DEVELOPMENT OF TEST METHODOLOGY FOR ANAEROBIC SCREENING STUDY BY BATCH ASSAY

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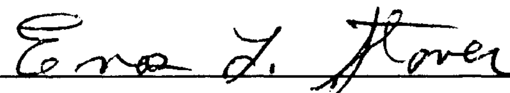
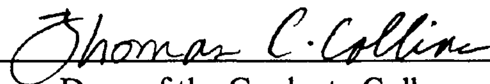
Thesis Adviser





Dean of the Graduate College

PREFACE

This study was performed to provide specific information and knowledge on a new test methodology for anaerobic toxicity and ways to control the toxicity. Healthly anaerobic bacteria produce methane, carbon dioxide, and when sulfate is present, hydrogen sulfide. Measuring gas production is critical to monitoring the performance of the bacteria. The goal of the study was to define the test conditions needed to properly run the experiment and to develop techniques to control the resultant toxicity in the glass syringes.

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CHAPTER I

INTRODUCTION

DEVELOPMENT OF TEST METHODOLOGY FOR ANAEROBIC SCREENING STUDY BY BATCH ASSAY

Anaerobic treatment processes have been used extensively for several years to stabilize domestic sludges and treat complex high strength industrial wastewaters. Their popularity has developed from the multiple advantages the various processes have to offer. Anaerobic treatment processes provide a significant advantage over aerobic processes since they produce methane gas, a usable by-product, have lower sludge production rates, and have proved to be less susceptible to organic shock loads than aerobic treatment.

In today's industrial wastewater streams and hazardous waste sites, chemical compounds and combinations of chemicals exist that could be toxic or inhibitory to the anaerobic bacteria. To determine wastewater characterics and the treatability of the

wastewater, small bench-scale continuous, semi-continuous, and batch systems have been developed.

Bench-scale tests on wastewater streams help develop treatability methods on a small scale before any large scale work is initiated. Stuckey *et al.* (1980) reported that biological assay procedures generally offer the most promise for determining whether or not digester imbalance is indeed caused by toxic materials and can aid in the evaluation of toxicity thresholds for suspect chemicals.

Either batch, continuous, or semi-continuous feed bioassays can be used to test toxicity thresholds, and each has advantages and disadvantages. Batch systems offer a quick and easy method of determining toxic or inhibitory effects of certain wastewaters. One means of determining these impacts is to measure the volume and rate of gas produced in anaerobic reactors (Young *et al*, 1991). Laboratory tests designed for the purpose of measuring gas and reactor performance are typically small, 50 to 500 ml, and contain anaerobic cultures that are dosed with various amounts of wastewater or specific chemicals. Since gas production relates to the performance of anaerobic bacteria, a decrease in the cumulative volume or rate of gas produced indicates an adverse effect of the test material. The results of such tests weigh heavily on the accuracy of gas measurements.

Methods available for measuring the rate and volume of gas produced by bench-scale anaerobic reactors include volume displacement devices, wet-test meters, lubricated syringes, automatic anaerobic respirometers, manometer-assisted syringes, and calibrated pressure manometers or transducers. Each of these methods has advantages and disadvantages that should be considered when performing a batch study versus a continuous or semi-continuous study.

Several bench-scale batch systems have evolved for evaluating anaerobic treatment of wastewaters. In this study, 125 ml glass syringes were used to evaluate their use for testing toxicity in anaerobic systems. The syringes are unique in that the test vessel also works to measure the gas production. A proven toxic substance to anaerobic bacteria, sulfides, was used in this experiment to evaluate the test methodology. Also tested were ways to control sulfide toxicity in the syringes using metal and magnesium salts for sulfide precipitation.

Goal of the Study

The goal of this study was to develop a test methodology for anaerobic toxicity assay using 125 ml glass syringes as reaction vessels.

Objectives

The specific research objectives were to:

a) Develop specific operating and environmental conditions for this methodology.

b) Define and correlate gas production as an indication of inhibition.

c) Determine the potential of controlling bulk liquid sulfide concentrations by metal and magnesium salt precipitation to reduce toxicity.

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

The test media used in this methodology (sulfide inhibition) required some knowledge and background of the requirements for sulfate reduction. The amount of COD to sulfate, F/M ratio, pH, temperature, and gas production are all important operating conditions to consider when a high sulfate waste stream is treated anaerobically. The anaerobic toxicity assay (ATA) is a general test method used to evaluate the effects of certain chemicals or conditions on anaerobic treatment. The test has been run using a variety of materials and reactor configurations; this test method used 125 ml glass luer-lock syringes. A general discussion of sulfur species and anaerobic treatment is needed to understand the test conditions that were considered for this particular methodology.

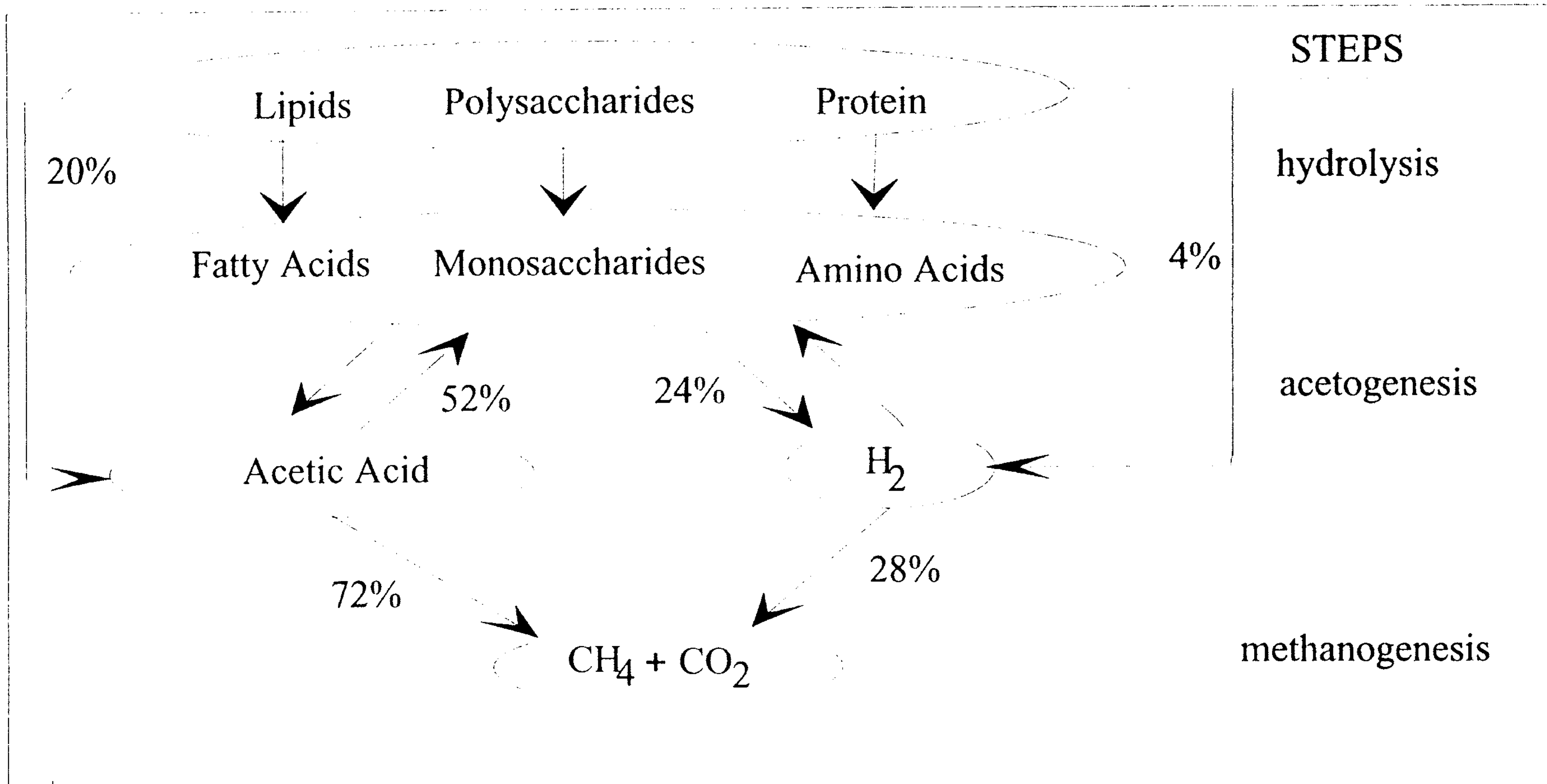
Anaerobic Treatment

Anaerobic digestion is one of the oldest processes used for the stabilization of sludges, and for about the past ten years, anaerobic processes have been developed commercially for the treatment of high strength organic wastes. Anaerobic waste

treatment involves the decomposition of organic and inorganic matter in the absence of oxygen.

The biological conversion of the organic matter in treatment plant sludges is known to occur in at least three steps. Figure 1 illustrates a simplified view of anaerobic metabolism of complex organic matter. The first step, hydrolysis, involves the breakdown of large-molecular-mass compounds into compounds suitable for use as a source of energy and cell carbon. The second step, acidogenesis, involves the bacterial conversion of the compounds resulting from the first step into identifiable lower-molecular-mass intermediate compounds. The third step, methanogenesis, involves the bacterial conversion of the intermediate compounds into simpler end products, principally methane and carbon dioxide. (Holland *et al*, 1987) (McCarty 1966).

In an anaerobic reactor, a multitude of anaerobic organisms work together to convert organic sludges and wastes to stable products. In the first step of conversion, one group of organisms is responsible for hydrolyzing organic polymers and lipids to basic structural building blocks such as monosaccharides, amino acids, and related compounds. A second group of bacteria fulfills the second stage of digestion, acidogenesis. This step breaks down the products of hydrolysis to simple organic acids, the most common of which is acetic acid. This group of microorganisms are non-methanogenic and consist of



Source: Metcalf and Eddy, Third Edition 1991

Figure 1. Anaerobic Conversion of Complex Organic Matter

facultative and obligate anaerobic bacteria.

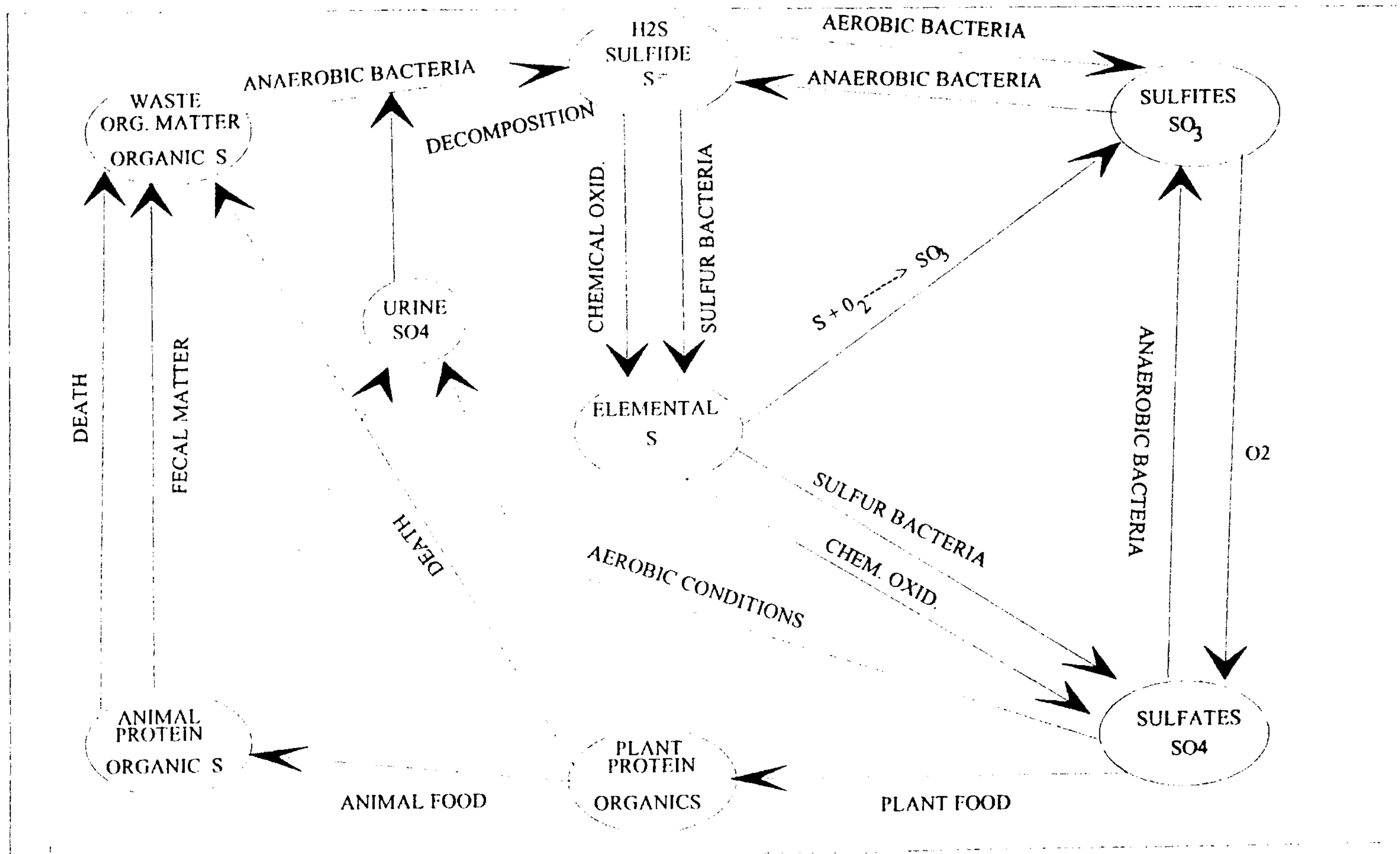
The third and final group in this consortium converts the hydrogen and acetic acid formed by the acid formers to methane gas and carbon dioxide. The bacteria responsible for this conversion are strict anaerobes and are called methanogens. The most important bacteria of the methanogenic group are the ones that utilize hydrogen and acetic acid. These bacteria have very slow growth rates and as a result their metabolism is usually considered rate-limiting in the anaerobic treatment of an organic waste. Waste stabilization in anaerobic treatment is accomplished when methane and carbon dioxide are produced. Methane gas is highly insoluble, and its departure from solution represents actual waste stabilization (Stover *et al*, 1992).

The primary environmental factors of concern for anaerobic treatment include temperature, pH, supplying adequate macronutrients/micronutrients, and minimizing and /or controlling toxic organic compounds. The most effective indicators of performance and system stability are pH, volatile acids, volatile acid to alkalinity ratio, biogas (methane) production, and COD/BOD removal efficiency. Generally, the volatile acid/alkalinity ratio should be maintained below 1.0 in order to obtain optimum system performance (Lawrence *et al*, 1966).

The use of pH alone is not an adequate indicator of reactor activity because environmental changes will have already taken place before a pH change is noticed. However, knowledge of the pH is important to good operation of the system and should be maintained between 6.5 and 7.5 for most applications. Therefore, maintaining acceptable pH, alkalinity, and buffering capacity in the bulk liquid of anaerobic treatment systems is critical to successful operations. Typical chemicals used for the addition of alkalinity to anaerobic systems include caustic, sodium bicarbonate, and lime. Magnesium hydroxide can also be used for alkalinity and buffering addition.

Sulfur Cycle

The sulfate ion is one of the major anions occurring in natural waters. It is of importance in public water supplies because of its cathartic effect upon humans when it is present in excessive amounts (Sawyer and McCarty, 1978). Sulfur generally enters into the microbial biosynthetic pathways at the oxidation levels of sulfate (SO_4-S) or sulfide ($S^=$). The biological interconversion of sulfate has been found to be a reversible process which has been termed the sulfur cycle. Figure 2 illustrates the sulfur cycle.



Source: Sawyer and McCarty, 1978

Figure 2. The Sulfur Cycle

The reduction of sulfate in the sulfur cycle can be broken down into two distinct processes. Sulfate reduction which eventually forms elemental sulfur and meets only the nutritional requirements of the bacteria is called assimilatory sulfate reduction. During the second type of sulfate reduction large amounts of sulfide accumulate in the environment, and this is termed dissimilatory (respiration) sulfate reduction. In the dissimilatory process, sulfide is produced as a result of the use of sulfate as the terminal electron acceptor in the oxidation of organic material and/or molecular hydrogen. This step is carried out by only a few obligate anaerobes.

Effects of Sulfides on Anaerobic Treatment

Numerous experiments evaluating sulfide inhibition have reported various concentrations of sulfides known to cause toxic effects on methanogenic bacteria. Early studies showed that around 200 mg/l $S^{=}$ caused inhibition. Experiments by Lawrence and McCarty (1966) concluded that concentrations of soluble sulfide up to 200 mg/l produced no significant toxic effects on anaerobic treatment. Some reports state that concentrations of 300 mg/l had no significant inhibitory effect on the performance of the anaerobic culture (Parkin *et al*, 1983).

The increased threshold of sulfides can be attributed to several factors. Foremost is the increased knowledge of the fate of sulfur, optimum pH, and gas production. etc. The acclimation of anaerobic bacteria to soluble sulfides is probably the best explanation for the higher threshold.

Throughout the study of the effects of soluble sulfides on anaerobic bacteria, some distinct response characteristics have been defined. Inhibitory concentrations of sulfides affect gas production first. This fact suggests that the methanogenic bacteria are the first to respond to inhibitory levels of sulfides. Methanogens are known to be the most sensitive of the consortium of anaerobic organisms in a digester which also fail first at the onset of organic shock loads. The accumulation of volatile fatty acids takes place much slower, and only after gas production has been severely retarded. Gas production rates have fallen as much as 70% before significant volatile acid accumulation occurs (McCarty 1966).

In a serum bottle study by Parkin *et al* (1983), a sulfide concentration as low as 50 mg/l $S^{=}$ resulted in some inhibition to unacclimated batch systems. According to their study, the 50 mg/l $S^{=}$ sample experienced a lag in gas production initially, yet produced the same amount of biogas overall as the control. The sulfur source in this study was

Na_2S-$9H_2O$. Using CSTR systems exposed to 100 to 500 mg/l $S^=$, a rapid decrease in gas production followed by a fairly rapid recovery was observed. Their experiments also focused on the reversibility of sulfide toxicity at concentrations of 250, 500, and 1500 mg/l. They reported that once the sulfide rich supernatant was removed, recovery accelerated. The authors reported no significant decrease in process performance up to 400 mg/l sulfide.

The presence of sulfates in the anaerobic environment can affect gas production (methanogenic populations) without sulfide inhibition taking place. Sulfates in an anaerobic environment result in the consumption of organic matter by sulfate reducing bacteria (SRB) at the expense of methane formation. The methane precursors, acetate and H_2, are competitively pursued by both sulfate reducing bacteria and methanogenic bacteria. In a study conducted by Winfrey and Zeikus (1977), it was demonstrated that methane production was completely inhibited in a freshwater sediment containing a sulfate concentration of 320 mg/l SO_4-S. The addition of acetate (60mg/l) or gaseous hydrogen (0.18 mg H_2/l) to these sediments reversed the methanogenic inhibition. This phenomenon is termed competitive inhibition.

This process would explain a drop of gas production in a reactor without elevated

volatile acid accumulation or low COD removal rates. Acetate and other volatile acids would be consumed by SRB, leaving the energy unavailable for methanogenesis. The advantage that the sulfate reducing bacteria have over methane producing microorganisms has been explained by thermodynamics or kinetics. Winfrey and Zeikus (1977) concluded that the greater free energy gain from sulfate reduction over methane production enabled sulfate reducers to out-compete methanogens for energy sources (i.e. the thermodynamic argument).

Although thermodynamically, sulfate reduction is energetically more favorable than methanogenesis, it cannot be used, in principle, to explain kinetic phenomena (McCarty, 1972). Thermodynamics can only predict the maximum amount of energy available to the cell from the given reaction. Kinetic phenomena (e.g. reaction rates, growth rates, etc.) depend on the efficiency of energy utilization by the particular organisms involved (Grady and Lim, 1980).

Since a more efficient utilization of energy by one group of organisms over another is reflected in the half velocity coefficient (Ks), this would seem to be a more useful predictor of bacterial competition than free energy analysis. It has been demonstrated that under substrate limiting conditions, sulfate reducing bacteria continue to be active while methanogenesis is hindered. An interesting

result of these studies is that under conditions of a non-limiting substrate, methane production and sulfate reduction are not mutually exclusive (Winfrey and Zeikus, 1977).

Anaerobic Toxicity Assays

The toxicity of a substance to anaerobic bacteria can be evaluated by either batch, continuous, or semi-continuous methods. Each has advantages and disadvantages, and the choice between the methods will depend on how much time, money, and extensive research will be needed. The continuous procedures closely simulate full scale anaerobic operation; however they are costly in terms of facilities, equipment, time, and personnel. Batch assay techniques do not have these limitations and thus permit the evaluation of a wide range of variables and scenarios (Owen *et al*, 1979). Batch techniques can evaluate the influence of shock loads while not being able to simulate full-scale systems in operation. Batch studies can be used as a preliminary step to a more efficient continuous-feed assay program.

The most popular devices used for batch studies are serum bottles or large syringes because they are inexpensive and provide a method for both analyzing and

monitoring gas quality and production rates. These devices are used for batch test procedures termed "anaerobic toxicity assays" (ATA) where, among other parameters, gas production can be easily monitored.

Another system, called the Warburg respirometer, has been widely used as a batch procedure to evaluate biodegradability and toxicity in anaerobic systems. However, the Warburg respirometer has several limitations: (1) it is costly and requires some degree of skill to operate, (2) a given instrument is limited in the number of samples that can be analyzed at one time, (3) sample size is limited, making subsequent analyses difficult, (4) it is difficult to sample the gas and liquid phase during the assay, and (5) extended incubation times are impractical and produce inconsistent results (Stuckey *et al*, 1980).

In one study monitoring the biochemical methane potential and ATA (Owen *et al*, 1979) the Warburg method was combined with serum bottle techniques to attempt to overcome the Warburg disadvantages. Their serum bottles contained liquid and gas phase sampling points for syringe extraction and subsequent analyses.

The study by Owen concluded that the anaerobic bioassay techniques they used were relatively rapid and accurate methods for assessing toxicity. By these methods several variables could be investigated thereby extending the more promising conditions to more detailed studies. Probably the best aspect of the method is its flexibility, enabling both liquid and gas phases to be monitored. In that way, the progress of substrate utilization and intermediate formation and utilization can be monitored simultaneously which are important considerations for identifying the cause and effect of toxicity.

In a later study, Stuckey *et al* (1980) evaluated anaerobic toxicity by both batch and semi-continuous assay methods using four different organic materials: methylene chloride, vinyl acetate, ethylene dichloride, and vinyl chloride. The batch assays utilized 125 ml serum bottles while 1.5 liter CSTR digesters were used in the semi-continuous assay. They observed two of the four chemicals exert similar threshold responses between the batch and semi-continuous assay. The authors noted that in both batch and semi-continuous digestion the ability to acclimate to toxic effects was apparent. In the batch studies acclimation was represented by increased gas production rates after a period of time.

The batch toxicity threshold tends to indicate the lower concentration where concern over possible toxic effects could begin. The method also provides a measure of the concentration of a given substance that would cause a toxic shock-load to a continuous operating system if it were added in a "slug".

Stuckey *et al* (1980) pointed out that with respect to the practical operation of the two assays, the batch method (using the ATA procedure) was considerably quicker, providing information within 5 to 10 days. Semi-continuous operation requires an acclimation period and a longer period of operation to gather data, or about 30 to 60 days.

Koster *et al* (1986) investigated sulfide inhibition of methanogens at various pH levels using a ATA method. The assays were conducted in 1.16 liter serum bottles with six activity measurements per test run. Koster *et al* (1986) reported that the serum bottle anaerobic toxicity assay was a relatively simple technique for quantifying the effect of certain chemicals on methanogenic bacteria.

The ATA method enabled the researchers to perform test runs at a constant pH without the necessity of a pH-controlling apparatus. The assays were

operated at an acetate conversion rate of approximately 30 $u$mol per hour per serum bottle. Such a very small conversion in relation with the amount of acetate present guarantees a nearly constant pH during the tests. In this assay, the authors were able to aquire accurate measurements of the specific methane production rate at a constant pH and a constant sulfide concentration.

The impact of toxic chemicals on an anaerobic system can be evaluated by the system performance. Monitoring the COD removal, volatile acid accumulation, and gas production of a system will assist in determining the toxic effects. Most of the previous research has focused on the volume and rate of gas production as the means of determining toxicity or inhibition. This is because a substance that causes toxicity will affect the methanogenic population first, reducing the amount of gas produced and increasing the accumulation of VFA's and soon thereafter, producing a possible failure of the system.

Inhibition can be quantified approximately by determining the concentration of the chemical that causes a 50% reduction in total gas production over a fixed period of time compared with a feed control. This is referred to as 50% inhibition (Stuckey *et al*, 1980). A study by Owen *et al* (1979) quantified

gas production by computing the ratios between respective rates for samples and the average of the controls, termed maximum rate ratio (MRR). A MRR of less than 0.95 suggested possible inhibition and one less than 0.90 suggested significant inhibition. Not all researchers agree that toxicity of a system is defined by a 50% reduction in gas production. To some that might indicate excessive toxicity while a loss of 10% or greater over a control would indicate an effect of that chemical has taken place. As discussed earlier, a loss of gas production does not solely indicate biological toxicity as in the case of sulfides. Competitive inhibition could explain a loss of gas production without the accumulation of volatile fatty acids. Therefore other performance parameters must be evaluated as well.

Several methods exist that measure the volume and rate of gas production in serum bottles or larger scale anaerobic reactors. The accuracy and precision of these methods is important because total gas production and the rate of production helps define toxicity in anaerobic toxicity assays. Various methods of measurement for gas production and the rate of production include volumetric displacement devices, wet test meters, calibrated pressure manometers, lubricated syringes, manometer-assisted syringes, manual removal with syringes, and automatic anaerobic respirometers.

A comparison study performed by Young *et al* (1991) reviewed the use of syringes as a gas measuring device, similar to the syringes used in this study. The authors reported the method to be reasonably accurate for measuring cumulative gas production rates greater than about 100 ml/d but erratic for hourly measurements and not amenable to automation. Some disadvantages noted were error due to resistance of the syringe to movement and loss of gas through the fluid seal.

The difference between the luer-lock syringes used for this test methodology herein and the comparison study by Young *et al* (1991) was that both the anaerobic seed culture and the resultant biogas produced was in the same syringe wereas the comparison study used a serum bottle for the anaerobic culture and a lubricated syringe as a separate entity, capturing the biogas. Using the vessel to house both the seed culture and the resultant biogas decreased the chances of leaks occurring by not having to connect the two vessels together with tubes.

pH Effects

The pH of an anaerobic reactor is an important process parameter which impacts both the biological metabolism and the equilibrium distribution of many chemical species.

Although methane producing bacteria show optimal growth in the neutral pH region (6.8 to 7.2), the growth of anaerobic acid forming microorganisms is best in the pH range of 5.5 to 6.0 (Grady and Lim, 1980). pH values outside these regions result in a sharp decrease in growth rate and performance.

Sulfide toxicity is very much dependent on pH because unionized hydrogen sulfide is able to pass through the cell membrane (Schlegel, 1981; Speece and Parkin 1983). Changes in enzyme activity as well as membrane permeability due to pH fluctuations represent possible mechanisms of pH effects on microbial growth. Sulfates entering the anaerobic environment are quickly reduced to sulfide as shown in the following equation:

$$8H^{+} + 8e^{-} + SO^{=}_{4} \text{--------}> S^{=} + 4H_2O$$

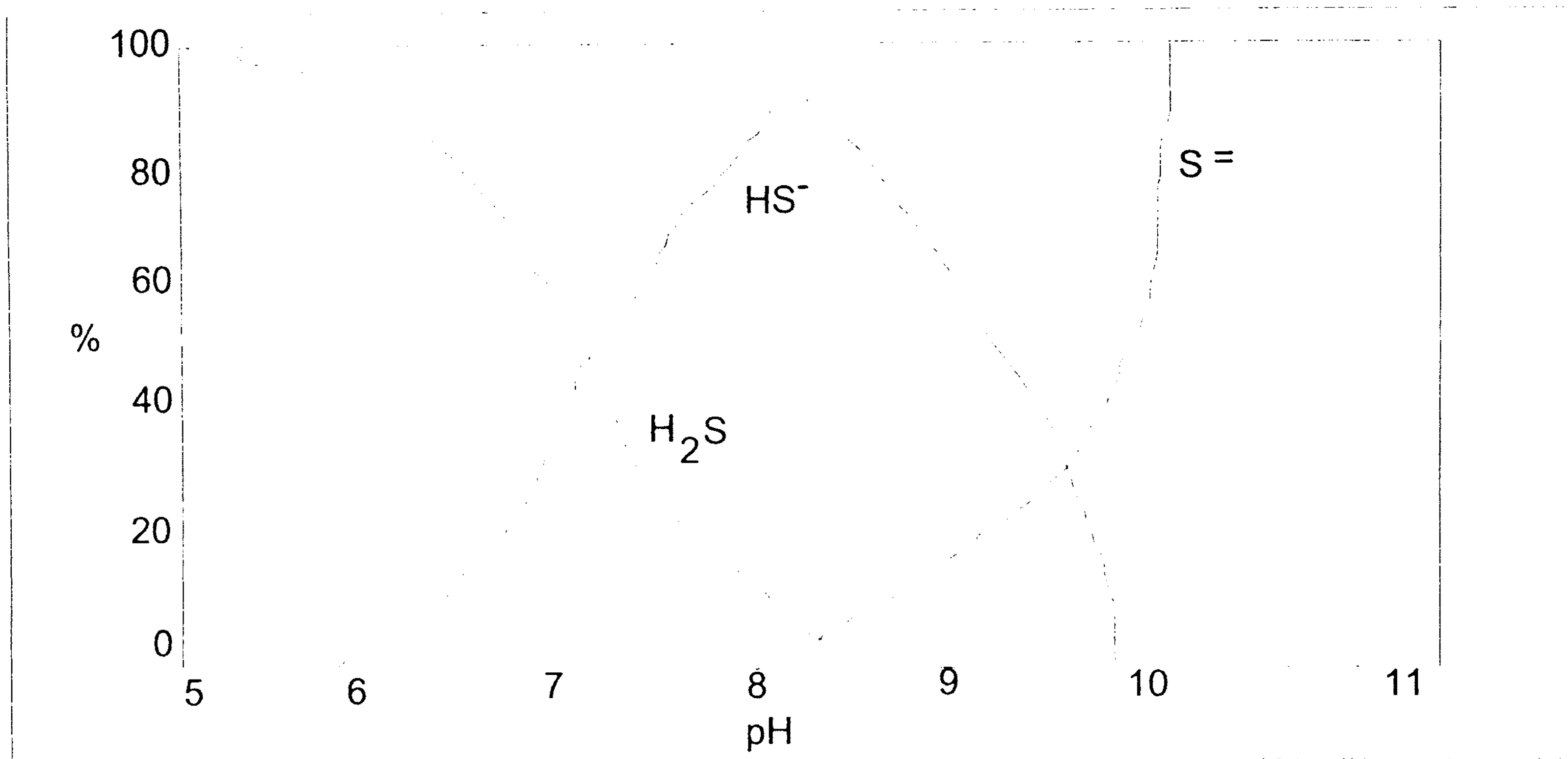
Sulfates serve as a source of an electron acceptor for biochemical oxidations. Under anaerobic conditions, the sulfate ion is reduced to sulfide ion, which establishes an equilibrium with hydrogen ion to form hydrogen sulfide in accordance with its primary ionization constant $K_1 = 9.1 \times 10^{-8}$. Under stable fermenter operation, the sulfide ions will hydrolyze resulting in the production of the sulfide species: HS^{-}, H_2S (aq), H_2S (g). The equilibrium distribution of these species is a function of pH and temperature (McFarland,1982).

The relationships existing between H_2S, HS^- and $S^=$ at various pH levels in a 10^{-3} molar solution are shown in Figure 3. At pH levels of 8.0 and above, most of the reduced sulfur exists in solution as HS^- and $S^=$ ions, while at pH levels below 8.0, the equilibrium shifts rapidly toward the formation of unionized H_2S and is about 80% complete at pH 7.0 (Sawyer and McCarty, 1978). Therefore, the stripping of H_2S with the biogas will be lower at higher pH and higher at lower pH (Stover *et al*, 1992). Hence, by operating the anaerobic reactor at a slightly acidic pH (6.7 to 6.9), more sulfides can be stripped in the biogas resulting in lower bulk liquid sulfide concentrations.

For many high sulfate wastewaters, there will be difficulties in controlling the bulk liquid sulfides below the toxic levels with pH control alone. A common strategy used for the control of bulk liquid sulfides is by precipitation of the sulfides in the reactor.

Temperature

Temperature is one of the most important environmental factors which influence both growth and survival of microorganisms. Temperature not only influences the metabolic activities, such as enzymatic reactions of the microbial population, but also has a profound effect on such factors as the diffusion rate of substrate into the cell (Grady and



Source: Sawyer and McCarty, 1978

Figure 3. Effect of pH on hydrogen sulfide-sulfide equilibrium

Lim, 1980).

It has been demonstrated that the microbial growth rate increases with increasing temperature (Grady and Lim,1980). The benefits of increased temperatures have limits because cellular components such as nucleic acids and proteins are very sensitive to temperature. A macromolecule like protein will actually denature when exposed to high temperatures. Therefore, there exists an optimum temperature at which maximum growth rate occurs (Brock *et al*, 1971).

According to the temperature range in which they function best, bacteria may be classified as psychrophilic, mesophilic, or thermophilic. Psychrophilic microorganisms are those organisms which operate in an optimum range of 12-18^{0}C. Mesophilic microorganisms have temperature optima in the range of 25 to 40^{0}C, while thermophiles display optimal growth in the 55 to 65^{0}C temperature range (Metcalf and Eddy, 1991).

Temperature also influences the equilibrium distribution of many aqueous species. Under equilibrium conditions, the concentrations of soluble species are governed by the equilibrium constant, K. The temperature dependence of K is described by the following

equation:

$$K = \exp(-AG/RT)$$

where, AG = Gibbs free energy at standard conditions
R = Gas constant
T = Absolute temperature

The equilibrium relationship between ionized (HS^-) and unionized ($H_2S(aq)$) soluble sulfide at 25°C was given by the equation $H_2S(aq) <\text{------}> HS^- + H^+$ ($K_1 = 10^{-7}$). Table 1 illustrates the influence temperature has on the absorption coefficient. If certain conditions were kept constant such as pH, gas production, and influent sulfate concentration while increasing the reactor temperature, one would be able to detect lower sulfide levels in the bulk liquid.

Although increasing the reactor temperature along with maintaining proper pH would reduce the level of sulfides in the bulk liquid, the cost of heating the reactor may prove to be more expensive than precipitating the sulfides with a metal salt.

Sulfide Production And Distribution

An evaluation of the possible effects of sulfides on anaerobic treatment must consider the quantity of sulfides either entering the reactor or produced during treatment

TABLE 1

VALUES FOR SULFIDE EQUILIBRIUM CONSTANTS

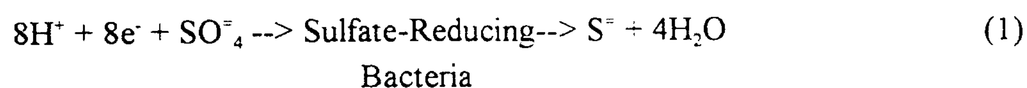
Temperature (^0C)	Ionization Constant K_1	Absorption Coefficient α
18	$9.1x10^{-8}$	2.72
25	$11.2x10^{-8}$	2.28
35	$14.9x10^{-8}$	1.83
45	$19.4x10^{-8}$	1.52

Source: Lawrence and McCarty, 1966

and how these sulfides are distributed within the reactor (Lawrence *et al*, 1966). Sulfides in anaerobic treatment can result from (1) introduction of sulfides with the raw wastewater and/or (2) biological production in the reactor from reduction of sulfates and other sulfur containing inorganic and organic compounds. Unlike most mammals, which are unable to reduce sulfate to sulfide for the biosynthesis of sulfur containing compounds (such as amino acids (e.g., cysteine and methionine), biotin, thiamine, and coenzyme A), most bacteria do have this ability (Muth and Oldfield, 1970).

The anaerobic environment fulfills the conditions necessary for sulfate reduction, namely, low oxidative reduction potential (ORP), presence of degradable organic matter, proper temperature, and sulfates (Heukelekian, 1948). The principal organism involved in sulfate reduction is believed to be *Desulfovibrio desulfuricans* under mesophilic conditions. This organism is a strict anaerobe. It derives energy for synthesis and maintenance from the metabolism of organic matter, and uses sulfate as its terminal electron acceptor.

The reduction of sulfates in this process is expressed by the following equation:



$$8H^+ + 8e^- + SO_4^= \xrightarrow[\text{Bacteria}]{\text{Sulfate-Reducing}} S^= + 4H_2O \qquad (1)$$

The reduction of one mole of sulfur (32g) by this process corresponds to the oxidation of eight equivalents of organic matter, or about 64 grams on a COD or oxygen-equivalent basis. Sulfides in the reactor may be present in soluble or insoluble form, depending upon the cations with which they are associated. The soluble sulfide forms a weak acid which ionizes in aqueous solution, the extent depending upon the pH. Thus, it is possible to have H_2S, HS^-, and $S^=$ in solution. Around the neutral pH conditions required for anaerobic treatment, only the first dissociation of hydrogen sulfide is of importance, as follows:

$$H_2S \longleftrightarrow H + HS^- \quad (2)$$

Because of the limited solubility of hydrogen sulfide, a certain portion of the hydrogen sulfide formed will escape with the biogas produced. The resulting equilibrium between the hydrogen sulfide remaining in the reactor bulk liquid and that existing in the biogas phase is governed by Henry's Law. Consideration of the relationship between pH and the different forms of soluble sulfides, as well as the solubility of hydrogen sulfide itself, allows prediction of the distribution of the sulfides between the biogas and aqueous phases. The equation developed by Lawrence *et al* 1966 to predict the distribution of the sulfides is as follows:

$$T*S*S/H_2S(g) = \alpha(1 + K_1/H^+) \qquad (3)$$

where,

T*S*S= Bulk liquid total concentration of soluble sulfides, mg/l
$H_2S(g)$= Biogas H_2S concentration, mg/l
α = Absorption coefficient
K_1= Equilibrium constant

The absorption coefficients, α, for sulfides are shown in Table 1 . From Equation 3, it is possible to calculate the equilibrium ratio for the concentration of soluble sulfides in the reactor bulk liquid to the concentration of hydrogen sulfide in the biogas, knowing only the reactor pH and temperature. The quantity of soluble sulfides in the reactor bulk liquid is related to several factors: (1) the total quantity entering and produced within the reactor, (2) the quantity lost through precipitation by heavy metals, and (3) the quantity which escapes with the biogas. The quantity of soluble sulfides which escapes with the biogas is considerable. The quantity of sulfides lost in the biogas is related to the relative quantity of biogas produced each day, as well as the relationship with aqueous soluble sulfide as represented by Equation 3.

The relationship between the quantity of soluble sulfides entering or formed within the reactor and the quantity which is lost in the biogas is as follows:

$$VwSw = VgSg + VwSe \qquad (4)$$

where,

Vw = volume of wastewater entering or leaving the reactor per day
Vg = volume of biogas produced per day
Sw = concentration in mg/l of soluble sulfides or soluble sulfide precursors in the raw wastewater
Sg = sulfide concentration in mg/l in biogas produced
Se = soluble sulfide concentration in mg/l in reactor effluent

The ratio of soluble sulfides in the reactor effluent to that in the biogas at equilibrium is related to equation (3) as follows:

$$Se/Sg = \frac{(T.S.S.)}{(H_2S\ g)} = \alpha \left[\frac{1 + K_1}{(H^+)} \right] = A \qquad (5)$$

A is a constant for any given pH and temperature. Combining Equations (4) and (5) gives:

$$VwSw = VgSe/A + VwSe \qquad (6)$$

or

$$Se/Sw = 1/\ 1 + Vg/AVw \qquad (7)$$

or

$$Sg/Sw = 1/\ A + Vg/Vw \qquad (8)$$

From the relationships expressed in equations (7) and (8), it is possible to calculate the total soluble sulfide concentrations and undissociated sulfide (H_2S) concentrations in the reactor bulk liquid as well as H_2S concentration in mg/l in the biogas produced. The

volume of wastewater to be treated per day, volume of biogas produced per day, and all forms of sulfide precursors (like sulfates) in the wastewater have to be known.

In the present test methodology using 125 ml luer-lock syringes, the volumes of sample for analysis were limited because of the total volume available in the syringe. Subsequently, there was not enough sample volume to analyze for bulk liquid or biogas sulfides. Sulfide balance calculations using the Lawrence and McCarty equations have been successful in predicting the partitioning of bulk liquid and gas hydrogen sulfide. In a study by Stover *et al* (1992) concerning the control of bulk liquid sulfide toxicity, measured sulfides and theoretical sulfides using these equations were compared. Table 2 represents the program used to balance sulfide partitioning.

Three 14.5 liter UASB continuous feed reactors were used in the study by Stover *et al* 1992. Based on the feed sulfate concentration, feed flow rate, and biogas production rate, theoretical sulfide partitioning analyses were conducted using equations 3, 7, and 8. One reactor was operated at an influent sulfate concentration of 4,000 mg/l with magnesium hydroxide as an alkalinity source and sulfide precipitating agent. A second reactor was operated at an influent sulfate concentration of 2,600 mg/l with sodium hydroxide as the alkalinity source. A control reactor received only 90 mg/l sulfate.

TABLE 2

ANAEROBIC REACTOR (SULFATE)

SULFIDE BALANCE CALCULATIONS BASED ON PARTITIONING

DESIGN CONDITION

Reactor operating temperature, OC	=	35
Absorption coefficient, alpha	=	1.83
Ionization constant, K1	=	1.4900000E-07
pH	=	6.8
Volume of wastewater (Vw), l/d	=	3
Volume of gas (Vg), l/d	=	75
Sulfate conc. of wastewater, mg/l	=	4000
Sulfide conc. of wastewater, mg/l	=	1333

CALCULATIONS

Ratio of soluble sulfides in the reactor liquid to gas at equillibrium

Se/Sg = alpha(1+(K1/(H+)))

Where,
alpha = absorption coefficient
K1 = ionization constant
Se = soluble sulfide in reactor liquid, mg/l
Sg = sulfide in gas, mg/l

Se/Sg = A	=	3.55

Se/Sw = 1/(1+(Vg/(A*Vw)))

Se/Sw	=	0.1244
Sulfide conc. in the reactor liquid, mg/l	=	166

Sg/Sw = 1/(A+(Vg/Vw))

Sg/Sw	=	0.0350
Sulfide conc. in the gas, mg/l	=	46.70
Percentage of H2S in the gas	=	3.27

Source: Stover et al, 1992

The results of this sulfide balance are presented in Table 3. In the reactor receiving 4,000 mg/l sulfates, the actual measurable bulk liquid sulfides were 100 mg/l. The theoretical bulk liquid total soluble sulfide concentration for this condition, assuming no sulfide precipitation in the reactor, was 210 mg/l. The average observed biogas hydrogen sulfide concentration for this condition was 21.2 mg/l. Using the measurable biogas hydrogen sulfide in the partitioning program, the bulk liquid sulfide concentration was calculated to be 96 mg/l. This sulfide value agreed very closely with the actual measured value of 100 mg/l in the bulk liquid. Since there were no sulfates detected in the reactor bulk liquid and magnesium was the only metal cation added in significant quantity to the reactor bulk liquid, it was apparent that around one-half of the sulfides were precipitating out of solution.

In order to verify the magnesium precipitation reaction hypothesis, the reactor operated at an influent sulfate concentration of 2,600 mg/l with sodium hydroxide as the alkalinity source was run through a sulfide partitioning study. The average measured total soluble sulfide in the reactor bulk liquid at this condition was 100 mg/l. The theoretical total soluble sulfides assuming no sulfide precipitation in the reactor were 110 mg/l. The average observed biogas hydrogen sulfide concentration was 27.0 mg/l. Using the measured biogas hydrogen sulfide

TABLE 3

SULFIDE BALANCE ANALYSES

Description	Reactor 1 (control)	Reactor 2	Reactor 3
Feed			
Flow Rate, l/d	2.8	2.9	2.9
Sulfates ($SO^{=}_4$), mg/l	90	4,000	2,600
Sulfide (S), mg/l	30	1,330	870
Biogas			
Biogas Production, l/d	69	66	67
Measured H_2S, mg/l	2.2	21.2	27.0
Reactor			
Bulk Liquid pH Range, s.u.	6.5-6.8	7.0-7.1	6.7-6.9
Theoretical Total soluble sulfides without sulfide precipitation, mg/l	13	210	110
Theoretical Total soluble sulfides based on actual biogas H_2S, mg/l	7	96	96
Measured Total Soluble Sulfides, mg/l	8	100	100

Source: Stover, Brooks, and Munirathinam, 1992

concentration in the partitioning program, a theoretical total soluble sulfide concentration in the bulk liquid was calculated to be 96 mg/l. The results of this test show that the partitioning program was able to predict the amount of sulfur in the bulk liquid and gaseous phase to some accuracy. This sulfide partitioning program is therefore a useful tool for this (ATA) method when sulfide toxicity is being tested because of the limited sample volume available.

Bulk liquid and gaseous hydrogen sulfide concentrations were predicted in this study by taking the difference between the initial and ending sulfates for each syringe. That sulfate concentration represented what was reduced. The biogas production, pH, temperature, and volume of wastewater were used to calculate sulfide partitioning for each syringe.

CHAPTER III

MATERIALS AND METHODS

Experimental Procedure

The experimental portion of this study was conducted in two phases: one, to define what influent sulfate concentration caused inhibitory conditions, and two, to control the sulfide inhibition by precipitating the soluble sulfides with either ferric chloride, ferrous chloride, magnesium hydroxide or a combination of ferric and magnesium. The first phase focused on defining operational conditions such as F/M ratios and also selecting stock solution concentrations that were appropriate for the total volume of the syringe. Six glass syringe reactors were used in this study. Figure 4 shows the syringes in their test position. The syringes had a total volume of 125 ml with a 100 ml measurable volume. The total liquid volume had to be restricted to about 80 ml to allow for measurable gas production. The syringes were equipped with lure-lock valves on one end which enabled the biogas produced to be evacuated from the vessel easily and also insured that the vessel

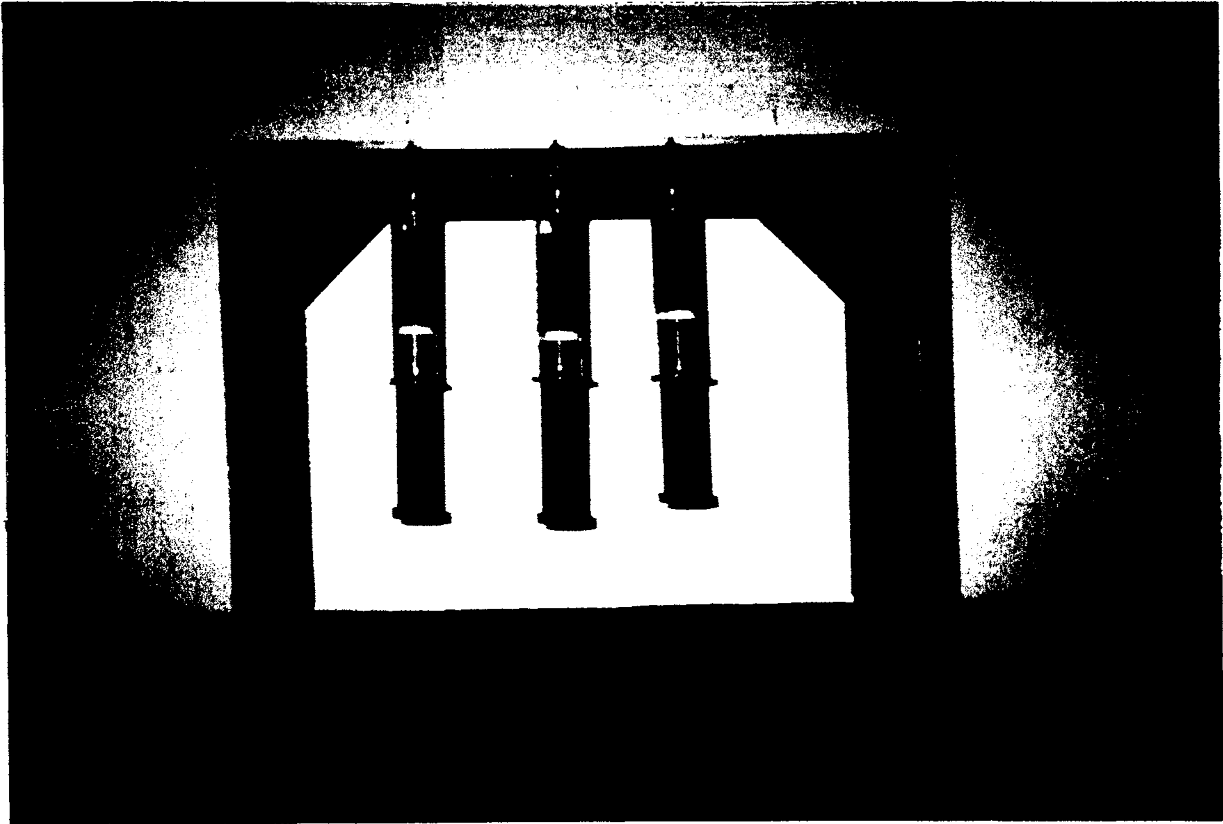


Figure 4. Luer-Lock Syringes

was air tight and could build pressure back up again. A synthetic sucrose wastewater (simulated high strength carbohydrate wastewater) was used as the principal carbon and energy source.

Table 4 presents the feed stock solutions and their concentrations used in this study. Sodium sulfate (Na_2SO_4) was used as the sulfate source. Sodium sulfate was chosen as the sulfate sulfur source for several reasons. First, sodium sulfate is soluble in the digester environment. Secondly, in the range of sulfate concentrations, sodium toxicity was not a concern. Finally, the use of sulfide precursors rather than sulfide containing compounds simulated the expected major source of sulfides in field digesters and also avoided the many problems inherent in the use of a solution of soluble sulfides. Adequate macronutrients (nitrogen and phosphorus) were added to all syringes to insure that these were not growth limiting factors. Micronutrients (Mo, Ni, Cu, Co, and Zn) were added in the amounts listed in Table 5 to insure that these micronutrients were not growth limiting factors.

The test syringes were designated as Reactor A through F according to the amounts of sodium sulfate and precipitation chemicals added. As noted earlier, there were two phases to the study. The first phase defined what level of influent sulfate

TABLE 4

FEED STOCK SOLUTION FOR THE ANAEROBIC BATCH STUDY

Chemical	Concentration, gm/l
Sucrose	3.3
Ammonium Chloride (NH_4Cl)	1.0
Ammonium Phosphate Dibasic (NH_4PO_4)	0.5
Sodium Sulfate (Na_2SO_4)	1.5

Note: The concentration of sodium in sodium sulfate was not enough to be considered as a possible source of toxicity as reported by Kugelman and McCarty (1964) to be 6g/L.

TABLE 5

MICRONUTRIENT ADDITIONS

Chemical	Concentration
$(NH_4)^{*}\ 6Mo_7O_{24}$	0.10 mg/l as Mo
$NiCl_2 * 6H_2O$	0.10 mg/l as Ni
$CuSO_4 * 5H_2O$	0.10 mg/l as Cu
$CoCl_2 * 6H_2O$	0.10 mg/l as Co
$ZnSO_4 * 7H_2O$	0.10 mg/l as Zn

concentrations showed signs of inhibition. The first five test runs were used to define these concentrations as well as other operating data. There were a total of seven tests performed to define the experimental data. Syringe A represented the control, receiving small quantities of sodium sulfate for stimulatory purposes. Syringe B was operated at 300 mg/l and 600 mg/l $SO^{=}_4$ in the first phase. Syringe C was operated at 450 mg/l and 900 mg/l $SO^{=}_4$ in the first phase. Syringe D was operated at 600 mg/l and 1200 mg/l $SO^{=}_4$ in the first phase. Syringe E was operated at 750 mg/l and 1500 mg/l $SO^{=}_4$ in the first phase. Syringe F was operated at 900 mg/l and 1800 mg/l $SO^{=}_4$ in the first phase. All syringes in tests number 6 and 7 were fed 1500 mg/l $SO^{=}_4$.

The anaerobic seed source for each test was from a 20 liter bench-scale upflow anaerobic sludge blanket hybrid reactor. The seed source reactor was operated in a continuous flow mode and was fed a synthetic sucrose wastewater low in sulfate concentrations. However, at one time this seed source reactor was fed high influent sulfate concentrations, around 4,000 mg/l. This fact will be taken into consideration in the results and discussion section of this paper. Before each test run, the feed to the seed source reactor was shut off allowing the anaerobic bacteria to reduce the majority of the organics, thus reaching endogenous conditions. The effluent of the bench scale reactor was analyzed for pH, sCOD, and alkalinity/volatile acids before each test to insure the culture was healthy.

In each syringe a mixture of sodium sulfate, sucrose, anaerobic bacteria, macronutrients, and micronutrients were added and mixed together. Table 6 presents a typical feed stock solution for a test run. Each syringe was analyzed for pH and sCOD once everything was mixed together. The total volume of each syringe varied, and the difference was made up with deionized water. The pH of each syringe was analyzed and adjusted to 6.8-7.0 s.u. before the impinger was placed inside the syringe which was pushed in to evacuate all the air out of the vessel. The luer-lock was then closed to allow gas to build up inside the syringe. The syringes were stored in a vertical position in a temperature controlled room at around 35^{0}C throughout the duration of the study. The syringes were inverted and mixed at least twice per day. Gas production was measured daily by taking the difference of the beginning volume from the final volume over a twenty four hour time period. Gas quality on each syringe was analyzed throughout each test run. Each test was run until gas production ceased or became virtually immeasurable over several days. Once gas production stopped, the contents of the syringes were analyzed for pH, sCOD, volatile acids, alkalinity, and sulfate.

The first phase of this study was used to define the operating conditions such as the influent sulfate concentration which causes inhibition, F/M ratio, and the concentration of NH_3-N, PO^{-3}_4-P, and sucrose stock solutions. The concentrations of the stock solutions were important because of the restricted volume of the syringe. In order to

TABLE 6

TYPICAL FEED STOCK SOLUTION FOR THE ANAEROBIC TOXICITY ASSAY

Syringe I.D.	NA_2SO_4 (ml)	SUCROSE (ml)	NH_4Cl (ml)	KH_2PO_4 (ml)	Micronutrients (ml)	Bacteria Seed (ml)	Make-up Water (ml)	Total Vol. (ml)
A	0.35	10	3	2	2	55	9.65	82
B	3.3	10	3	2	2	55	6.7	82
C	5.0	10	3	2	2	55	5.0	82
D	6.6	10	3	2	2	55	3.4	82
E	8.3	10	3	2	2	55	1.7	82
F	10	10	3	2	2	55	0.0	82

successfully reduce the sulfates to sulfides, the syringes were operated under similar organic loading conditions. The F/M ratio needed to be high enough to achieve sulfate reduction yet low enough to avoid the possibility of shocking the bacteria with an organic load in a batch system. Once a concentration of influent sulfate that exhibited signs of inhibition and a F/M ratio that supplied plenty of carbon for sulfate reduction was defined, the second phase began.

Sulfide Precipitation Study

During this second phase, three different chemical compounds were evaluated for their sulfide complexing abilities: ferric chloride, ferrous chloride, and magnesium hydroxide. Table 7 lists the concentration of each chemical compound. The chemical compounds were calculated stoichiometrically to complex approximately 50% of the resultant sulfides, or just below known inhibition levels (200 mg/l).

Test runs 6 and 7 began much like the previous five tests. Stock solutions of nitrogen, phosphorous, sucrose, sodium sulfate, deionized water and anaerobic seed were combined together in the glass syringes. Calculated volumes of the precipitating chemical compounds were added to the syringes last, and the contents were mixed and analyzed for pH. The pH of each syringe was adjusted with either 10% sodium hydroxide (NaOH) or 5N hydrochloric acid (HCl). The syringes were then sealed with the impinger,

TABLE 7

CHEMICAL COMPOUNDS FOR SULFIDE PRECIPITATION STUDY

Chemical	Concentration gm/l
Ferric Chloride, $FeCl_3$	10.0
Ferrous Chloride, $FeCl_2$	26.0
Magnesium Hydroxide, $Mg(OH)_2$	14.5

purged of all air, mixed, and hung vertically in a temperature controlled room at 35° C.

Wet Chemistry Analysis

All samples for wet chemistry analysis were obtained from the syringes before and after each test run. The contents of the syringes were mixed well before a sub-sample was removed for testing. Samples which required filtration before testing were poured through a 4.25 micron filter to remove suspended solids.

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 was 0-1500mg/l. In this method, 5ml aliquots of anaerobic effluent were filtered. The test volume of each COD vial was 1.5 ml consisting of concentrated sulfuric acid (H_2SO_4), mucuric sulfate ($HgSO_4$), and silver sulfate. The total sample volume added to each COD vial was 2ml. This 2ml volume could be a combination of undiluted volumes or dilutions of the anaerobic effluent. Once the correct dilution factors were determined to detect COD within the range of the method, the vials were analyzed colormetrically by measuring the absorbency at 620nm by a HACH DR/3 spectrophotometer. In each batch of tests a standard COD reagent was analyzed to check the accuracy of the test, and samples were run in duplicate to verify reproducibility.

Total And Volatile Suspended Solids (TSS/VSS)

The total and volatile suspended solids determinations were made according to Standard Methods 18th Edition (1992). Total suspended solids (TSS) were dried in a Fisher Isotemp 500 oven at 103°C-105°C for over two hours. Volatile suspended solids (VSS) were ignited in a Linberg furnace at 550°C + 50°C for fifteen minutes. All solids analyses were run in duplicate to check reproducibility. The weight of each sample was determined on an Ohaus GA200D balance. The balance accuracy was checked each day with type S weights in the range tested.

Sulfate

Sulfate ($SO^{=}_4$) was determined turbidimetrically using HACH sulfaver 4 reagent and methods. Filtered samples were mixed with the contents of one sulfaver 4 sulfate reagent powder pillow and measured on a HACH DR/3 spectrophotometer at a wavelength of 450nm. A white turbidity developed when sulfate was present. A HACH sulfate standard solution, 100 mg/l as $SO^{=}_4$, was used to check the accuracy of the test. Samples were also run in duplicate to check reproducibility.

Alkalinity

Total alkalinity was determined titrametrically according to Standard Methods 18th Edition (1992). The initial pH of the anaerobic effluent (50ml) was lowered to 4.5s.u. using 0.5N H_2SO_4. That volume of acid was recorded and used to calculate alkalinity.

Volatile Fatty Acids

Volatile fatty acids (VFA) were determined titrametrically according to Standard Methods 18th Edition (1992). The pH of a 50ml sample of anaerobic effluent, previously lowered to 3.5 s.u.,boiled for three minutes and cooled after alkalinity determinations, was raised from approximately 3.5s.u. to 4.5s.u. using 0.05 N NaOH. The sample pH was then raised from a pH of 4.5 to 7.0 with 0.05N NaOH and the volume titrated was used for VFA determination.

pH

The pH of all liquids in this study was determined by a Fisher Accumet meter 900. The pH meter was calibrated each day with pH buffers to insure accuracy.

Gas Analysis: Volume Production rate

The gas production rate and cumulative volumes for each test were measured in the glass syringe vessel by monitoring the volume displaced every twenty-four hours. Biogas quality samples were obtained from the luer- lock end of the syringe.

Methane Content (%CH_4)

The methane content of the biogas was determined by removing a representative sample of the biogas and injecting it into a Hewlett Packard gas chromatograph model 9100. The biogas was prepared for analysis by inserting a silicone tube over the end of the lure- lock. The lock was then opened and gas was forced through the tube by pushing on the impinger. As the biogas was flowing through the tube one end was clamped off to capture the gas. Using a small syringe and needle, one milliliter of biogas was removed from the tube and injected into the chromatograph. A 100 percent methane standard was run before each batch of samples were analyzed to calibrate the instrument.

Gaseous Hydrogen Sulfide

Gaseous hydrogen sulfide (H_2S) concentrations (mg/l) and percentages (%) were determined theoretically using Lawrence and McCarty (1966) equations. The initial and final sulfate concentrations were determined turbidimetrically, and

the difference was used for the partitioning. The hydrogen sulfide gas was not analyzed, but rather predicted with the help of a computer model.

CHAPTER IV

RESULTS

The results of this study are reported in two sections. The first is the set of tests that defined what level of influent sulfate concentration caused biological inhibition. This phase also defined certain test conditions. The second phase experimented with sulfide complexing compounds.

Sulfide inhibition study

Tests one through five were devoted entirely to defining specific test conditions and what concentration of influent sulfates would reduce to sulfides and cause biological inhibition. The seed source for the reactors was taken from a 20 liter hybrid reactor which was fed a simulated high strength wastewater. The syringes were operated at a consistent temperature around 35^{0}C. Table 8 presents the operational conditions for the anaerobic toxicity assay.

TABLE 8

OPERATIONAL CONDITIONS FOR THE ANAEROBIC TOXICITY ASSAY

TEST #	TEMP. C	pH s.u.	Soluble COD Initial (mg/l)	Org. Load gm COD/L	F/M gmCOD/gm VS	SO4 Initial (mg/l)
1	35	6.8	1850	1.7	1.25	50-900
2	35	6.85-6.9	1375-1500	1.3	0.63-0.74	50-900
3	35	6.7-7.5	2125-2525	2.3	1.2-1.4	50-900
4	35	6.7-6.8	5575-5800	4.9	3.1-3.2	50-1800
5	35	6.8	4250-5200	4.1	1.0-1.24	50-1800
6	35	6.8	3450-4400	4.7	0.7-0.9	1500
7	35	6.9-7.0	4000-5300	4.7	0.8-1.0	50-1500

TEST 1

The average syringe reactor operating conditions and performance summaries are presented in Table 9. Trend plots of the syringes for test one are presented in Figure 5. Tables 5 and 9 are duplicated in Appendix A and B respectively. The average F/M ratio in test 1 was 1.25 mg COD/mg VSS. The initial sulfate concentrations ranged from 50mg/l to 900mg/l. The COD removal for the control syringe was 74% with an average 66% methane content in the biogas. The average biogas production rate was 0.547L/g COD removed. Syringe B was fed 150mg/l $SO^{=}_4$. The COD removal was 73% with an average 65% methane content in the biogas. Gas production was 128% of the control. The average biogas production rate was 0.713L/g COD removed. Syringe C was fed 300mg/l $SO^{=}_4$. The COD removal was 71% with an average 67% methane content in the biogas. Gas production was 131% of the control with an average gas production rate of 0.750L/g COD removed.

Syringe D was fed 450mg/l $SO^{=}_4$. The COD removal was 70% with an average 50% methane content in the biogas. Gas production was 123% of the control with an average gas production rate of 0.711L/g COD removed. Syringe E was fed 600mg/l $SO^{=}_4$. The COD removal was 69% with an average 64% methane content in the biogas.

TABLE 9

TEST # 1
ANAEROBIC BATCH STUDY
SUMMARY WORKSHEET

DESCRIPTION	SYRINGE I.D. A	B	C	D	E	F
AVERAGE OPERATING CONDITIONS						
pH, s.u.	6.8	6.8	6.8	6.8	6.8	6.8
sCOD, mg/l	1850	1850	1850	1850	1850	1850
F/M	1.25	1.25	1.25	1.25	1.25	1.25
SO4, mg/l	50	150	300	450	600	900
ALKALINITY, mg/l	3200	3200	3200	3200	3200	3200
REACTOR VFA, mg/l	675	675	675	675	675	675
PERFORMANCE DATA						
pH, s.u.	7.6	7.4	7.4	7.5	7.5	7.5
EFFLUENT sCOD,mg/l	480	500	540	550	575	560
COD Removal, %	74	73	71	70	69	70
SO4, mg/l	0	100	150	220	290	410
LIQUID SULFIDE, mg/l	16	16	49	75	102	161
GAS SULFIDE, mg/l	4.6	4.6	14	21	29	46
GAS PROD., ml	60*	77*	79	74	65	50
GAS PROD., L/g CODr	0.547	0.713	0.750	0.711	0.637	0.485
GAS PROD., % OF CONTROL	-	128	131	123	108	83
H2S, %	0.32*	0.32*	1	1.5	2	3
CH4, %	66	65	67	50	64	67
ALKALINITY,mg/l	2990	2340	2766	2857	3010	3281
REACTOR VFA, mg/l	350	350	350	350	350	350

* Data does not correlate

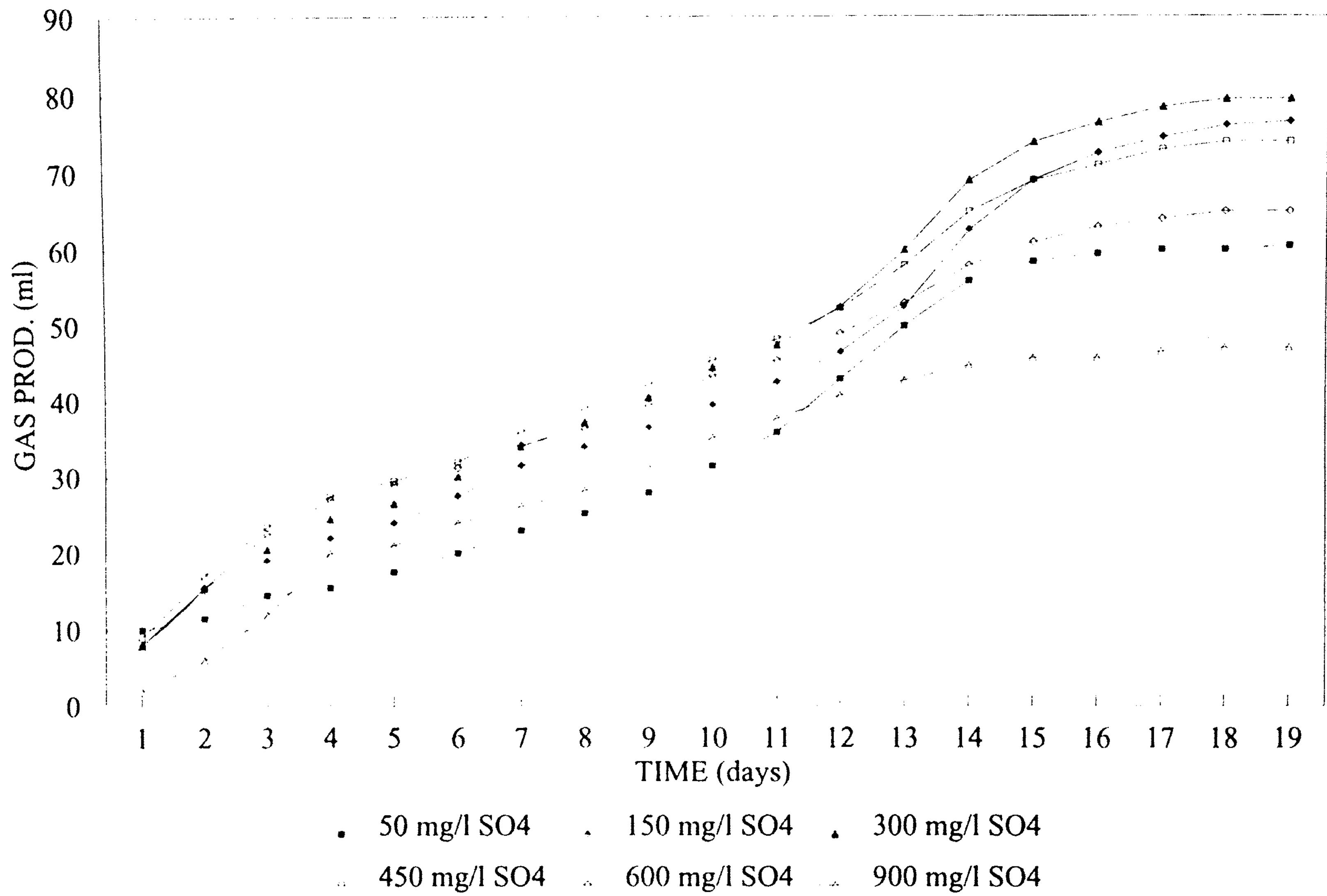


Figure 5. Cumulative Gas Production For Test 1

Gas production was 108% of the control. The average gas production rate was 0.637L/g COD removed. Syringe F was fed 900mg/l $SO^{=}_{4}$. The COD removal was 70% with an average 67% methane content in the biogas. Gas production was 83% of the control. The average gas production rate was 0.485L/g COD removed.

TEST 2

Average syringe reactor operating conditions and performance summaries are presented in Appendix A, Table A-2. Trend plots of the syringes for test two are presented in Appendix B, Figure B-2. In test two the influent sulfate concentration varied somewhat in the syringes but did not increase above 900mg/l. An important change in test two was lowering the F/M ratio from 1.25 in test one to around 0.7. This ratio continued to provide enough carbon source to reduce the sulfates to sulfides and lower the chances of organic shock to the bacteria in the batch system. Syringe A was operated as the test control receiving only 50mg/l $SO^{=}_{4}$. The COD removal was 45% with an average 66% methane content in the biogas. The average gas production rate was 0.820L/g COD removed. Syringe B was fed 300mg/l $SO^{=}_{4}$. The COD removal was 52% with an average 65% methane content in the biogas. Gas production was 102% of the control. The average gas production rate was 0.689L/g COD removed.

Syringe C was fed 450 mg/l $SO^{=}_{4}$. The COD removal was 52% with an average 67% methane content in the biogas. Gas production was 88% of the control and the production rate was 0.581L/g COD removed. Syringe D influent sulfate concentration was 600mg/l. The COD removal was 42% with an average 50% methane content in the biogas. Gas production was 66% of the control syringe with a gas production rate of 0.614L/g COD removed. Syringe E was fed 750mg/l $SO^{=}_{4}$. The COD removal was 53% with an average 64% methane content in the biogas. Gas production was 78% of the control and the gas production rate was 0.485L/g COD removed. Syringe F was fed the highest sulfate concentration, 900mg/l. The COD removal was 49% with an average 67% methane content in the biogas. Gas production was 80% of the control and the gas production rate was 0.550L/g COD removed.

TEST 3

Test number three was run using similar concentrations of influent sulfate as test two, however the F/M ratio was increased to around 1.3 mg COD/mgVSS. The average syringe operating conditions and performance summaries are presented in Appendix A, Table A-3. Trend plots of the syringes for test three are presented in Appendix B, Figure B-3. Syringe A was operated as the test control receiving only 50 mg/l $SO^{=}_{4}$ in the stock feed solution. The COD removal was 83% with an average 80% methane content in the

biogas. The gas production rate was 0.789L/g COD removed. Syringe B was fed 300mg/l $SO^{=}_4$. The COD removal was 76% with an average 76% methane content in the biogas. Gas production was 91% of the control and the gas production rate was 0.936L/g COD removed. Syringe C was fed 450mg/l $SO^{=}_4$. The COD removal was 72% with an average 79% methane content in the biogas. Gas production was 89% of the control. The gas production rate was 0.949L/g COD removed.

Syringe D was fed 600mg/l $SO^{=}_4$. The COD removal was 76% with an average 75% methane content in the biogas. Gas production was 93% of the control and the gas production rate was 0.819L/g COD removed. Syringe E was fed 750 mg/l $SO^{=}_4$. The COD removal was 73% with an average 77% methane content in the biogas. The gas production rate was 0.927L/g COD removed. Gas production was 91% of the control. Syringe F was fed 900 mg/l $SO^{=}_4$. The COD removal was 70% with an average 71% methane content in the biogas. Gas production was 84% of the control and the gas production rate was 0.899L/g COD removed..

<u>TEST 4</u>

Test number four was operated at higher influent sulfate concentrations and higher F/M ratios. The soluble COD in each syringe averaged 2281 mg/l in test three and

5500 mg/l in test four. The higher CODs resulted in an F/M of around 3.0 mg COD/mg VSS, an F/M ratio which was higher than planned. The conditions created an organic shock load in the batch syringes and the results are reported for information reasons. Average syringe operating conditions and performance summaries are presented in Appendix A, Table A-4. Trend plots of the syringes for test four are presented in Appendix B, Figure B-4. Gas production rates were not reported because they were erroneously high. The syringes that performed poorly had unusually high calculated gas production rates. Syringe A was operated as the test control receiving only 50 mg/l $SO^{=}_4$ in the stock solution. The COD removal was 8% with an average 24% methane content in the biogas. Syringe B was fed 600 mg/l $SO^{=}_4$. The COD removal was 10% with an average 22% methane content in the biogas. Gas production was 108% of the control. Syringe C was fed 900 mg/l $SO^{=}_4$. The COD removal was 8% with an average 24% methane content in the biogas. Gas production was 85% of the control.

Syringe D was fed 1200 mg/l $SO^{=}_4$. The COD removal was 6% with an average 26% methane content in the biogas. Gas production was 104% of the control. Syringe E was fed 1500 mg/l $SO^{=}_4$. The COD removal was 12% with an average 25% methane content in the biogas. Gas production was 107% of the control.

removed. Syringe F was fed 1800 mg/l $SO^{=}_{4}$. The COD removal was 5.5% with an average 24% methane content in the biogas. Gas production was 96% of the control. The alkalinity concentration at the end of the test also confirmed the shock load conditions. At the beginning of the test the volatile fatty acid (VFA) concentration was 95 mg/l. At the end of the test the VFA in each syringe measured between 615 mg/l and 690 mg/l indicating a halt in the methanogenic bacteria activity.

TEST 5

Test number five was operated similarly to test four with respect to influent sulfate concentrations, however the F/M ratio was lowered to around 1.2 mg COD/mg VSS. The average syringe operating conditions and performance summaries are presented in Appendix A, Table A-5. Trend plots of the syringes for test five are presented in Appendix B, Figure B-5. Syringe A was operated as the test control receiving only 50 mg/l $SO^{=}_{4}$. The COD removal was 87% with an average 64% methane content in the biogas. The gas production rate was 0.690L/g COD removed. Syringe B was fed 600 mg/l $SO^{=}_{4}$. The COD removal was 84% with an average 70% methane content in the biogas. Gas production was 99% of the control and the gas production rate was 0.830L/g COD removed. Syringe C was fed 900 mg/l $SO^{=}_{4}$. The COD removal was 80% with an

average 69% methane content in the biogas. Gas production was 100% of the control and the gas production rate was 0.920L/g COD removed..

Syringe D was fed 1200 mg/l $SO^{=}_{4}$. The COD removal was 83% with an average 54% methane content in the biogas. Gas production was 93% of the control and the gas production rate was 0.714L/g COD removed. Syringe E was 1500 mg/l $SO^{=}_{4}$. The COD removal was 77% with an average 56% methane content in the biogas. Gas production was 96% of the control. The gas production rate was 0.872L/ g COD removed. Syringe F was fed 1800 mg/l $SO^{=}_{4}$. The COD removal was 77% with an average 57% methane content in the biogas. Gas production was 96% of the control and the gas production rate was 0.858L/g COD removed.

Metal Precipitation Study

TEST 6

Test number six represented the initial test run experimenting with various compounds that were used to complex with the bulk liquid sulfides. The compounds used were magnesium hydroxide $Mg(OH)_2$, ferrous chloride ($FeCl_2$), ferric chloride ($FeCl_3$), and a combination of magnesium hydroxide and ferric chloride. The average syringe operating conditions and performance summaries are presented in Appendix A, in Table

A-6. Trend plots of the syringes for test six are presented in Appendix B, Figure B-6. All syringes were fed 1500 mg/l $SO^{=}_{4}$ because it was determined from the previous test runs that this concentration created inhibitory effects. Gas production rates for Syringes B and D were not reported for similar reasons as in test four.

Syringe B was operated as the control receiving the 1500 mg/l $SO^{=}_{4}$ but no chemicals to complex the sulfides. The COD removal was 2% with a 24% methane content in the biogas. The volatile acids in Syringe B increased from 60 mg/l to 600 mg/l by the end of the run. Syringe C contained small amounts of magnesium hydroxide (725 mg/l) as its sulfide complexing agent. The COD removal was 74% with an average 68% methane content in the biogas. Gas production was 194% of the control and the gas production rate was 0.437L/g COD removed.

Syringe D contained ferric chloride (2000 mg/l) as its sulfide complexing agent. It had a COD removal of 13% and a methane content of 36% in the biogas. Gas production was 152% of the control. Syringe E contained a combination of magnesium hydroxide (363 mg/l) and ferric chloride (1000 mg/l) to precipitate sulfides. The COD removal was 79% and the average methane content in the biogas was 62%. Gas production was 278% of the control and the gas production was 0.521L/g COD removed. Syringe F contained

only ferrous chloride (1600 mg/l) to complex with the resultant sulfides. The COD removal was 78% and had an average 58% methane content in the biogas. Gas production was 398% of the control and the gas production rate was 0.768L/g COD removed.

TEST 7

Test number seven represented the last test run of the study and the second of the sulfide precipitation test runs. The influent sulfate concentration remained the same as in test number six. All six syringes were operated in this test run with the first , A, operated as one of two controls. Syringe A was fed low concentrations of sulfate while syringe B was operated receiving high concentrations of influent sulfate with no complexing agents added. The average operating conditions and performance summaries are presented in Appendix A, Table A-7. Trend plots of the syringes for test seven are presented in Appendix B, Figure B-7. Syringe A was fed 50 mg/l $SO^{=}_4$ and removed 79% of the COD. The average methane content of the biogas was 70% . The gas production was 10% higher than the rest of the syringes while the gas production rate was 0.629L/g COD removed. Syringe B was operated as the sulfate control receiving 1500 mg/l $SO^{=}_4$ without sulfide complexing compounds. The COD removal was 82% with an average

24% methane content in the biogas. Gas production was 84% of the control and the gas production rate was 0.531L/g COD removed. Syringe C received magnesium hydroxide (725 mg/l) as the sulfide complexing agent. The COD removal was 79%, and methane content averaged 68% in the biogas. Gas production was 78% of the control and the gas production rate was 0.517L/g COD removed.

Syringe D received ferric chloride (2000 mg/l). The COD removal was 83% with a 36% methane content in the biogas. Gas production was 86% of the control and the gas production rate was 0.708L/g COD removed. Syringe E received both magnesium hydroxide (363 mg/l) and ferric chloride (1000 mg/l) as sulfide complexing agents. The COD removal was 83% with a 62% methane content in the biogas. Gas production was 91% of the control and the gas production rate was 0.761L/g COD removed. Syringe F received ferrous chloride (1600 mg/l) as the sulfide complexing agent. The COD removal was 59% with a 58% methane content in the biogas. Gas production was 45% of the control and the gas production rate was 0.453L/g COD removed.

CHAPTER V

DISCUSSION

This test methodology for anaerobic toxicity assays using 125 ml glass syringes with luer-locks is the first to be used, to this author's knowledge, for the purpose of evaluating anaerobic toxicity and performance. The uniqueness of this apparatus is its ability to house the anaerobic culture and simultaneously measure the biogas produced by the microorganisms. The syringes also provide easy access to discrete gas and liquid samples for analyses.

One of the critical aspects to the test method was the feed and nutrient stock solution concentrations. Since the total measurable volume of the syringe is 100 ml, the total liquid volume had to be restricted to about 80 ml to allow for measurable gas production. The anaerobic seed (VSS) concentration was also an important factor in creating a reasonable F/M ratio that allowed as complete sulfate reduction as possible.

The proper F/M ratio was the most important operational parameter to define. In order for sulfide inhibition to occur, sulfates had to be reduced to sulfides to a

certain concentration. As discussed earlier, sulfate reduction requires 2 mg of COD for every 1 mg of sulfate reduced, thus if the carbon source is not sufficient, sulfates will not reduce to sulfides.

However, if too high an F/M is applied to a batch system the system will fail due to the organic shock. Test run number four represents this condition. An F/M ratio of around 3.0 mg COD/mg VSS was applied to the syringes. After five days the gas production dropped in every vessel. Once the test was determined to be complete, additional analysis confirmed that an organic shock load had occurred. The highest COD percent removal in this test was 12%. Volatile acids increased from 95 mg/l at the beginning of the test to over 600 mg/l at the end. The overall methane content dropped drastically in test four compared to the previous tests. Gas production rates were erroneously high and were not considered for comparison to the other tests.

In tests one through three, percent methane in the biogas ranged from 50% to 80% while all syringes in test four were around 25% methane. Data from past research and from the results of the first few tests in this study indicated that an F/M ratio of around 0.8 to 1.3 mg COD/mg VSS is sufficient to provide organic

carbon for sulfate reduction yet low enough not to cause an organic shock in batch systems.

Observation of the data showed that incomplete sulfate reduction took place in every test in every syringe except for the control which received minimal amounts of sulfate. The anaerobic bacteria had excess organic carbon for the requirement of sulfate reduction and therefore should not have been limited by carbon availability. One possible explanation for the incomplete reduction is insufficient mixing and /or contact time. The syringes were mixed well before each test for representative sampling purposes and at least twice per day for maintenance reasons throughout the test.

As discussed earlier, the syringes were not mixed twenty-four hours per day but rather mixed twice per day and hung vertically. A mixing device such as a shaker table may have improved sulfate reduction by allowing better contact time between the bacteria and the sulfates.

A small amount of sulfate (50 mg/l) was fed to each control reactor in order to stimulate activity and avoid a sulfur deficiency. The cumulative gas

production curves for tests one through seven indicate that a higher concentration of sulfate may have been needed to stimulate activity. Only in tests three and seven did the control syringe produce more gas over the test period than any other syringe. A concentration of 100 mg/l to 150 mg/l appears stimulatory yet low enough to avoid sulfide inhibition.

Generally, the toxicity of the sulfides in this assay was found to occur at higher concentrations of soluble sulfide and with less drastic effects as were seen in earlier studies. Instead of defining toxicity as gas production dropping fifty percent of the control as in the Stuckey *et al* (1980) research, variations of 10 to 20 percent of the control in this study were distinct enough to conclude that there was something inhibiting gas production.

Test runs one through three had a predicted liquid sulfide concentration of around 160 mg/l for the syringes with the highest influent sulfate concentration (Syringe F). The COD removal for these syringes ranged from 4 to 13 percent lower than the control. The gas production for the same reactors was 4 to 20 percent below the control while the percent methane content was 71 to 67 percent compared to 80 to 66 percent for the control. Test number five had three syringes

with predicted liquid sulfide concentrations of over 350 mg/l, yet most analytical parameters were similar to reactors with 160 mg/l liquid sulfides. For example, among the three syringes, COD removal was 4 to 10 percent lower than the control. Gas production was only 4 to 7 percent lower than the control and the percent methane content range was between 54 and 57 percent compared to 65 percent for the control.

The data suggest that the performance of the bacteria does not drastically change when liquid sulfide concentrations fluctuate between 160 mg/l and 447 mg/l. To explain this difference it is necessary to go back and research the anaerobic bacteria seed. The 20 liter hybrid reactor, which supplied the anaerobic bacteria for the assay, was at one time operated with an influent sulfate concentration of 4000 mg/l in a separate study. Two months before this research started, the influent sulfate concentration was lowered to around 100 mg/l. Since this seed source was subjected to liquid sulfide concentrations of around 200 mg/l continuously, it may have become somewhat acclimatized to certain concentrations. In this case it would take large concentrations of sulfides to affect performance.

Although the inhibition was not as obvious as in previous studies, tests one through five defined what was inhibitory to this seed at various concentrations. Gas production and COD removal were consistently lower in each test (excluding test four) for the syringes experiencing the highest concentration of sulfides. Gas production rates (L/g CODr) did not always correlate, which may be an error due to the low production rates.

Based on the first five test runs, it was decided that an influent sulfate concentration of 1500 mg/l would be used in the next phase of the test, sulfide precipitation. Test runs number six and seven, sulfide precipitation, experienced variations in the data such as incomplete sulfate reduction and reproducibility. In test number six the control syringe receiving high concentrations of sulfate (1500 mg/l) but no precipitating chemicals failed drastically removing only 2% of the COD, containing 24% methane in the biogas, and producing far less biogas than other syringes. However, identical operating conditions in test seven resulted in performance data similar to the syringes in tests one, two, and three. The COD removal was 82% with a methane content of 24%. The biogas production was

16 % lower than the control. The pH of the control syringe in test six at the end of the study was 5.0. The gas production and overall performance of the syringe may have been impacted by a rapid production of volatile acids thus lowering the pH. The low pH inhibited the methanogens before they could convert the volatile acids to methane.

The sulfide precipitation study did produce some encouraging results with the use of the three chemical compounds. In test number six the syringe with ferric chloride performed better than the control, removing 74 percent of the COD, producing biogas volume 194 percent of the control with a 68 percent methane content. The syringe in test seven also receiving ferric chloride performed similarly removing 79 percent COD, 68 percent methane but with only 78 percent biogas production compared to the control.

The syringe that received magnesium hydroxide in tests six and seven did not have consistent results. In test six only one third of the sulfates were reduced, resulting in a predicted 131 mg/l liquid sulfide concentration. An interesting result at the end of the study in test six was the final pH of 5.3. The low pH would not be expected since an alkaline compound, Na_2SO_4, was added. The drop in pH affected the performance, removing only 13 percent of the COD and containing 36 percent methane. The volatile

acid concentration increased from 60 mg/l to 540 mg/l. The drop in pH was also observed in the control reactor lowering from 6.8 to 5.0. The volatile acid concentration rose from 60 mg/l to 600 mg/l, similar to Syringe D, magnesium hydroxide.

Since Syringe B in test six experienced severe sulfide inhibition (i.e. low pH, accumulation of acids and low gas production), Syringe D in test six could have experienced sulfide inhibition even though magnesium hydroxide was present. The inhibition could have occurred before the precipitating chemicals had time to work. This again appears to be caused by a mixing problem.

The syringe receiving magnesium hydroxide in test seven performed better although different than in test six. The COD removal was 83 percent yet had 36 percent methane in the biogas. The volatile acid concentration only increased a marginal amount. Similar results were found in the syringe receiving ferrous chloride. In test six the reactor performed well, removing 78 percent of the COD and producing more biogas than any other syringe. The methane content in the biogas was slightly lower at 58 percent. Syringe F in test seven receiving ferrous chloride performed poorly, removing only 59 percent of the COD. A drop in the bulk liquid pH to 5.8 also occurred, hindering performance. Again, these were signs of sulfide inhibition taking place before the chemicals had a chance to work.

Test Syringe E, receiving a combination of ferric chloride and magnesium hydroxide, produced consistent results in both tests six and seven. The COD removal was 79 and 83 percent respectively, while the methane content in the biogas was 62 percent in both test runs. Biogas production values were also similar. Overall, there was a small improvement in the performance of the syringes that received sulfates with some type of precipitation chemical than those that did not. The COD removal was between 78 to 83 percent for the precipitation study and 69 to 83 percent for the inhibition tests.

The preliminary studies of this research developed environmental and operating conditions specifically for the glass luer-lock syringes. Although defining sulfide toxicity and attempting to control it was part of the study, the main emphasis was creating optimal environmental and other operating conditions for the bacteria so that the only limiting factor affecting the biological system was the wastewater and/or toxicant.

Important environmental conditions to anaerobic bacteria were adequately supplied namely, pH, temperature, alkalinity, macronutrients, and micronutrients. Operating conditions were developed which lead to successful batch operations, they included food to microorganism ratio and organic loading.

Other important factors surrounding the operation of the glass syringes were the stock solution concentrations of nutrients, seed material, and sucrose. The restricted

liquid volume of the syringe (~80 ml) required concentrated stock solutions to meet the volume limit. Overall, the screening studies using the luer-lock syringes was simple to setup and maintain. The development of this test methodology discovered specific advantages and limitations of the luer-lock syringes.

CHAPTER VI

CONCLUSIONS

This study has led to the following conclusions regarding the test methodology for anaerobic sulfide toxicity assays using 125 ml glass luer-lock syringes.

1. The syringes were accessible for both gas and liquid samples during and after test runs.

2. Volumes of feed stock solutions were able to be reduced down to restricted levels to allow for gas production readings.

3. Food to microorganism ratio (F/M) operating levels were found to be best between 0.8 to 1.3 mg COD/mg VSS.

4. Several test conditions were able to be run at one time. Preparation time was minimal with results from one test in about three weeks.

5. Biogas production rates in terms of L/gram COD removal were reproducible and correlated to signs of inhibition.

6. COD removal rates were lower when compared to higher influent sulfate concentrations.

7. An F/M ratio of 3 mg COD/mg VSS caused reactor failure, inhibiting gas production, accumulating volatile acids, and lowering the pH.

8. Measurable effluent sulfate concentrations indicated that complete reduction to sulfides was not accomplished for sulfate concentrations above 50 mg/l.

9. Variable reproducibility in the sulfide precipitation study and incomplete sulfate reduction in the syringes indicates that improper mixing may have occurred.

10. Sulfide inhibition was found to occur at 160 to 447 mg/l soluble sulfide with no drastic performance changes between the two concentrations.

11. A previously acclimated biological seed source to sulfide concentrations of 200 mg/l may have influenced the high tolerable sulfide concentrations observed in this study.

12. The use of a sulfide partitioning computer program to predict the amount of sulfides in the gas and bulk liquid based on actual data correlated to other parameters that indicated inhibition.

CHAPTER VII

RECOMMENDATIONS FOR FUTURE RESEARCH

Future work with the luer-lock syringe test method should attempt to overcome the mixing and contact time problem that was experienced in this research. The use of a shaker table or other mixing device should provide adequate mixing and better contact between the bacteria and the wastewater. It is also suggested that with this method a biological source is used which is unacclimated to the test media under investigation so that large variations in gas production and removal rates can be observed.

BIBLIOGRAPHY

1. Brock, T.D., Brock, M.L., and Edwards, M.R. (1981). "Microbial Life at 90°C: The Sulfur Bacteria of Boulder Spring. J. Bacteriol. Vol. 107, pp. 303 to 314.

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

3. Heukelekian, H. (1948). "Some Bacterial Aspects of Hydrogen Sulfide Production from Sewage". Sewage Works Journal, Vol. 20, pp. 490.

4. Holland, J.C., Knapp, J.S., and Shoesmith, J.G. (1987). "Anaerobic Bacteria". Chapman and Hall, New York.

5. Koster, I.W., Rinzema, A., De Vegt, A.L., and Lettinga, G., (1986). "Sulfide Inhibition of the Methanogenic Activity of Granular Sludge at Various pH-levels. Water Res. Vol. 20. No. 12. pp. 1561 to 1567.

6. Kugelman, I.J., and McCarty, P.L. (1964). "Cation Toxicity And Stimulation in Anaerobic Waste Treatment II. Daily Feed Studies". Proceedings from the 19th Industrial Waste Conference. Purdue University.

7. Lawrence, A.W., McCarty, P.L., and Guerin, F.J.A., (1966). "The Effects of Sulfides on Anaerobic Treatment". Water Pollution. Vol. 10. pp. 207 to 221.

8. McCarty, P.L. (1964). "Anaerobic Waste Treatment Fundamentals. Toxic Materials and their Control". Publ. Works 95, pp 91 to 94.

9. McCarty, P.L. (1966). "Kinetics of Waste Assimilation in Anaerobic Treatment, Developments in Industrial Microbiology". American Institute of Biological Sciences Vol. 7, Washington, DC.

10. McCarty, P.L. (1972). "Stoichometry of Biological Reactions". Presented at the International Conference Towards A United Concept of Biological Waste Treatment Design. Atlanta, Georgia.

11. McFarland, M.J., (1982). "Fate of Sulfate During the Thermophilic Methane Fermentatiion Process". PhD. Thesis. Cornell University

12. Metcalf and Eddy, Inc. (1991). "Wastewater Engineering Treatment, Disposal, and Reuse".

13. Muth, OH., and Oldfield, J.E., (1970). "Sulfur in Nutrition". Avi. Publishing Co., Inc.

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

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

16. Rudolfs, W., and Amberg, H.R., (1952)." Factors Affecting Design Efficiency". Sewage and Industrial Waste. Vol. 24. pp.1402 to 1409.

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

18. Schlegel, H.G. (1981). "Allgemeine Mikrobiologie 5. Auflage, Thieme Verlag". Stuttgart, West Germany.

19. Speece, R.E., and Parkin, G.F., (1983). "The Response of Methane Bacteria to Toxicity". Third International Symposium on Anaerobic Digestion. pp.23 to 35.

20. Standard Methods for the Examination of Water and Wastewater, 18th Edition (1992).

21. Stover, E.L., Brooks, S., Munirathinam, K. (1992). "Control of Bulk Liquid Sulfide Toxicity During Anaerobic Treatment". Presented at the Georgia Tech Industrial Food Conference.

22. Stuckey,D.C., Owen,W.F., McCarty,P.L., and Parkin, G.F., (1980)."Anerobic Toxicity Evaluation byBatch and Semi-continuous Assays". Journal WPCF, Vol. 52, No. 4.

23. Winfrey, M.R., and Zeikus, J.G. (1977). "Effect of Sulfate on Carbon and Electron Flow During Microbial Methanogenesis in Freshwater Sediments". Appl. Environmental Microbiology. Vol. 33,pp. 99 to 127.

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

APPENDIXES

APPENDIX A

SUMMARY WORKSHEET FOR ANAEROBIC TEST

OPERATING AND PERFORMANCE DATA

TABLE A-1

TEST # 1
ANAEROBIC BATCH STUDY
SUMMARY WORKSHEET

DESCRIPTION	SYRINGE I.D.					
	A	B	C	D	E	F
AVERAGE OPERATING CONDITIONS						
pH, s.u.	6.8	6.8	6.8	6.8	6.8	6.8
sCOD, mg/l	1850	1850	1850	1850	1850	1850
F/M	1.25	1.25	1.25	1.25	1.25	1.25
SO4, mg/l	50	150	300	450	600	900
ALKALINITY, mg/l	3200	3200	3200	3200	3200	3200
REACTOR VFA, mg/l	675	675	675	675	675	675
PERFORMANCE DATA						
pH, s.u.	7.6	7.4	7.4	7.5	7.5	7.5
EFFLUENT sCOD,mg/l	480	500	540	550	575	560
COD Removal, %	74	73	71	70	69	70
SO4, mg/l	0	100	150	220	290	410
LIQUID SULFIDE, mg/l	16	16	49	75	102	161
GAS SULFIDE, mg/l	4.6	4.6	14	21	29	46
GAS PROD., ml	60*	77*	79	74	65	50
GAS PROD., L/g CODr	0.547	0.713	0.750	0.711	0.637	0.485
GAS PROD., % OF CONTROL	-	128	131	123	108	83
H2S, %	0.32*	0.32*	1	1.5	2	3
CH4, %	66	65	67	50	64	67
ALKALINITY,mg/l	2990	2340	2766	2857	3010	3281
REACTOR VFA, mg/l	350	350	350	350	350	350

* Data does not correlate

TABLE A-3

TEST # 3
ANAEROBIC BATCH STUDY
SUMMARY WORKSHEET

DESCRIPTION	SYRINGE I.D. A	B	C	D	E	F
AVERAGE OPERATING CONDITIONS						
pH, s.u.	6.7	6.7	6.7	6.7	6.7	6.75
sCOD, mg/l	2525	2125	2150	2500	2188	2200
F/M	1.4	1.2	1.2	1.4	1.23	1.23
SO4, mg/l	50	300	450	600	750	900
ALKALINITY, mg/l	1450	1450	1450	1450	1450	1450
REACTOR VFA, mg/l	150	150	150	150	150	150
PERFORMANCE DATA						
pH, s.u.	6.75	6.8	6.8	6.9	6.9	6.9
EFFLUENT sCOD,mg/l	440	515	600	625	580	660
COD Removal, %	83	76	72	76	73	70
SO4, mg/l	50	200	200	300	300	400
LIQUID SULFIDE, mg/l	16	80	121	160	202	248
GAS SULFIDE, mg/l	4.5	25	38	50	63	70
GAS PROD., ml	127	116	113	118	115	107
GAS PROD., L/g CODr	0.789	0.936	0.949	0.819	0.927	0.899
GAS PROD., % OF CONTROL	-	91	89	93	91	84
H2S, %	0.32	0.6	1.6	1.9	3	3.2
CH4, %	80	76	79	75	77	71
ALKALINITY,mg/l	875	1063	1063	1375	1375	1500
REACTOR VFA, mg/l	110	105	107	95	100	100

TABLE A-4

TEST # 4
ANAEROBIC BATCH STUDY
SUMMARY WORKSHEET

	SYRINGE I.D.					
DESCRIPTION	A	B	C	D	E	F
AVERAGE OPERATING CONDITIONS						
pH, s.u.	6.7	6.75	6.8	6.8	6.8	6.8
sCOD, mg/l	5800	5800	5750	5575	5750	5500
F/M	3.2	3.2	3.2	3.1	3.2	3.1
SO4, mg/l	50	600	900	1200	1500	1800
ALKALINITY, mg/l	1250	1250	1250	1250	1250	1250
REACTOR VFA, mg/l	95	95	95	95	95	95
PERFORMANCE DATA						
pH, s.u.	4.8	4.8	4.7	4.8	4.8	4.8
EFFLUENT sCOD,mg/l	5350	5200	5300	5250	5050	5200
COD Removal, %	8	10	8	6	12	5.5
SO4, mg/l	0	575	838	1050	1075	1275
LIQUID SULFIDE, mg/l	16	8	20	48	136	169
GAS SULFIDE, mg/l	4.5	2.3	5.6	14	38	48
GAS PROD., ml	54	59	46	56	58	51
GAS PROD., L/g CODr	1.60	1.34	1.39	2.33	1.11	2.31
GAS PROD., % OF CONTROL	-	108	85	104	107	96
H2S, %	0.31	0.16	0.4	1	2.7	3.3
CH4, %	24	22	24	26	25	24
ALKALINITY,mg/l	375	250	250	375	375	375
REACTOR VFA, mg/l	615	585	623	615	653	690

TABLE A-5

TEST # 5
ANAEROBIC BATCH STUDY
SUMMARY WORKSHEET

	SYRINGE I.D.					
DESCRIPTION	A	B	C	D	E	F
AVERAGE OPERATING CONDITIONS						
pH, s.u.	6.8	6.8	6.8	6.8	6.8	6.8
sCOD, mg/l	5200	4450	4250	4900	4450	4550
F/M	1.24	1.1	1.0	1.2	1.1	1.1
SO4, mg/l	50	600	900	1200	1500	1800
ALKALINITY, mg/l	1600	1600	1600	1600	1600	1600
REACTOR VFA, mg/l	120	120	120	120	120	120
PERFORMANCE DATA						
pH, s.u.	6.9	7.1	7.2	7.2	7.3	7.3
EFFLUENT sCOD,mg/l	685	718	835	835	1010	1035
COD Removal, %	87	84	80	83	77	77
SO4, mg/l	0	30	40	30	150	400
LIQUID SULFIDE, mg/l	16	182	274	374	431	447
GAS SULFIDE, mg/l	4.5	51	77	105	121	126
GAS PROD., ml	257	255	258	238	246	247
GAS PROD., L/g CODr	0.690	0.830	0.920	0.714	0.872	0.858
GAS PROD., % OF CONTROL	-	99	100	93	96	96
H2S,%	0.31	3.6	5.4	7.4	8.5	8.8
CH4, %	64	70	69	54	56	57
ALKALINITY,mg/l	1125	1750	1813	2125	2125	2250
REACTOR VFA, mg/l	140	130	115	135	120	135

TABLE A-6

TEST # 6
ANAEROBIC BATCH STUDY
SUMMARY WORKSHEET

DESCRIPTION	SYRINGE I.D. B	C	D	E	F
AVERAGE OPERATING CONDITIONS					
pH, s.u.	6.8	6.8	6.8	6.8	6.8
sCOD, mg/l	4250	4000	3450	4500	4400
F/M	0.82	0.8	0.7	0.9	0.9
SO4, mg/l	1500	1500	1500	1500	1500
ALKALINITY, mg/l	1600	1600	1600	1600	1600
REACTOR VFA, mg/l	60	60	60	60	60
PERFORMANCE DATA					
pH, s.u.	5.0	7.3	5.3	7.2	7.1
EFFLUENT sCOD,mg/l	4188	1038	3000	938	950
COD Removal, %	2	74	13	79	78
SO4, mg/l	900	30	1100	125	150
LIQUID SULFIDE, mg/l	198	481	131	447	443
GAS SULFIDE, mg/l	55.8	135	37	126	122
GAS PROD., ml	50	97	76	139	199
GAS PROD., L/g CODr	10.75	0.437	2.25	0.521	0.768
GAS PROD., % OF CONTROL	-	194	152	278	398
H2S, %	3.9	9.5	2.6	8.8	8.5
CH4, %	24	68	36	62	58
ALKALINITY,mg/l	375	3500	750	2250	2200
REACTOR VFA, mg/l	600	25	540	85	110

TABLE A-7

TEST # 7
ANAEROBIC BATCH STUDY
SUMMARY WORKSHEET

	SYRINGE I.D.					
DESCRIPTION	A	B	C	D	E	F
AVERAGE OPERATING CONDITIONS						
pH, s.u.	7.0	6.9	6.9	7.0	7.0	7.0
sCOD, mg/l	5250	5300	5225	4000	4000	4600
F/M	1.0	1.0	1.0	0.8	0.8	0.9
SO4, mg/l	50	1500	1500	1500	1500	1500
ALKALINITY, mg/l	1600	1600	1600	1600	1600	1600
REACTOR VFA, mg/l	60	60	60	60	60	60
PERFORMANCE DATA						
pH, s.u.	7.0	7.3	7.3	7.2	7.2	5.8
EFFLUENT sCOD,mg/l	890	950	1080	675	700	1900
COD Removal, %	79	82	79	83	83	59
SO4, mg/l	0	40	31	13	15	1250
LIQUID SULFIDE, mg/l	16	470	474	478	476	82
GAS SULFIDE, mg/l	4.5	132	134	135	134	23
GAS PROD., ml	195	164	152	167	178	87
GAS PROD., L/g CODr	0.629	0.531	0.517	0.708	0.761	0.453
GAS PROD., % OF CONTROL	-	84	78	86	91	45
H2S, %	0.31	9.3	9.3	9.4	9.4	1.6
CH4, %	68	24	68	36	62	58
ALKALINITY,mg/l	1125	2500	3250	2000	2250	1125
REACTOR VFA, mg/l	95	105	50	115	80	413

APPENDIX B

CUMULATIVE GAS PRODUCTION CURVES

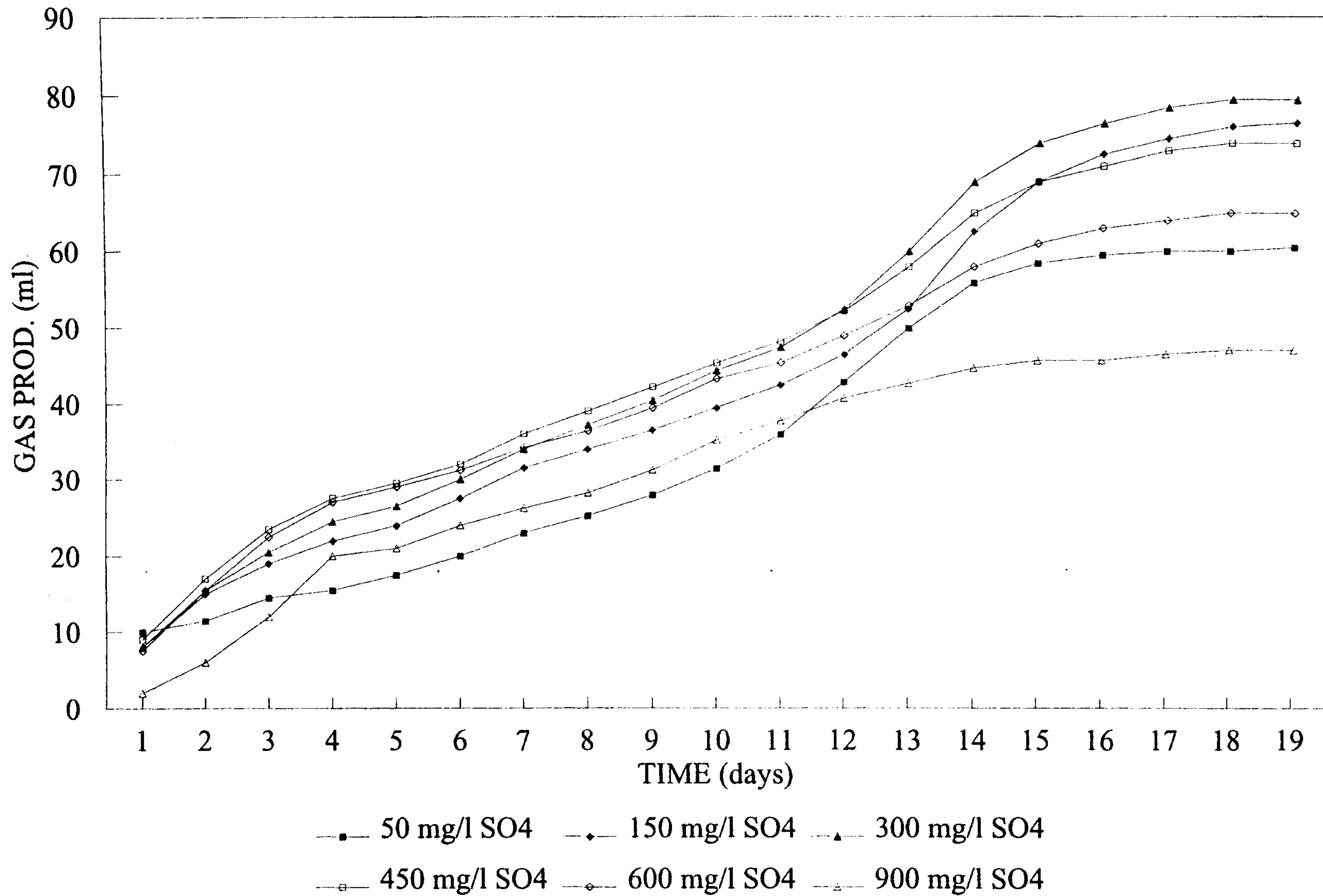


Figure B-1. Cumulative Gas Production For Test 1

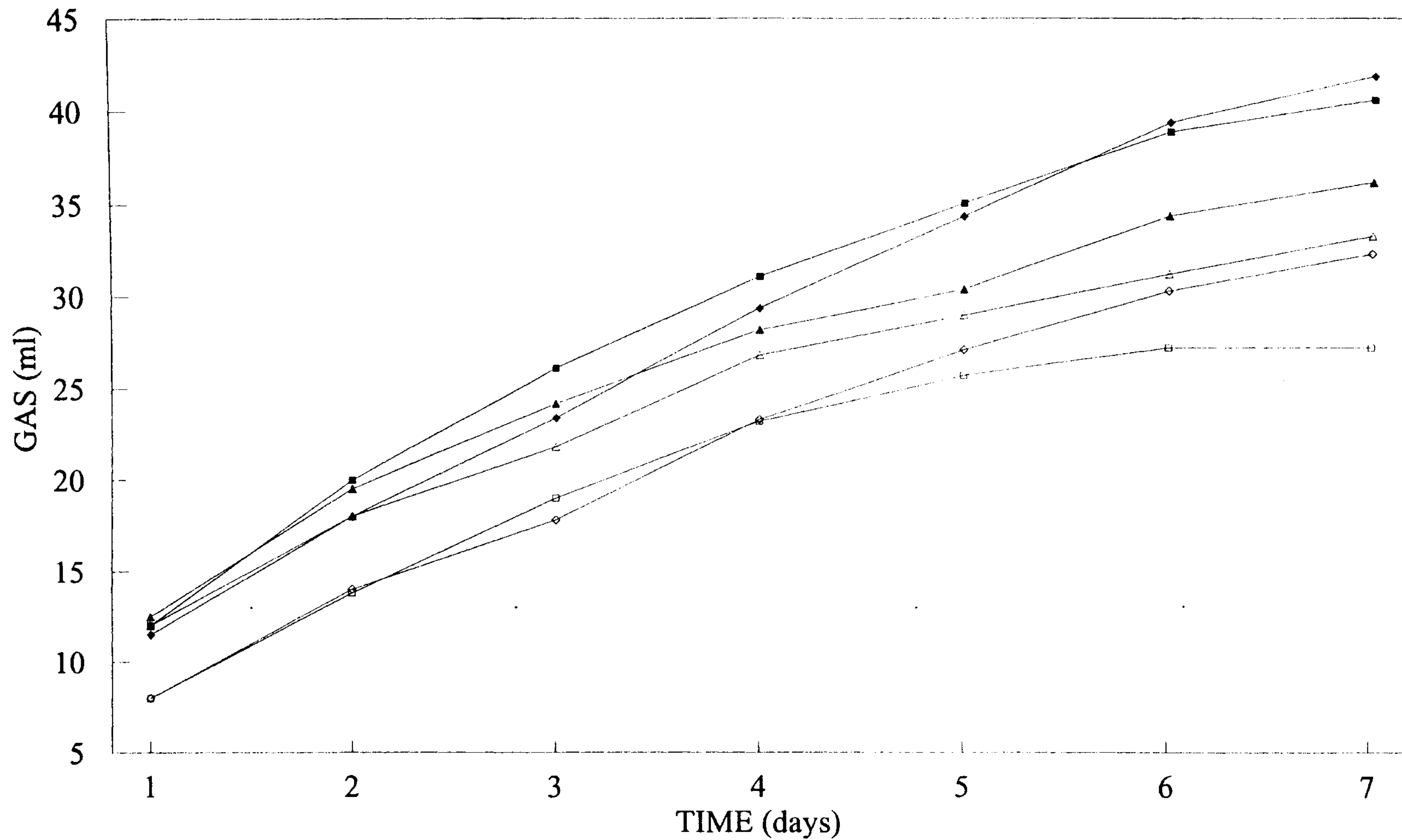


Figure B-2. Cumulative Gas Production For Test 2

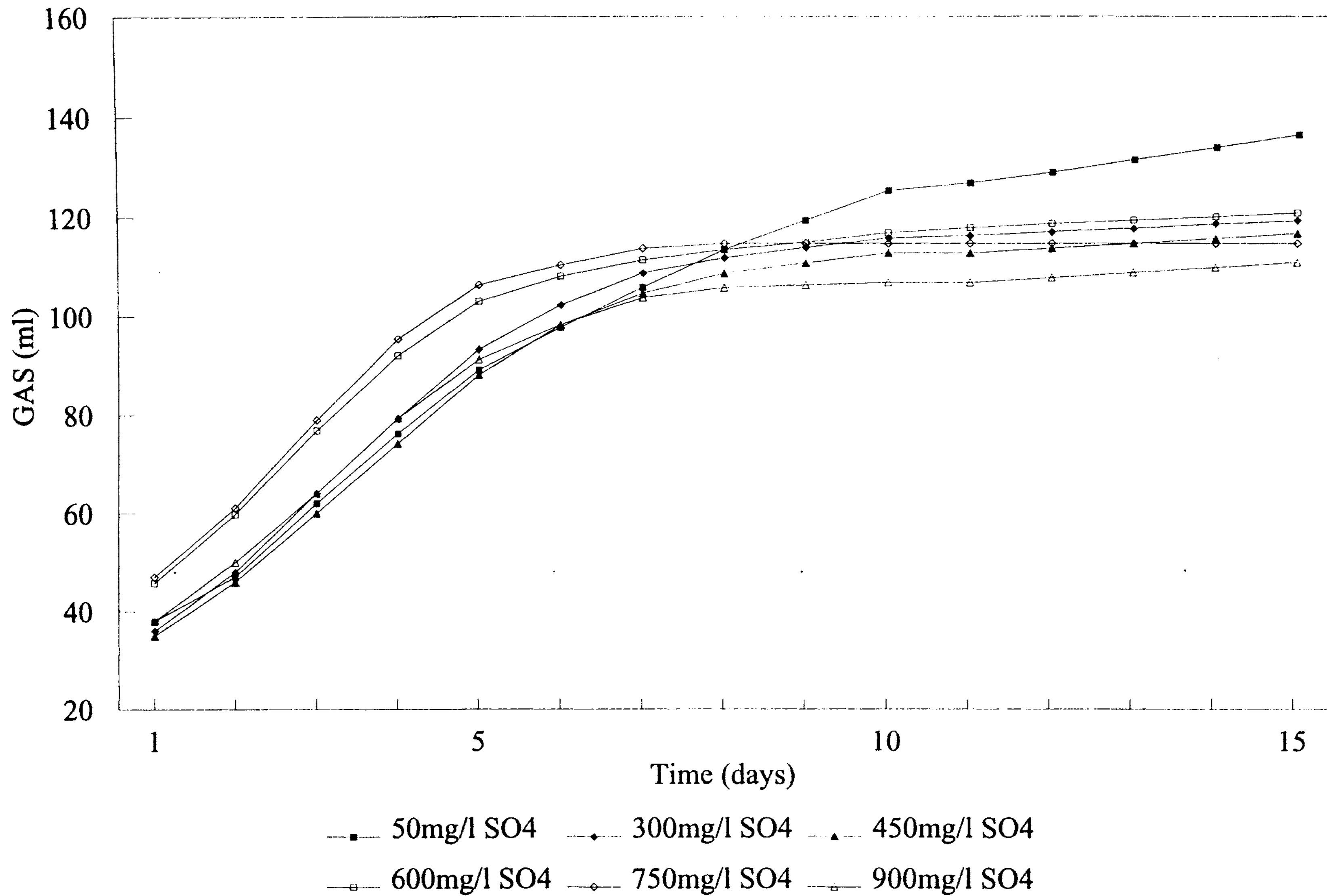


Figure B-3. Cumulative Gas Production For Test 3

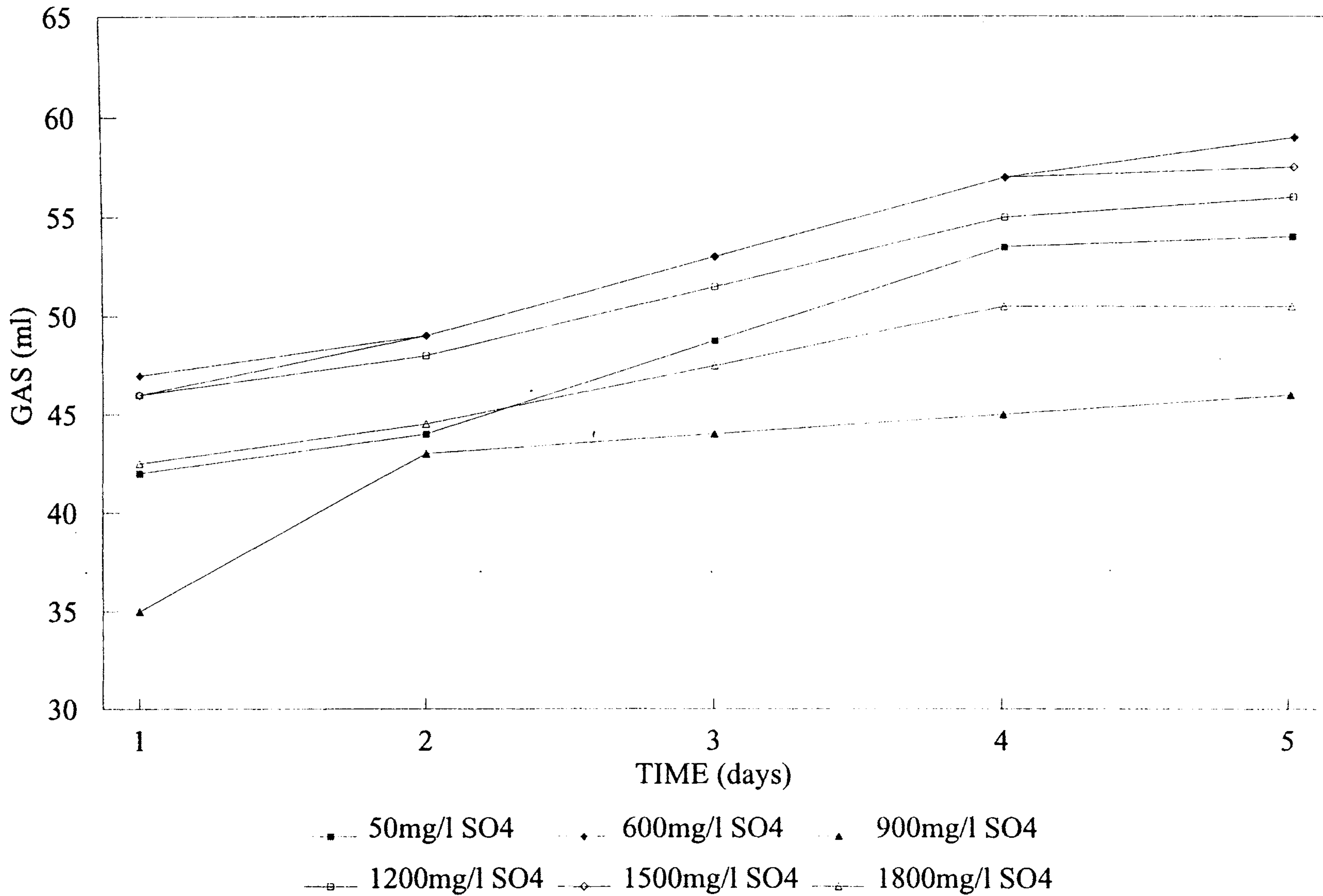


Figure B-4. Cumulative Gas Production For Test 4

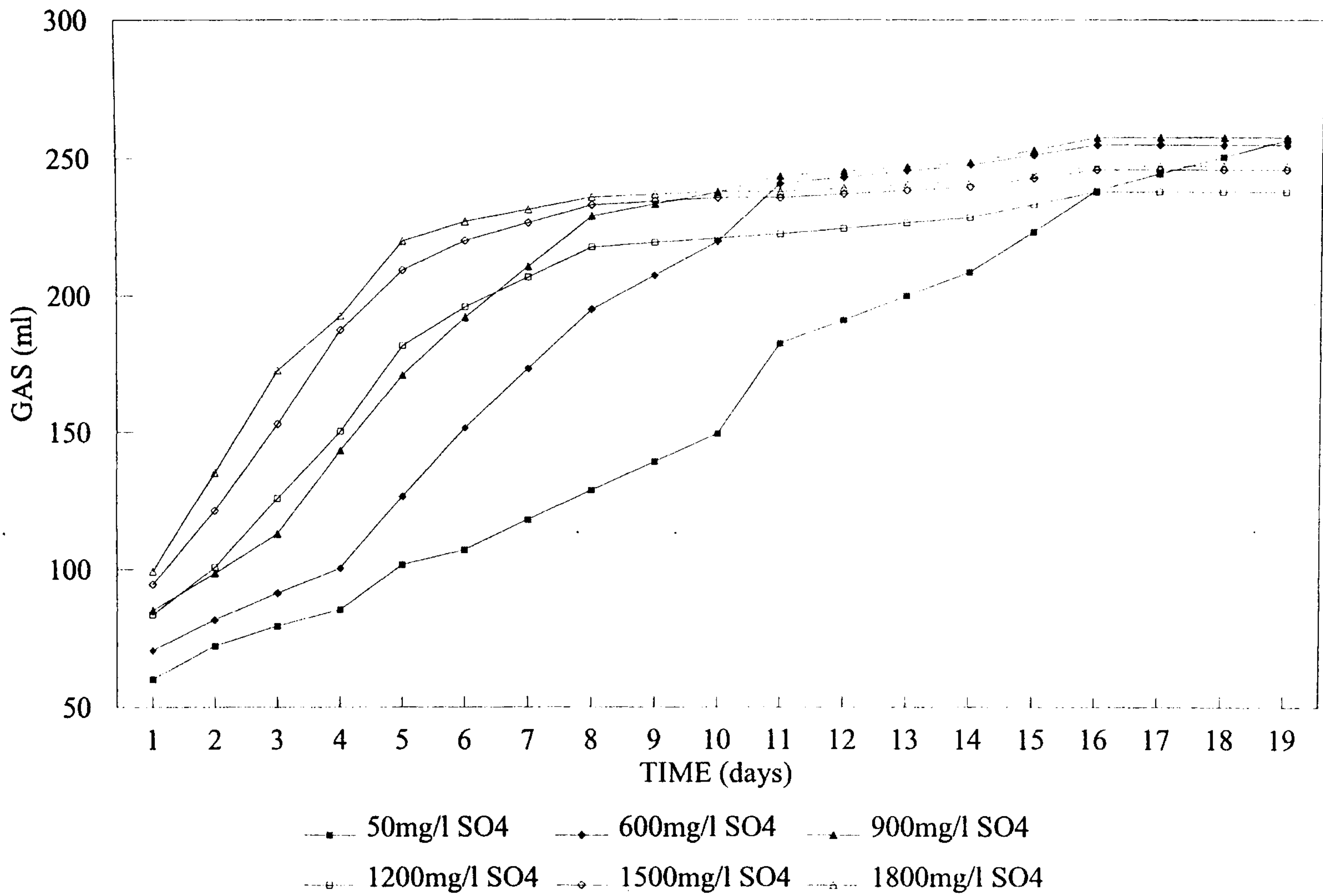


Figure B-5. Cumulative Gas Production For Test 5

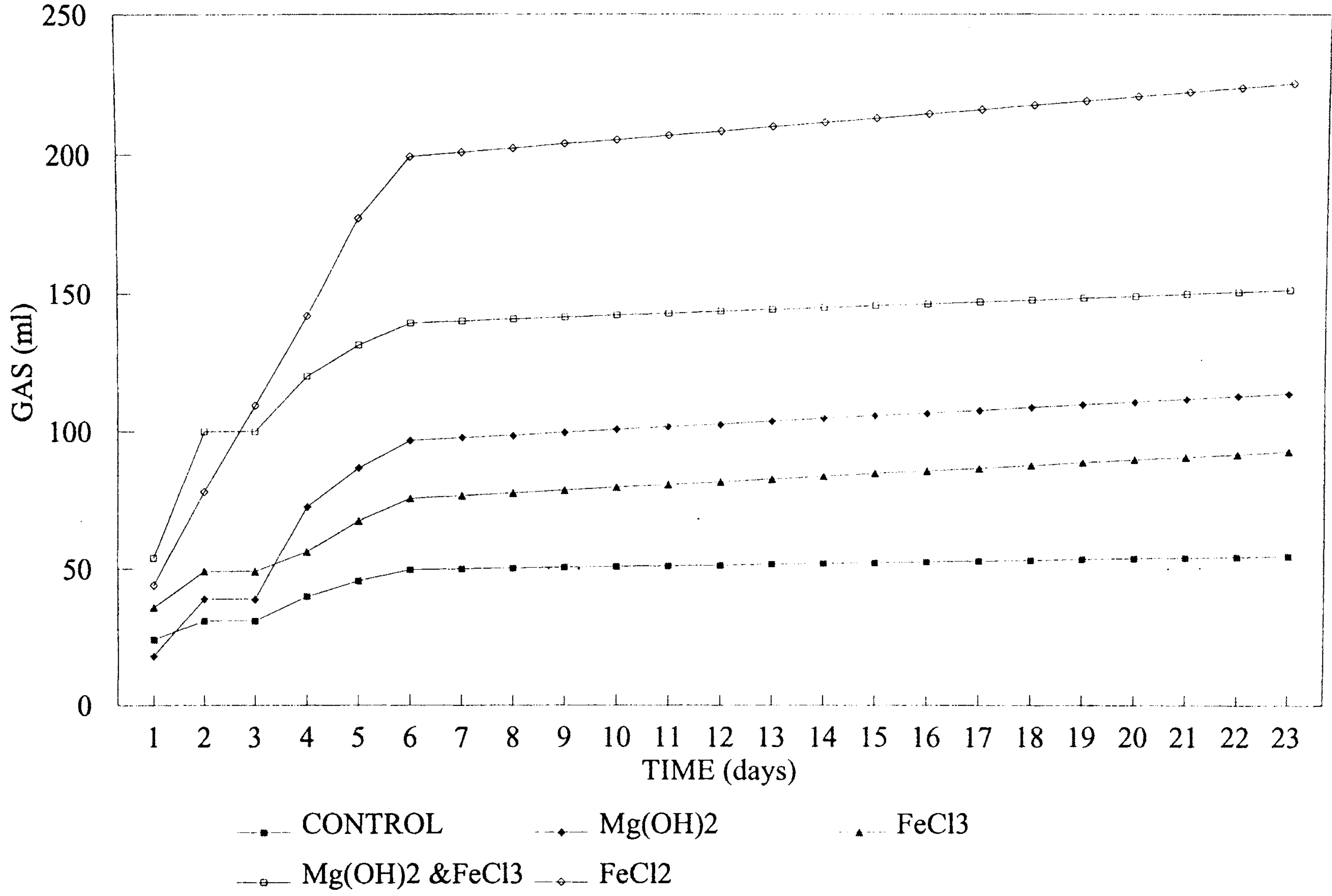


Figure B-6. Cumulative Gas Production For Test 6

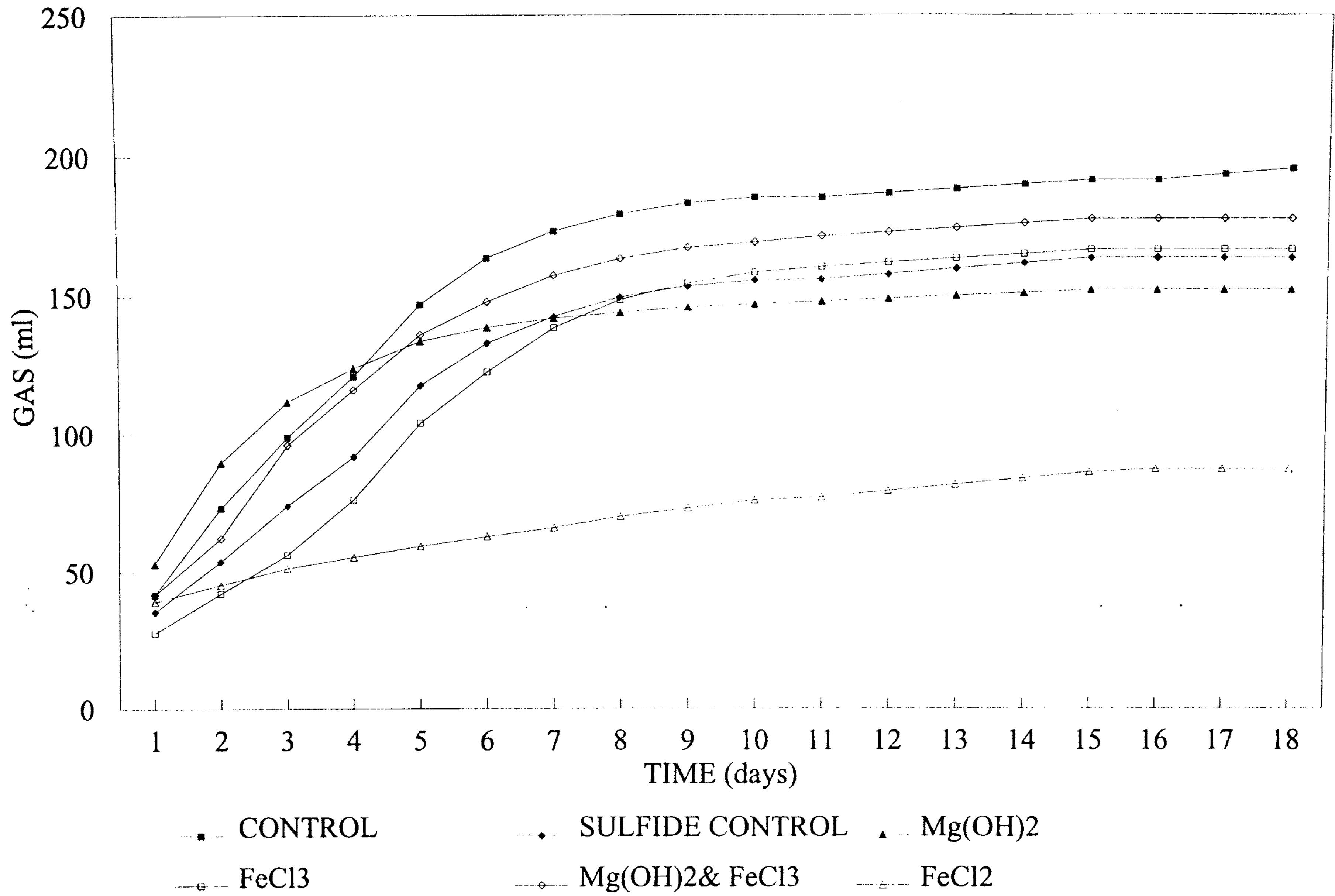


Figure B-7. Cumulative Gas Production For Test 7

VITA 2

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