe B 10 ml higher and syringes C and D lower then the results of Test A by 9 and 25 ml, respectively.

One possible explanation for the lag periods has been discussed with reference to lag periods in hydrolyzing bacteria due to nickel similar to a study by Ashley *et al.* (1982). A second explanation is the precipitation of nickel as NiS,

$NiCO_3$ and $NiPO_4$. Gould and Genetelli (1978) reported lower pH values result in higher soluble metal concentrations. This may be dependent on the anion species present at low pH; in this study HS^-, HCO_3^- and HPO_4^{2-} and $H_2PO_4^-$ were the dominant species. The authors noted that there might be competition between metals and hydrogen ions for sites on ligands. Metal toxicity is caused by the free or soluble form which is directly related to the concentration of divalent sulfur (Mosey, 1976). An effective procedure for reducing metal toxicity is to add sodium sulfide or a sulfate salt which will be reduced in an anaerobic environment (McCarty, 1964). Along with precipitation, metals may bind to sludge solids (Gould and Genetelli, 1978).

Distribution of nickel in Test B was examined to determine what form was dominant. Two conditions were analyzed, total and soluble (filtered) nickel, to determine the quantity of soluble (filtered) nickel and the nickel fraction absorbed by the sludge. Results of the nickel distribution test are listed in Table 12. The majority of the nickel was associated with the solids. However, approximately 40% was found in the soluble form. The soluble form is the most toxic. The nickel complexed with solids or possibly absorbed by solids may have caused the toxic effects. MacNicol and Beckett (1989) found the majority of nickel and other metals in the biofloc and the particulate fraction less than 3.3 gm/cm^3, which was similar to the results in this study.

One change was made in the procedure in Test B. Syringe C was spiked from 136 to 226 mg/l nickel approximately four days into the test. The fourth day was chosen due to an observed increase in gas production. The 226 mg/l concentration was chosen because at that time 226 mg/l nickel was the largest concentration that permitted gas production and gas production rates and lag periods were similar to lower concentrations. The theory was, if the bacteria were acclimated the increase in concentration would produce no effect and gas production would continue. If the lag periods were due to precipitation, gas production would cease until the spiked nickel was precipitated or bound and no longer bioavailable.

Gas production rates in syringe C continued after the spike to 226 mg/l $NiCl_2$. Syringe E (226mg/l) produced a greater volume of gas with a difference of 9 ml compared to syringe C. Though the final nickel concentration in syringes C and E were similar, the syringes were different in that syringe C was fed under a step feed procedure. This procedure made the syringes difficult to compare. Syringe D (181 mg/l) produced less with difference of 6 ml of gas compared to syringe C. Gas production rates in Syringe E and D were similar to syringe C after the spike, (refer to Figure 13). The results weakly suggest that the lag period was an acclimation period. If the gas production in syringe C had terminated after the spike to 226 mg/l $NiCl_2$ then the data would suggest precipitation or complexation of nickel must occur, rendering the nickel non-bioavailable. However, this sludge contained a small amount of sulfate (assumption), which

will be reduced to sulfide and could precipitate nickel. The data developed in this study is insufficient and the most likely explanation of the lag periods is a combination of both precipitation and acclimation, provided S^{2-} was present in the sludge used in this study (S^{2-} was not analyzed). If precipitation and acclimation were occurring, the question becomes; which is the most dominate mechanism?

Table 12. Distribution of Nickel in Experiment 5; Test B

Syringe	Total Nickel (mg/l)	Soluble Nickel (mg/l)	Solids Fraction (mg/l)	Solids Fraction (%)	Soluble Nickel (%)
A	1.5	0.62	0.84	57	43
B	90	18	72	80	20
C	220	107	113	52	48
D	164	72	92	56	44
E	216	92	124	57	43
F	250	102	147	59	41

All values are as nickel

The threshold dose in this study was 45 mg/l nickel which corresponds to the Parkin *et al.* (1983) study, which reported a threshold of 50 mg/l Ni. Parkin and Miller (1982) reported 138 to 208 mg/l Ni^{2+} as the maximum tolerable concentration in a study performed under semi-continuous feed conditions. Distribution of nickel was not analyzed in either study. The authors noted that the ability of methanogens to become acclimated to nickel was very strong. This is a possible explanation for the lag periods observed in this study.

The results of this study indicated that the syringes exhibit low variability and good reproducibility under mixed conditions. Knowledge of VSS and F/M ratios is critical in developing test conditions that are practical. Volatile suspended solids concentrations above 4,000 mg/l and F/M ratios above 1 create unfavorable conditions requiring intense supervision of the syringes during the test. Gas production is essential in monitoring the effect of a chemical component or wastewater on anaerobic sludge. Thus, a sufficient volume of gas needs to be produced so that gas composition may be analyzed and toxic effects may be observed. Volatile suspended solids concentrations below 2,000 mg/l and F/M ratios below 0.5 produce inconclusive (small) volumes of gas, creating more uncertainty when forming comparisons. Improvements in the syringe methodology were observed when provided continuously mixed conditions, which improved gas production rates, produced low variability and reduced the test duration.

There are other ATA methods which make use of serum bottles as the reactor vessel and syringes are used to determine gas production rates. This method is widely accepted. However, when examining several conditions in one test the syringe method would be much more convenient and generate less human error than inserting a needle into each serum bottle to measure gas production. Also, withdrawing gas from the serum bottle requires the purging with an inert gas (N_2) to replace the void space. This procedure is not necessary when syringes

are used as the reactor vessel. A disadvantage to this method was the impediment of the luer-lock valve by solids in the test media, however this was not a frequent observation. Gas measurements and evacuations become impossible if the valve is not cleared.

CHAPTER VI

CONCLUSION

The goal of this study was to develop operating conditions for an improved anaerobic treatability/toxicity screening test procedure, using 125 ml glass syringes. The primary objectives of this study were to 1) determine the optimum range of volatile suspended solids and F/M ratio, 2) examine the variability and reproducibility, 3) evaluate the effects of mixing and 4) review the syringe method in evaluating ammonia-nitrogen and nickel toxicity. The study led to the following conclusion regarding the methodology and application to ammonia-nitrogen and nickel toxicity presented in this paper.

Methodology

1. The optimum volatile suspended solids concentration was determined to be 3,000 to 4,000 mg/l for the seed sludge used in this study.

2. The optimum F/M ratio was determined to be 0.5 to 1.0 mg COD/mg VSS for the seed sludge in this study. These values coupled with above VSS values resulted in manageable gas production.

3. Reactor failure occurred at an F/M of 1.0 and 1.5, which was most probably due to improper buffering.

4. Variability among the syringes was minimal. After two test runs the standard deviation in gas production among the syringes with approximately identical loadings was 3.3 ml.

5. Mixing conditions improved (increased) gas rates, reducing the test duration by approximately five days.

6. A control chart may be used to effectively determine syringe variability.

7. The pH should be the first parameter analyzed due to CO_2 partitioning to the gas phase.

8. Daily gas measurements and analysis were quick and easy, requiring minimal time.

Ammonia-Nitrogen Toxicity Tests

1. Ammonia-nitrogen concentrations of 800 to 2,000 mg/l inhibited gas production rates. All other parameters such as COD removal, pH, percent CO_2 and VFA showed no evidence of toxicity.

2. An ammonia-nitrogen concentration of 262 mg/l exhibited greater gas production rates than the control.

3. Significant reduction in gas production occurred at 2,550 mg/l NH_3-N, where gas production was 61% of the control syringe. All other parameters scuh as increased VFA concentration, lower COD removal and higher CO_2 production indicated toxicity.

Nickel Toxicity Tests

1. A nickel concentration of 45 mg/l nickel reduced gas production rates.

2. Lag periods were observed with nickel concentrations beginning at 90 mg/l nickel.

3. The extent of gas produced was reduced by 60% of the control in the syringe containing 90 mg/l nickel.

4. Lag periods increased with increasing concentrations of nickel.

5. Lag periods were reproducible, where 136 and 181 mg/l nickel exhibited lag periods similar in length in Tests A and B.

6. Gas production continued after syringe C (136 mg/l nickel) was spiked to 226 mg/l nickel, suggesting that the lag periods were more of a function of acclimation than precipitation. More studies using this procedure are required to develop more conclusive results.

CHAPTER VII

RECOMMENDATIONS FOR FUTURE RESEARCH

Future research should focus on the versatility of the syringe test procedure. In addition to, convenient gas measurements, samples may be withdrawn for analysis during the test, without introducing oxygen. Which would allow for a study evaluating the kinetics of waste streams and other chemical compounds. A study should be performed comparing this method with the serum bottle method to further evaluate the syringe method.

This study has pointed out questions about nickel toxicity. The lag periods observed in this study should be investigated further. A possible avenue would be to evaluate nickel toxicity with a variety of sludges, mainly municipal.

Other topics of research which would provide useful information to the industry are, studies focusing on using data developed from the syringe methodology for scaling up to full scale systems and micronutrient studies.

BIBLIOGRAPHY

Ashley, N.V., Davies, M., and Hurst, T.J. (1982). "The Effect of Increased Nickel Ion Concentrations on Microbial Populations in the Anaerobic Digestion of Sewage Sludge". *Water Res.* Vol. 16 pp. 963 to 971.

Brooks, S., Stover, E.L., and Clarkson, W.W., (1994). "Anaerobic Treatment/Toxicity Screening Study Procedure". Presented at the 1994 Food Industry Environmental Conference, Atlanta, Georgia.

Brooks, S., (1994). " Development of Test Methodology for Anaerobic Screening Study by Batch Assay". Master's Thesis. Oklahoma State University.

Callander, I.J., and Barford, J.P., (1983). "Precipitation, Chelation, and the Availability of Metals as Nutrients in Anaerobic Digestion. I. Methodology. " *Biotechnology and Bioengineering.* Vol. 25. p.1,947.

Dague, R.R., Hopkins, R.L., and Tonn, R.W., (1970). " Digestion Fundamentals Applied to Digestion Recovery: Two Case Studies." *Journal WPCF.* Vol. 42. p. 1,666.

Drosite, R.L., (1997). "Theory and Practice of Waster and Wastewater Treatment". John Wiley and Sons, Inc. New York, NY.

EPA, (1986) "Test Methodology for Evaluating Solid Waste; Lab Manual for Physical/Chemical Methods". Vol 1A, Third Edition. SW 846.

Fox, P. and Pohland F.G. (1994). "Anaerobic Treatment applications and Fundamentals: Substrate Specificity During Phase Separation". *Water Env. Res.* Vol. 66, No. 5.

Grady, C.P.L. Jr., and Lim, H.C. (1980). " Biological Wastewater Treatment: Theory and Application". Marcel Dekker, New York.

Gould, M.S., and Genetelli, E.J., (1978). "Heavy Metal Complexation Behavior in Anaerobically Digested Sludges". *Water Research.* Vol. 12 pp. 505 to 512.

Hays, T.D., and Theis, T.L., (1978). "The Distribution of Heavy Metals in Anaerobic digestion". *Journal WPCF.*, Vol. 50 p. 61.

Hobson , P.N., and Shaw, B.G., (1976). "Inhibition of Methane Production in Methanobactermum Formicicum. *Water Research.* Vol. 10. P. 849.

Kroeker, E.J., Shulte, D.D., Sparling, A.B., and Lapp H.M., (1979). "Anaerobic Treatment Process Stability". *Journal WPCF.* Vol. 51. p. 718.

MacNicol, R.D., and Beckett, P.H.T., (1989). "The Distribution of Heavy Metals Between the Principal Components of Digested Sludge". *Water Research.* Vol. 23 No. 2, pp. 199 to 206.

McCarty, P.L. (1964). "Anaerobic Waste Treatment Fundamentals. Toxic Materials and Their Control". *Publ. Works* pp 91 to 94.

Melbinger, N.R., and Donnellon, J., (1971). "Toxic Effects of Ammonia-Nitrogen in High-Rate Digestion". *Journal WPCF.* Vol. 43, p. 1,658.

Metcalf and Eddy, Inc. (1991). "Wastewater Engineering Treatment, Disposal and Reuse". Third Edition. McGraw-Hill. St. Louis, MO.

Mosey, F.E., (1971). "The Toxicity of Cadmium to Anaerobic Digestion: its Modification by Inorganic Ions". *Wat. Pollut. Control.* p. 584.

Mosey, F.E., Swanwick J. D., and Hughes D.A., (1971). "Factors Affecting the Availability of Heavy Metals to Inhibit Anaerobic Digestion". *Wat. pollut. Control.* p. 668.

Mosey, F.E., (1976). "Assessment of the Maximum Concentration of Heavy Metals of Crude Sludge which will not Inhibit the Anaerobic Digestion of Sludge" *Water Pollution Control.* Vol. 75, p.10.

Owen, W.F, Stucky, D.C., Healy, J.B., Young, L. Y., and McCarty, P.L., (1979). "Bioassay for Monitoring Biochemical Methane Potential and Anaerobic Toxicity". Water Research. Vol. 13 pp. 485 to 492.

Parkin, G.F., and Miller S.W., (1982). "Response of Methane Fermentation to Continuous Addition of Selected Industrial Toxicants". *Proceedings of the 37th Purdue Industrial Waste Conference*, West Lafayette, Ind.

Parkin, G.F., Speece, R.E., Yang, C.H.J., and Kocher, W.M. (1983). "Response of Methane Fermentation Systems to Industrial Toxicants". *Jounal WPCF*, Vol. 55, No. 1.

Sawyer, McCarty and Parkin, (1978). "Chemistry for Environmental Engineering". Fourth Edition. McGraw-Hill Publishing Co.

Snoeyink, V.L., and Jenkins, D., (1980). "Water Chemistry" John Wiley and Sons, Inc.

Standard Methods for the Examination of Water and Wastewater, 18th Edition (1992). American Public Health Association. Washington D.C.

Stover, E.L., Brooks, S., and Nunirathinam, K. (1992). "Control of Bulk Liquid Sulfide Toxicity Evaluation by Batch and Semi-continuous Assays". *Journal WPCF*, Vol. 52, No. 4.

van Velsen, A.F.M., (1979). "Adaptation of Methanogenic Sludge to High Ammonia-Nitrogen Concentrations". *Water Research*. Vol. 13 PP. 995 to 999.

Young, J.C., Kuss, M.L., and Nelson, M.A. (1991). "Use of Anaerobic Respirometers for Measuring Gas Production in Toxicity and Treatability Test". Presented at the 84th Air and Waste Management Assoc. Vancouver, B.C.

APPENDIXES

APPENDIX A

RAW DATA

Table A-1. Alkalinity Data for Experiment 1; Test A, Initial Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N Vol. (ml)	Total Alkalinity (mg/l as $CaCO_3$)
A	50	8.4	0.5	1	500
B	50	6.8	0.5	1	500
C	50	6.8	0.5	1.6	800
D	50	6.9	0.5	2.3	1,150
E	50	7.0	0.5	2.5	1,450
F	50	7.1	0.5	3.6	1,800

Table A-2. Alkalinity Data for Experiment 1; Test A, Final Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N Vol. (ml)	Total Alkalinity (mg/l as $CaCO_3$)
A	50	7.1	0.5	0.51	500
B	50	7.0	0.5	1.7	500
C	50	7.1	0.5	2.4	800
D	50	7.1	0.5	3.3	1,150
E	50	7.3	0.5	4.1	1,450
F	50	7.4	0.5	4.8	1,800

Table A-3. COD Data for Experiment 1; Test A, Initial Conditions

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	300	300
A	2	0	1	350	350
A	2	0	1	350	350
B	2	0	1	450	450
B	2	0	1	470	470
C	2	8	5	150	750
C	2	8	5	150	750
D	4	6	2.5	360	900
D	4	6	2.5	360	900
E	4	6	2.5	500	1,250
E	4	6	2.5	500	1,250
F	4	6	2.5	580	1,450
F	4	6	2.5	580	1,450

Table A-4. COD Data for Experiment 1; Test A, Initial Conditions

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	310	310
A	1	1	2	40	80
A	1	1	2	50	100
B	1	1	2	140	280
B	1	1	2	140	280
C	1	1	2	50	100
C	1	1	2	50	100
D	1	1	2	40	80
D	1	1	2	50	100
E	1	1	2	40	80
E	1	1	2	50	100
F	1	1	2	50	100
F	1	1	2	60	120

Table A-5. Total Volatile Suspended Solids Data for Experiment 1; Test A, Initial Conditions

Syringe	Tared Wt. (g)	Sample Vol/Wt	Solids			Volatile		
			Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)	Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)
A	1.0928	2	1.948	0.002	1,000	1.0930	0.0018	900
B	1.0885	2	1.0922	0.0037	1,850	1.0890	0.0032	1,600
C	1.0879	2	1.0941	0.0067	3,100	1.0887	0.0054	2,700
D	1.0813	2	1.0903	0.009	4,500	1.0826	0.0077	3,850
E	1.0932	2	1.1042	0.011	5,500	1.0949	0.0093	4,650
F	1.0866	2	1.1002	0.0136	6,800	1.0886	0.0116	5,800
DUP D	1.0924	2	1.1014	0.009	4,500	1.0937	0.0077	3,850

Table A-6. CO_2 Data for Experiment 1; Test A, Final Conditions

Syringe	Sample Vol. (ml)	Total Inorganic Carbon (TIC)
Standard 10% CO_2	1.4	94.7
Standard 10% CO_2	1.4	89.9
Standard 10% CO_2	1.4	92.2
A	1.4	86.4
A	1.4	83.6
B	1.4	147.1*
B	0.7	71.8
B	0.7	71.2
C	0.7	70.7
C	0.7	71.3
D	0.7	75.7
D	0.7	63.1
E	0.7	63.1
E	0.7	69.2
F	0.7	63.9
F	0.7	63.8
CO_2 % = Ave. TIC of a syringe * (% CO_2 Standard)/ Ave. Standard TIC		

* Was not used due to IR overrange

Table A-7. Alkalinity Data for Experiment 1; Test B, Initial Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
A	50	6.8	0.05	14.1	705
B	50	6.9	0.1	7.2	720
C	17	7.0	0.1	6.3	670
D	48	6.9	0.1	8.7	906
E	48	7.0	0.1	8.8	916
F	50	7.0	0.1	9.5	950

Table A-8. Alkalinity Data for Experiment 1; Test B, Final Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
A	50	7.3	0.1	9.9	990
B	50	5.7	0.1	7.6	760
C	-	-	-	-	-
D	48	7.4	0.1	12.7	1,322
E	50	5.8	0.1	9.8	980
F	-	-	-	-	-

Table A-9. Volatile Fatty Acids Data for Experiment 1;
Test B, Initial Conditions

Syringe	NaOH N	NaOH Vol. (ml)	VFA (mg/l)
A	0.5	0.4	300
B	0.5	1.5	1,125
C	-	-	-
D	0.5	0.2	100
E	0.5	1.7	1,275
F	-	-	-

Table A-10. COD Data for Experiment 1; Test B, Initial Conditions

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	310	310
A	2	8	5	200	1,000
A	2	8	5	210	1,050
B	2	8	5	475	2,375
B	2	8	5	490	2,450
C	2	8	5	600	3,000
C	2	8	5	600	3,000
D	2	8	5	250	1,250
D	2	8	5	260	1,300
E	2	8	5	620	3,100
E	2	8	5	620	3,100
F	2	8	5	1,000	5,000
F	2	8	5	1,000	5,000

Table A-11. COD Data for Experiment 2; Test B, Final Conditions

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	325	325
A	1	1	2	325	650
A	1	1	2	310	620
B	1	1	2	1,350	2,700
B	1	1	2	1,400	2,800
C	-	-	-	-	-
C	-	-	-	-	-
D	1	1	2	50	100
D	1	1	2	50	100
E	1	1	2	1,500 +	3,000 +
E	1	1	2	1,500 +	3,000 +
F	-	-	-	-	-
F	-	-	-	-	-

Table A-12. Total Volatile Suspended Solids Data for Experiment 1; Test A, Initial Conditions

Syringe	Tared Wt. (g)	Sample Vol/Wt	Solids			Volatile		
			Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)	Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)
A	1.0896	2	1.0960	0.0064	3,200	1.0906	0.0054	2,700
B	1.0844	2	1.0915	00.71	3,550	1.0853	0.0062	3,100
C	-	-	-	-	-	-	-	-
D	1.0897	2	1.0982	0.0085	4,250	1.0909	0.0073	3,650
E	1.0849	2	1.0942	0.0093	4,650	1.0858	0.0084	4,200
F	-	-	-	-	-	-	-	-
DUP E	1.0859	2	1.0952	0.0093	4,650	1.0868	0.0084	4,200

Table A-13. CO_2 Data for Experiment 1; Test A.

Syringe	Sample Vol. (ml)	Total Inorganic Carbon (TIC)
Standard 20%	0.7	63.9
Standard 20%	0.7	57.5
Standard 20%	0.7	61.9
A	0.7	95.8
A	0.7	97.1
B	0.7	141
B	0.5	108
D	0.5	100
D	0.5	99
E	0.5	128
E	0.5	127
CO_2 % = Ave. TIC of a syringe * (% CO_2 Standard)/ Ave. Standard TIC		

* Must convert the 0.5 ml sample volume TIC to a 0.7 ml TIC for the above equation

Table A-14. Alkalinity data for Experiment 2; Test A, Initial (Stock Solution) and Final Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
Stock Solution	50	7.5	0.2	8	1,600
Final Conditions					
A	50	7.4	0.5	3.7	1,850
B	50	7.4	0.5	3.4	1,700
C	45	7.4	0.5	2.9	1,611
D	50	7.5	0.5	3.4	1,700
E	50	7.4	0.5	3.5	1,750
F	50	7.5	0.5	3.5	1,750

Table A-15. COD Data for Experiment 2; Test A, Initial (Stock Solution) Conditions and Final Conditions

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1		
Stock Solution	2	8	5	425	2,175
Dup	2	8	5	425	2,175
Final Conditions					
Blank	0	2	1	0	0
Standard	2	0	1	300	300
A	1	1	2	50	100
A	1	1	2	60	120
B	1	1	2	90	180
B	1	1	2	50	100
C	1	1	2	110	220
C	1	1	2	50	100
D	1	1	2	50	100
D	1	1	2	40	80
E	1	1	2	40	80
E	1	1	2	50	100
F	1	1	2	40	80
F	1	1	2	50	100

Table A-15. COD Data for Experiment 2; Test A, Initial (Stock Solution) Conditions and Final Conditions

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1		
Stock Solution	2	8	5	425	2,175
Dup	2	8	5	425	2,175
Final Conditions					
Blank	0	2	1	0	0
Standard	2	0	1	300	300
A	1	1	2	50	100
A	1	1	2	60	120
B	1	1	2	90	180
B	1	1	2	50	100
C	1	1	2	110	220
C	1	1	2	50	100
D	1	1	2	50	100
D	1	1	2	40	80
E	1	1	2	40	80
E	1	1	2	50	100
F	1	1	2	40	80
F	1	1	2	50	100

Table A-16. Total Volatile Suspended Solids Data for Experiment 2; Test A, Initial (Stock Solution) and Final Conditions

Syringe	Tared Wt. (g)	Sample Vol/Wt	Solids			Volatile		
			Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)	Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)
Stock Solution	1.0939	2	1.1035	0.0096	4,800	1.0953	0.0082	4,100
DUP	1.0904	2	1.100	0.0096	4,800	1.0918	0.0082	4,100
Final Conditions								
DUP	1.0904	2	1.1000	0.0096	4,800	1.0918	0.0082	4,100
A	1.1043	2	1.1128	0.0085	4,250	1.1059	0.0069	3,450
B	1.1000	2	1.1085	0.0085	4,250	1.1016	0.0069	3,450
C	1.0988	2	1.1071	0.0083	4,150	1.1002	0.0069	3,450
D	1.0906	2	1.0993	0.0087	4,350	1.0923	0.007	3,500
E	1.0945	2	1.1029	0.0084	4,200	1.0960	0.0069	3,450
F	1.1026	2	1.1111	0.0085	4,250	1.1041	0.007	3,500
DUP F	1.0903	2	1.0818	0.0085	4,250	1.0748	0.007	3,500

Table A-17. CO_2 Data for Experiment 2; Test A.

Syringe	Sample Vol. (ml)	Total Inorganic Carbon (TIC)
Standard 30%	0.7	111
Standard 30%	0.7	107
Standard 30%	0.7	104
Standard 30%	0.7	113
A	0.7	88
A	0.7	85
B	0.7	94
B	0.7	96
C	0.7	86
C	0.7	79
D	0.7	86
D	0.7	90
E	0.7	84
E	0.7	82
F	0.7	81
F	0.7	80
CO_2 % = Ave. TIC of a syringe * (% CO_2 Standard)/ Ave. Standard TIC		

Table A-16. Total Volatile Suspended Solids Data for Experiment 2; Test A, Initial (Stock Solution) and Final Conditions

Syringe	Tared Wt. (g)	Sample Vol/Wt	Solids			Volatile		
			Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)	Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)
Stock Solution	1.0939	2	1.1035	0.0096	4,800	1.0953	0.0082	4,100
DUP	1.0904	2	1.100	0.0096	4,800	1.0918	0.0082	4,100
Final Conditions								
DUP	1.0904	2	1.1000	0.0096	4,800	1.0918	0.0082	4,100
A	1.1043	2	1.1128	0.0085	4,250	1.1059	0.0069	3,450
B	1.1000	2	1.1085	0.0085	4,250	1.1016	0.0069	3,450
C	1.0988	2	1.1071	0.0083	4,150	1.1002	0.0069	3,450
D	1.0906	2	1.0993	0.0087	4,350	1.0923	0.007	3,500
E	1.0945	2	1.1029	0.0084	4,200	1.0960	0.0069	3,450
F	1.1026	2	1.1111	0.0085	4,250	1.1041	0.007	3,500
DUP F	1.0903	2	1.0818	0.0085	4,250	1.0748	0.007	3,500

Table A-17. CO_2 Data for Experiment 2; Test A.

Syringe	Sample Vol. (ml)	Total Inorganic Carbon (TIC)
Standard 30%	0.7	111
Standard 30%	0.7	107
Standard 30%	0.7	104
Standard 30%	0.7	113
A	0.7	88
A	0.7	85
B	0.7	94
B	0.7	96
C	0.7	86
C	0.7	79
D	0.7	86
D	0.7	90
E	0.7	84
E	0.7	82
F	0.7	81
F	0.7	80
CO_2 % = Ave. TIC of a syringe * (% CO_2 Standard)/ Ave. Standard TIC		

Table A-18. Alkalinity Data for Experiment 2; Test B, Initial Conditions (Stock Solution) and Final Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
Stock Solution	50	8.0	0.5	4.5	2,250
Final Conditions					
A	50	7.5	0.5	3.6	1,800
B	50	7.5	0.5	3.7	1,850
C	50	7.5	0.5	3.4	1,700
D	49	7.4	0.5	3.5	1,750
E	50	7.5	0.5	3.5	1,750
F	50	7.5	0.5	3.7	1,850

Table A-19. COD data for Experiment 2; Test B, Initial Conditions (Stock Solution) and Final Conditions

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	300	300
Stock Solution	2	8	5	450	1,900
Dup	2	8	5	450	1,900
Final Conditions					
Blank	0	2	1	0	0
Standard	2	0	1	305	305
A	1	1	2	75	150
A	1	1	2	60	120
B	1	1	2	50	100
B	1	1	2	40	80
C	1	1	2	45	90
C	1	1	2	45	90
D	1	1	2	50	100
D	1	1	2	50	100
E	1	1	2	50	100
E	1	1	2	50	100
F	1	1	2	50	100
F	1	1	2	50	100

Table A-18. Alkalinity Data for Experiment 2; Test B, Initial Conditions (Stock Solution) and Final Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
Stock Solution	50	8.0	0.5	4.5	2,250
Final Conditions					
A	50	7.5	0.5	3.6	1,800
B	50	7.5	0.5	3.7	1,850
C	50	7.5	0.5	3.4	1,700
D	49	7.4	0.5	3.5	1,750
E	50	7.5	0.5	3.5	1,750
F	50	7.5	0.5	3.7	1,850

Table A-19. COD data for Experiment 2; Test B, Initial Conditions (Stock Solution) and Final Conditions

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	300	300
Stock Solution	2	8	5	450	1,900
Dup	2	8	5	450	1,900
Final Conditions					
Blank	0	2	1	0	0
Standard	2	0	1	305	305
A	1	1	2	75	150
A	1	1	2	60	120
B	1	1	2	50	100
B	1	1	2	40	80
C	1	1	2	45	90
C	1	1	2	45	90
D	1	1	2	50	100
D	1	1	2	50	100
E	1	1	2	50	100
E	1	1	2	50	100
F	1	1	2	50	100
F	1	1	2	50	100

Table A-20. Total Volatile Suspended Solids Data for Experiment 2; Test B, Initial Conditions (Stock Solution) and Final Conditions.

Syringe	Tared Wt. (g)	Sample Vol/Wt	Solids			Volatile		
			Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)	Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)
Stock Solution	1.0943	2	1.1033	0.009	4,500	1.0958	0.0075	3,750
DUP	1.1009	2	1.1095	0.0086	4,500	1.1024	0.0071	3,550
Final Conditions								
A	1.1001	2	1.1082	0.0081	4,050	1.1015	0.0067	3,350
B	1.0909	2	1.0988	0.0079	3,950	1.0921	0.0067	3,350
C	1.0975	2	1.1057	0.0082	4,100	1.0988	0.0069	3,450
D	1.1000	2	1.1078	0.0078	3,900	1.1012	0.0066	3,300
E	1.0799	2	1.0878	0.0079	3,950	1.0812	0.0066	3,300
F	1.0907	2	1.0986	0.0079	3,950	1.0917	0.0069	3,450
DUP C	1.0893	2	1.0971	0.0078	3,900	1.0904	0.0067	3,350

Table A-21. CO_2 Data for Experiment 2; Test B.

Syringe	Sample Vol. (ml)	Total Inorganic Carbon (TIC)
Standard 30%	0.7	98.5
Standard 30%	0.7	101
Standard 30%	0.7	103
Standard 30%	0.7	94
Standard 30%	0.7	107
A	0.7	104
A	0.7	107
B	0.7	100
B	0.7	104
C	0.7	100
C	0.7	110
D	0.7	109
D	0.7	104
E	0.7	98
E	0.7	91
E	0.7	94
F	0.7	101
F	0.7	95
F	0.7	91
CO_2 % = Ave. TIC of a syringe * (% CO_2 Standard)/ Ave. Standard TIC		

Table A-22. Alkalinity Data for Experiment 3; Initial Conditions (Stock Solution) and Final Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
Stock Solution	50	7.8	0.5	4.5	2,250
Final Conditions					
A; Mixed	50	7.4	0.5	4	2,000
B; Static	49	7.3	0.5	3.7	1,887
C; Mixed	50	7.4	0.5	3.6	1,800
D; Static	50	7.3	0.5	3.7	1,850
E; Mixed	49	7.4	0.5	3.6	1,800
F; Static	50	7.3	0.5	3.7	1,850

Table A-23. COD data for Experiment 1; Test A, Initial Conditions (Stock Solutions) and Final Conditions.

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	300	300
Stock Solution	2	8	5	400	2,000
DUP	2	8	5	400	2,000
Final Conditions					
A; Mixed	1	1	2	40	80
A	1	1	2	40	80
B; Static	1	1	2	50	100
B	1	1	2	50	100
C; Mixed	1	1	2	75	150
C	1	1	2	75	150
D; Static	1	1	2	75	150
D	1	1	2	50	100
E; Mixed	1	1	2	50	100
E	1	1	2	50	100
F; Static	1	1	2	50	100
F	1	1	2	60	120

Table A-24. Total Volatile Suspended Solids Data for Experiment 2; Test B, Initial Conditions (Stock Solutions) and Final Conditions.

Syringe	Tared Wt. (g)	Sample Vol/Wt	Solids			Volatile		
			Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)	Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)
Stock Solution	1.0790	2	1.0875	0.0085	4,250	1.0803	0.0071	3,600
DUP	1.0971	2	1.1056	0.0085	4,250	1.0985	0.0072	3,550
Final Conditions								
A	1.0993	2	1.1075	0.0082	4,100	1.1007	0.0068	3,400
B	1.0866	2	1.0941	0.0075	3,750	1.0881	0.0060	3,000
C	1.0920	2	1.0996	0.0076	3,800	1.0933	0.0063	3,150
D	1.0903	2	1.0979	0.0075	3,750	1.0916	0.0063	3,150
E	1.0921	2	1.0999	0.0078	3,900	1.0932	0.0067	3,350
F	1.0908	2	1.0983	0.0075	3,750	1.0920	0.0063	3,150
DUP E	1.0905	2	1.0986	0.0078	3,900	1.0922	0.0064	3,200

Table A-25. CO_2 Data for Experiment 2; Test B.

Syringe	Sample Vol. (ml)	Total Inorganic Carbon (TIC)
Standard 30%	0.7	99
Standard 30%	0.7	96
Standard 30%	0.7	108
Standard 30%	0.7	98
A; Mixed	0.7	79
A	0.7	76
B; Static	0.7	86
B	0.7	87
C; Mixed	0.7	78
C	0.7	74
D; Static	0.7	84
D	0.7	80
E; Mixed	0.7	79
E	0.7	73
F; Static	0.7	83
F	0.7	94
F	0.7	85
CO_2 % = Ave. TIC of a syringe * (% CO_2 Standard)/ Ave. Standard TIC		

Table A-26. Alkalinity Data for Experiment 4; Test A, Initial Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
A	50	7.4	0.5	4.0	2,000
B	50	7.5	0.5	3.8	1,900
C	49	7.5	0.5	3.8	1,900
D	48	7.5	0.5	3.6	1,800
E	50	7.6	0.5	3.9	1,950
F	50	7.6	0.5	3.9	1,950

Table A-27. Alkalinity data for Experiment 4; Test A, Final Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
A	25	7.4	0.1	9.1	1,820
B	25	7.5	0.1	9.6	1,920
C	25	7.5	0.1	9.5	1,900
D	25	7.5	0.1	9.7	1,940
E	25	7.6	0.1	9.6	1,920
F	25	7.6	0.1	9.4	1,880

Table A-28. Volatile Fatty Acids Data for Experiment 4; Test A, Final Conditions.

Syringe	NaOH N	NaOH Vol. (ml)	VFA (mg/l)
A	0.1	0.4	40
B	0.1	0.5	50
C	0.1	0.5	50
D	0.1	0.5	50
E	0.1	0.5	50
F	0.1	0.5	50

Table A-28. COD Data for Experiment 4; Test A, Initial Conditions (Data was not used in this study due to interference's with chloride).

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	300	300
A	2	8	5	350	1,750
A	2	8	5	350	1,750
B	2	8	5	360	1,800
B	2	8	5	700	3,500
C	2	8	5	400	2,000
C	2	8	5	390	1,950
D	2	8	5	700	3,500
D	2	8	5	700	3,500
E	2	8	5	375	1,875
E	2	8	5	375	1,875
F	2	8	5	420	2,100
F	2	8	5	500	2,500

Table A-29. COD Data for Experiment 4; Test A, Final Conditions (Data was not used in this study due to interference's with chloride).

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	300	300
A	1	1	2	25	50
A	1	1	2	25	50
B	1	1	2	110	220
B	1	1	2	1,000	2,000
C	1	1	2	1,000	2,000
C	1	1	2	1,000	2,000
D	1	1	2	700	1,400
D	1	1	2	1,000	2,000
E	1	1	2	1,200	2,400
E	1	1	2	1,200	2,400
F	1	1	2	350	700
F	1	1	2	900	1,800

Table A-30. Total Volatile Suspended Solids Data for Experiment 4; Test A, Initial Conditions

Syringe	Tared Wt. (g)	Sample Vol/Wt	Solids			Volatile		
			Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)	Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)
A	1.0990	2	1.1072	0.0082	4,100	1.1007	0.0065	3,250
B	1.0898	2	1.0983	0.0085	4,250	1.0916	0.0067	3,350
C	1.0915	2	1.0997	0.0082	4,100	1.0931	0.0066	3,300
D	1.0900	2	1.0983	0.0083	4,150	1.0916	0.0067	3,350
E	1.0916	2	1.1001	0.0084	4,200	1.0932	0.0068	3,400
F	1.1007	2	1.1093	0.0086	4,300	1.1024	0.0069	3,450
DUP F	1.0855	2	1.0940	0.0085	4,250	1.0868	0.0072	3,600

Table A-31. CO_2 Data for Experiment 4; Test A.

Syringe	Sample Vol. (ml)	Total Inorganic Carbon (TIC)
Standard 30%	0.7	107
Standard 30%	0.7	95
Standard 30%	0.7	98
Standard 30%	0.7	98
A	0.7	100
A	0.7	83
B	0.7	90
B	0.7	92
C	0.7	84
C	0.7	97
D	0.7	96
D	0.7	94
E	0.7	97.4
E	0.7	103
F	0.7	110
F	0.7	81
CO_2 % = Ave. TIC of a syringe * (% CO_2 Standard)/ Ave. Standard TIC		

Table A-32. Alkalinity Data for Experiment 4; Test B, Initial Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
A	30	7.9	0.5	2.0	1,666
B	50	7.7	0.5	3.2	1,600
C	50	7.6	0.5	3.3	1,650
D	50	7.5	0.5	3.3	1,650
E	50	7.5	0.5	3.2	1,600
F	50	7.5	0.5	3.5	1,750

Table A-33. Alkalinity Data for Experiment 4; Test B, Final Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
A	48	7.3	0.5	3.4	1,770
B	49	7.1	0.5	3.3	1,650
C	50	7.0	0.5	3.0	1,500
D	50	7.0	0.5	3.2	1,600
E	50	6.9	0.5	2.7	1,350
F	50	6.9	0.5	2.7	1,350

Table A-34. Volatile Fatty Acids Data for Experiment 4; Test B, Final Conditions.

Syringe	NaOH N	NaOH Vol. (ml)	VFA (mg/l)
A	0.05	202	110
B	0.05	7.1	532
C	0.1	4.4	660
D	0.1	4.9	735
E	0.1	4.9	735
F	0.1	5.0	750

Table A-35. TOC Data for Experiment 4; Test B, Initial Conditions

Syringe	Sample Vol. (ml)	Total Organic Carbon (TOC)
Standard 1,000 mg/l	0.7	1,084
A	0.7	824
B	0.7	887
C	0.7	777
D	0.7	519
E	0.7	813
F	0.7	906

Table A-36. TOC Data for Experiment 4; Test B, Final Conditions.

Syringe	Total Volume. (ml)	D.F	Total Organic Carbon	
			Reading	Conc. (mg/l)
Standard 100 mg/l	20	1	105	105
A	20	4.4	10	44
B	20	6.6	78.5	518
C	20	10	60	602
D	20	10	74	741
E	20	10	79	793
F	20	10	83	833

Table A-37. Total Volatile Suspended Solids Data for Experiment 41; Test B, Initial Conditions (VSS were measured at the end of test but represent initial condition).

Syringe	Tared Wt. (g)	Sample Vol/Wt	Solids			Volatile		
			Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)	Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)
A	1.0806	2	1.0897	0.0091	4,550	1.0827	0.007	3,500
B	1.1005	2	1.1100	0.0095	4,750	1.1024	0.0076	3,800
C	1.0988	2	1.1084	0.0096	4,800	1.1003	0.0081	4,050
D	1.0982	2	1.0986	0.0094	4,700	1.0912	0.0074	3,700
E	1.1026	2	1.1130	0.0104	5,200	1.1045	0.0085	4,250
F	1.0892	2	1.1008	0.0116	5,800	1.0904	0.0104	5,200
DUP B	1.0926	2	1.1014	0.0088	4,400	1.0934	0.0075	3,750

Table A-38. CO_2 Data for Experiment 4; Test B.

Syringe	Sample Vol. (ml)	Total Inorganic Carbon (TIC)
Standard 30%	0.7	119
Standard 30%	0.7	104
Standard 30%	0.7	102
Standard 30%	0.7	100
A	0.7	73
A	0.7	73*
A	0.7	73
B	0.7	145^
B	0.5	99
C	0.5	116
C	0.5	104
D	0.5	106
CO_2 % = Ave. TIC of a syringe * (% CO_2 Standard)/ Ave. Standard TIC Must Convert 0.5 ml Sample Volume TIC Values to 0.7 ml Sample VolumeTIC Values.		

* Time out error

^ IR overrange

Table A-39. Alkalinity Data for Experiment 5; Test A, Initial Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
A	50	7.3	0.5	3.6	1,800
B	50	7.4	0.5	3.8	1,900
C	50	7.4	0.5	3.7	1,850
D	50	7.4	0.5	3.8	1,900
E	50	7.4	0.5	4.0	2,000
F	50	7.3	0.5	3.7	1,850

Table A-40. Alkalinity Data for Experiment 5; Test A, Final Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
A	50	7.2	0.5	4.3	2,150
B	50	7.4	0.5	4.4	2,200
C	50	7.2	0.5	4.3	2,150
D	50	7.0	0.5	3.3	1,650
E	50	6.7	0.5	2.9	1,450
F	50	6.8	0.5	3.2	1,600

Table A-41. Volatile Fatty Acids Data for Experiment 5; Test A, Final Conditions

Syringe	NaOH N	NaOH Vol. (ml)	VFA (mg/l)
A	0.1	1.2	120
B	0.1	1.0	100
C	0.1	1.3	130
D	0.1	3.6	540
E	0.1	4.9	735
F	0.1	4.1	615

Table A-42. COD Data for Experiment 5; Test A, Initial Conditions

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	250	250
A	2	8	5	420	2,100
A	2	8	5	450	2,100
B	2	8	5	400	2,000
B	2	8	5	400	2,000
C	2	8	5	400	2,000
C	2	8	5	-	-
D	2	8	5	400	2,000
D	2	8	5	400	2,000
E	2	8	5	400	2,000
E	2	8	5	-	-
F	2	8	5	400	2,000
F	2	8	5	400	2,000

- Represents Broken COD Vial

Table A-43. COD Data for Experiment 5 ; Test , Final Conditions

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	300	300
A	1	1	2	75	150
A	1	1	2	150	225
B	1	1	2	50	100
B	1	1	2	50	100
C	2	8	5	75	375
C	2	8	5	75	375
D	2	8	5	300	1,500
D	2	8	5	300	1,500
E	2	8	5	300	1,500
E	2	8	5	300	1,500
F	2	8	5	300	1,500
F	2	8	5	300	1,500

Table A-44. Total Volatile Suspended Solids Data for Experiment 5; Test A, Initial Conditions

Syringe	Tared Wt. (g)	Sample Vol/Wt	Solids			Volatile		
			Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)	Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)
A	1.0897	2	1.0995	0.0098	4,900	1.0912	0.0083	4,150
B	1.0918	2	1.1009	0.0091	4,550	1.0932	0.0077	3,850
C	1.0909	2	1.1002	0.0093	4,650	1.0924	0.0078	3,900
D	1.0955	2	1.1050	0.0095	4,750	1.0974	0.0076	3,800
E	1.0936	2	1.1034	0.0098	4,900	1.0952	0.0082	4,100
F	1.0983	2	1.1079	0.0096	4,800	1.1000	0.0079	3,950
DUP D	1.0914	2	1.1013	0.0099	4,950	1.0933	0.0080	4,000

Table A-45. CO_2 Data for Experiment 5; Test A.

Syringe	Sample Vol. (ml)	Total Inorganic Carbon (TIC)
Standard 30%	0.7	106
Standard 30%	0.7	104
Standard 30%	0.7	97
Standard 30%	0.7	103
A	0.7	111
A	0.7	115
B	0.7	1120
B	0.7	120
C	0.7	145
C	0.7	142
D	0.7	185*
D	0.5	120
D	0.5	137
D	0.5	137
E	0.5	143
E	0.5	127
F	0.5	133
F	0.5	121

CO_2 % = Ave. TIC of a syringe * (% CO_2 Standard)/ Ave. Standard TIC
Must Convert 0.5 ml Sample Volume TIC Values to 0.7 ml Sample Volume TIC Values

* IR overrange

Table A-46. Alkalinity Data for Experiment 5; Test B, Initial Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
A	50	7.3	0.5	3.9	1,950
B	54	7.4	0.5	4.3	2,150
C	50	7.4	0.5	3.9	1,950
D	50	7.4	0.5	3.9	1,950
E	50	7.3	0.5	3.9	1,950
F	50	7.2	0.5	4.0	2,000

Table A-47. Alkalinity Data for Experiment 5; Test B, Final Conditions.

Syringe	Sample Vol. (ml)	Initial pH	H_2SO_4 N	H_2SO_4 N (Vol.)	Total Alkalinity (mg/l as $CaCO_3$)
A	35	7.6	0.2	7.4	2,114
B	36	6.9	0.2	5.7	1,585
C	35	7.1	0.2	5.6	1,600
D	37	7.0	0.2	6.0	1,621
E	37	7.1	0.2	6.0	1,621
F	37	7.1	0.2	5.8	1,568

Table A-48. Volatile Fatty Acids Data for Experiment 5; Test B, Final Conditions

Syringe	NaOH N	NaOH Vol. (ml)	VFA (mg/l)
A	0.1	0.8	80
B	0.1	3.6	540
C	0.1	2.9	435
D	0.1	3.1	465
E	0.1	2.8	420
F	0.1	2.5	375

Table A-49. COD Data for Experiment 5; Test B, Initial Conditions.

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	300	300
A	1	1	2	1,200	2,400
A	1	1	2	1,200	2,400
B	1	1	2	1,000	2,000
B	1	1	2	1,000	2,000
C	1	1	2	1,100	2,200
C	1	1	2	1,100	2,200
D	1	1	2	1,000	2,000
D	1	1	2	1,000	2,000
E	1	1	2	1,000	2,000
E	1	1	2	1,000	2,000
F	1	1	2	1,000	2,000
F	1	1	2	1,000	2,000

Table A-50. COD Data for Experiment 5 ; Test B , Final Conditions

Syringe	Sample (Vol.) (ml)	Water (Vol.) (ml)	Dilution Factor	Meter Reading	COD (mg/l)
Blank	0	2	1	0	0
Standard	2	0	1	300	300
A	2.5	7.5	4	50	200
A	2.5	7.5	4	50	200
B	2.5	7.5	4	450	1,800
B	2.5	7.5	4	450	1,800
C	2.5	7.5	4	400	1,600
C	2.5	7.5	4	400	1,600
D	2.5	7.5	4	400	1,600
D	2.5	7.5	4	400	1,600
E	2.5	7.5	4	400	1,600
E	2.5	7.5	4	400	1,600
F	2.5	7.5	4	350	1,400
F	2.5	7.5	4	350	1,400

Table A-51. Total Volatile Suspended Solids Data for Experiment 5; Test B, Initial Conditions

Syringe	Tared Wt. (g)	Sample Vol/Wt	Solids			Volatile		
			Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)	Dried Wt. (g)	Delta Wt. (g)	Conc. (mg/l)
A	1.0917	2	1.1013	0.0096	4,800	1.0933	0.0080	4,000
B	1.0919	2	1.0992	0.0073	3,650	1.0932	0.0060	3,000
C	1.0951	2	1.1043	0.0092	4,600	1.0969	0.0074	3,700
D	1.1001	2	1.1090	0.0089	4,450	1.1018	0.0072	3,600
E	1.0980	2	1.0989	0.0099	4,950	1.0911	0.0078	3,900
F	1.1001	2	1.1103	0.0102	5,100	1.1023	0.0080	4,000
DUP C	1.0965	2	1.1057	0.0092	4,600	1.0984	0.0073	3,650

Table A-52. CO_2 Data for Experiment 5; Test B.

Syringe	Sample Vol. (ml)	Total Inorganic Carbon (TIC)
Standard 30%	0.7	98
Standard 30%	0.7	90
Standard 30%	0.7	97
Standard 30%	0.7	108
A	0.7	105
A	0.7	99
B	0.5	109
B	0.5	92
C	0.5	120
C	0.5	98
D	0.5	130*
D	0.3	88
E	0.5	110
E	0.3	106
F	0.5	132*
F	0.3	69

CO_2 % = Ave. TIC of a syringe * (% CO_2 Standard)/ Ave. Standard TIC
Must Convert 0.5 ml Sample Volume TIC Values to 0.7 (0.3) ml Sample Volume TIC Values

* IR overrange

VITA

Thomas Wynn

Candidate for the Degree of

Master of Science

Thesis: IMPROVED RAPID ANAEROBIC TREATABILITY-TOXICITY SCREENING TEST PROCEDURE

Major Field: Environmental Engineering

Biographical:

Personal Data: Born in Grand Haven, Michigan, on June 26, 1970, the son of Jack and Judy Wynn.

Education: Graduated from Edison High School, Tulsa, Oklahoma in May, 1988; received Bachelor of Science degree in Wildlife and Fisheries Ecology from Oklahoma State University, Stillwater, Oklahoma May 1993. Completed the requirements for the Master of Science degree at Oklahoma State University in July 1997.

Experience: Begin work in the environmental field as a fisheries field technician at the Oklahoma Cooperative Fish and Wildlife Research Unit. Recent experience as a lab technician performing various biomonitoring test, wet chemistry analysis and anaerobic screening/troubleshooting test.