

**A PRE-SCREENING TOOL FOR THE ANAEROBIC
TREATMENT OF COMPLEX INDUSTRIAL
EFFLUENTS AND WASTEWATERS**

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ABSTRACT

The objective of this investigation was to assess the potential of a titration bioassay i.e.: The Methanogenic Activity and Inhibition Analyser (MAIA), to determine the biodegradability of complex industrial effluents and wastewaters. Specifically, the project aimed to provide an alternative experimental method to the serum bottle method so that hazardous effluents can be pre-screened for treatment in under-utilised anaerobic digesters at sewage treatment plants in KwaZulu-Natal, South Africa. This study also aimed to provide a protocol and a simple mathematical model as experimental tools that could contribute to the development of future pre-screening studies.

MAIA was used to conduct biodegradability and toxicity studies on semi-hazardous landfill leachate and textile size effluent. Thereafter, selected studies were repeated using a conventional screening method i.e.: serum bottle method. The investigation with MAIA revealed that both effluent substrates had potential for anaerobic treatment. However, the studies highlighted certain intrinsic limitations of the MAIA apparatus to effectively pre-screen complex substrates. The existing titrimetric system is too coarse to accurately track the biochemical pathways leading from the breakdown of complex compounds to methane gas production. Further, temperature interferences and gas phase diffusion limitations associated with the existing design make the assessment of activity difficult.

The titrimetric method is comparable to the serum bottle method only if a qualitative assessment of toxicity and biodegradability is needed. However, the titrimetric method produces results in a much shorter period of time compared to the serum bottle method. Evaluated in this way the titrimetric method is the better alternative. However, the current system cannot challenge the reliability of the serum bottle method to provide good quantitative results.

A mathematical model was developed which is much less detailed than the existing one provided by Remigi (2001). It comprises only two significant anaerobic processes namely hydrolysis and acetogenesis. Simulation trials have suggested that the model is a necessary and beneficial component of the titrimetric pre-screening protocol.

This investigation has also led to the development of a more refined operating manual for MAIA. The manual provides a step-wise method for the preparation and conduction of pre-screening tests. Specifically, it highlights the need for a suitable biomass acclimation period and the importance of nutrient use for better pre-screening assessments.

I dedicate this work to the spirit and vision of my grandparents

Perumal Govender

Velliamma Moodley

“To know even one life has breathed easier because you have lived, this is to have succeeded”

(Ralph Waldo Emerson)

DECLARATION

I declare that this dissertation, unless indicated, is my own work and that it has not been submitted, in whole or in part, for a degree at another University or Institution.

.....

Dinesh Naidoo

June 2003

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CHAPTER ONE

INTRODUCTION

This chapter provides a brief background to the application of anaerobic treatment to industrial effluents and the need for anaerobic pre-screening studies. It also outlines the scope of this study and the manner in which the dissertation has been organised.

1.1. Anaerobic Treatment

Anaerobic treatment technologies were initially developed for the treatment of readily biodegradable fractions of municipal wastewater, manures and sludge. With the development of high rate systems, the technology was applied to agro-industrial effluents (Field, 2002). Increased knowledge on toxicity and biodegradability enabled applications to include effluents containing toxic and recalcitrant compounds from the chemical, petro-chemical and pulp/paper industries. Today, anaerobic processes are being applied to bioremediation.

1.2. Effluent Treatment

The KwaZulu Natal region has the potential to attract a significant amount of industry. Some of these industries could be those that produce effluents that have a high concentration of organic compounds. Industry of this type, within the region, encounters difficulties in safe disposal of their effluents. Common disposal solutions have been co-disposal at landfill sites and marine outfall. However, with increasing government emphasis on cleaner production, alternative disposal solutions need to be investigated.

Cleaner production is the continuous application of an integrated preventative environmental strategy, applied to processes, products and services to increase eco-efficiency and to reduce risks for humans and the environment (Sacks, 1997). In an effort to work cleaner, waste minimisation techniques could be implemented. However, waste minimisation techniques could lead to the production of more concentrated effluents. Anaerobic digestion has the potential to treat these concentrated wastewaters.

Investigations have identified anaerobic digestion facilities at existing Waste Water Treatment Plants that can accept high-strength organic effluents (Sacks, 1997). However, the characteristics of industrial effluent can be highly variable and could be a potential threat to the anaerobic micro-organisms that facilitate biodegradation and bio-transformation in conventional digesters. Therefore, it is imperative that suitable preliminary screening be conducted prior to an industrial effluent being introduced into an anaerobic digester.

1.3. Preliminary Screening

Biodegradation and toxicity assays have been used extensively as preliminary studies for the treatment of organic compounds in environmental wastes including wastewaters, hazardous wastes, and contaminated groundwater and soils. Much time and effort is expended in collecting experimental data on the biodegradability of organic compounds and their inhibitory effect on anaerobic processes. The establishment of kinetic models to describe the biodegradation processes and the estimation of the kinetic parameters can help us understand the intrinsic characteristics of the processes and predict the fate of the organic compounds in certain systems thereby saving significant experimental work and minimising labour-intensive undertakings (Suidan et al., 1988).

Considerable work has been successfully performed using serum bottle assays to determine the biodegradability and toxicity of organic compounds, however the assay is time-consuming. Recently, work was successfully conducted with the MAIA (Methanogenic Activity and Inhibitor Analyser) pH-stat titration biosensor to estimate kinetic constants for an anaerobic sludge (D'Ambrosio, 2000). Further, MAIA was used to assess whether a specific chemical or wastewater was harmful to methanogens (Castellazzi, 1998). The body of work performed with MAIA has highlighted the potential of this instrument to be used as a pre-screening apparatus.

1.4. Project Outline

This project will investigate the potential of MAIA as a screening tool to rapidly assess the toxicity of effluents to an acclimated bacterial consortium. A reliable pre-screening method would be necessary if effluent treatment is to be conducted in anaerobic digesters with an established biomass. This method would form an integral part of other WRC sponsored projects that are investigating aspects of the proposed treatment process at the laboratory and pilot (full) scale. At the full scale it is envisaged that the effluent will be transported to a designated wastewater treatment site and co-digested anaerobically.

Therefore, the aims of this project are to:

- evaluate MAIA i.e.: the titrimetric technique, as a rapid pre-screening tool for assessing anaerobic biodegradability and toxicity of complex industrial effluents and wastewaters;
- compare the results of the titrimetric method to an existing screening method, the serum bottle method
- highlight the importance of mathematical modelling to plan and improve experimental work conducted with MAIA.

Specifically the thesis would attempt to provide a:

- more refined operational manual for the use of MAIA;
- recommendations for the improvement of the titrimetric system if it is found that the system can be exploited as a pre-screening tool.

1.5. Thesis Outline

The thesis consists of four chapters following this one:

- CHAPTER TWO:
This is a discursive review of general literature. It presents information about anaerobic digestion, co-metabolism and kinetic models. In also includes literature covering the development and operation of pH-stat and related devices.
- CHAPTER THREE:
This chapter details the methodology of both the titrimetric and serum bottle studies. In addition, it describes how experimental information had been acquired and interpreted.
- CHAPTER FOUR:
This chapter contains the results of the experimental study. It was designed, so that the results from each experimental study could be independent of each other except where comparison and/or reference to other results or literature were made. **Figure [1-1]**, depicts the way in which the experimental work was incorporated into the thesis. The body of experimental work is divided into two parts: experiments with MAIA and the serum bottles respectively. In addition, this chapter contains a discussion on the mathematical modelling of the MAIA system.

- CHAPTER FIVE:
In this chapter, conclusions and recommendations are presented.

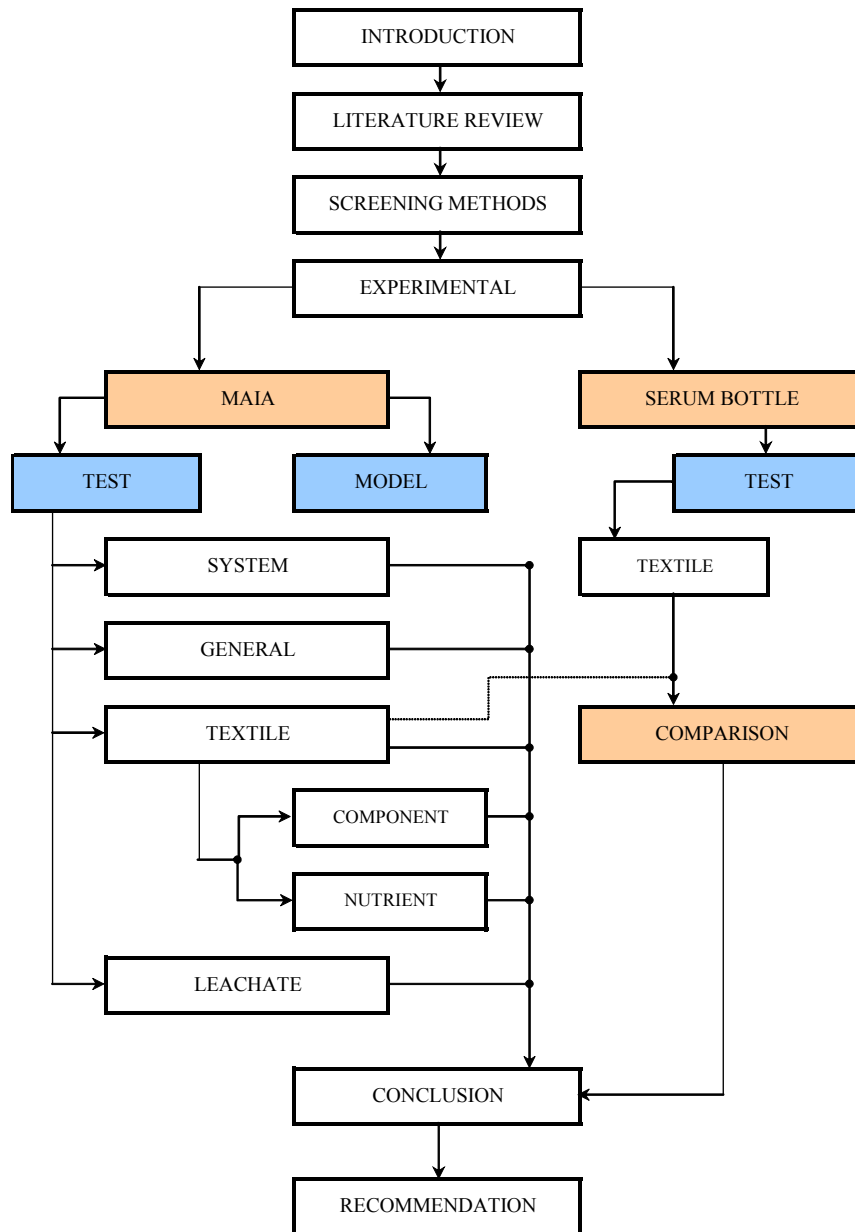


Figure [1-1]: Thesis Outline. The schematic shows how the thesis has been structured and highlights how different sections are related to each other.

CHAPTER TWO

LITERATURE REVIEW

This chapter provides background to certain theoretical concepts referred to in this dissertation. It includes a description of the anaerobic process; the chemistry associated with the breakdown of complex substrates; anaerobic cometabolism; kinetic models and the instrumentation used in this study.

2.1. The Anaerobic Process

Anaerobic waste treatment is one of the major biological waste treatment processes in use. It has been used for many years in the stabilization of municipal wastewater sludge and more recently in the treatment of high and medium strength industrial wastes. Other complex feedstock to which the anaerobic digestion process has been applied, include agricultural wastes and food-processing wastewaters, all of which are considered concentrated wastes i.e. high content of biodegradable organics. Anaerobic degradation of complex, particulate organic materials can be described as a multistage biochemical process consisting of series and parallel reactions (Kaspar and Wuhrmann, 1978; Bryant, 1979; Zehnder et al., 1982; Gujer and Zehnder, 1983 and Zinder, 1984).

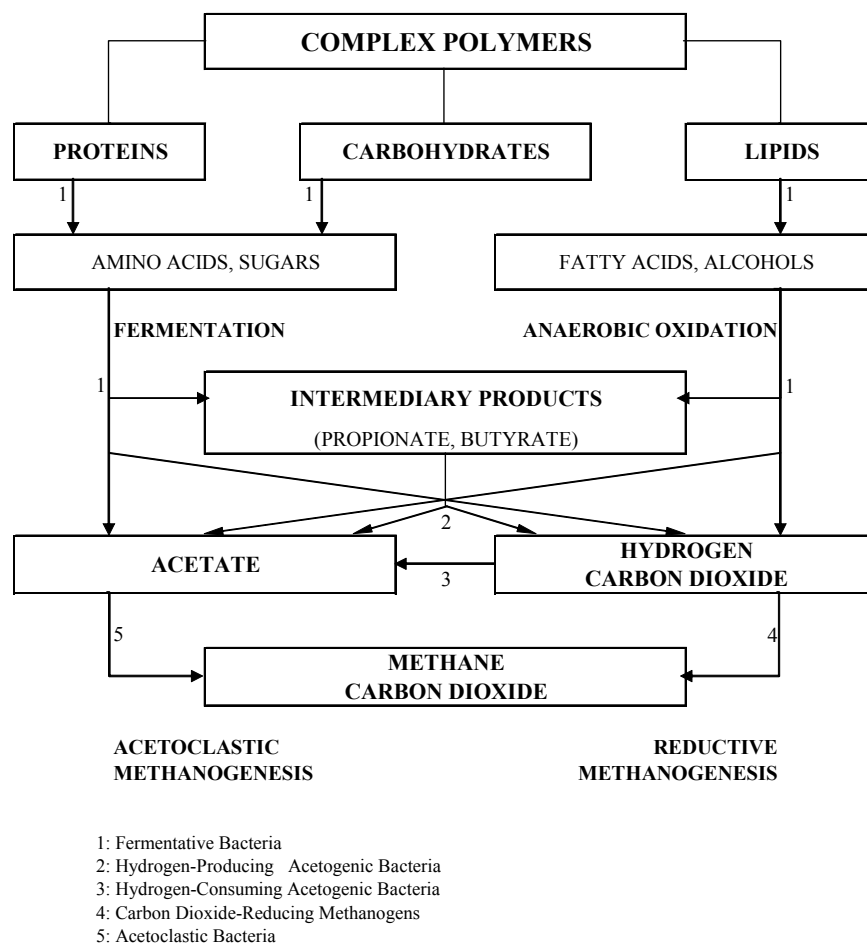


Figure [2-1]: The Anaerobic Process (Speece, 1996). The figure illustrates how complex substrates are degraded into simpler substrates and the micro-organisms which facilitate that process.

From **Figure [2-1]**, it can be seen that the main anaerobic microbial groups that are relevant for anaerobic process design and control are: hydrolysing bacteria or fermentative bacteria; acidogens or fermentative bacteria; acetogens or hydrogen consuming bacteria; acetotrophic methanogens and hydrogenotrophic methanogens. However, from a kinetic viewpoint, anaerobic treatment may be generally described as a three-step process (Remigi, 2001).

2.1.1 Stage One: Hydrolysis

In the first stage, complex compounds are converted to less complex soluble organic compounds by enzymatic hydrolysis in the extra-cellular environment. This is important since micro-organisms cannot utilize polymeric organic material unless it is broken down to soluble compounds that can pass the cell membrane. Therefore solubilisation is the first step in the anaerobic degradation of complex polymeric organic material. During hydrolysis, acid forming bacteria (acidogens) colonize the surface of the particles. The bacteria secrete hydrolytic enzymes that are responsible for the extra-cellular hydrolysis of the particulate and complex material. In terms of chemical composition, three groups of compounds are considered as the major components of complex molecules: carbohydrates, proteins and lipids. The following reactions are expected to occur:

- The hydrolysis of the glucoside bonds of polysaccharides (carbohydrates) to yield dimeric and monomeric sugars.
- The hydrolysis of the amide bonds of proteins to yield amino acids.
- The hydrolysis of ester bonds of lipids to yield long chain fatty acids, glycerol and alcohols.

Further, the rate of hydrolysis has been shown to be dependant on a large number of factors. These include:

- pH: Hydrolysis reactions are faster in an approximately neutral pH environment as opposed to acid conditions.
- Microbial biomass: The level of hydrolytic enzymes increases as biomass increases.
- Temperature: An increase in temperature results in an exponential increase in reaction rate.
- Particle geometry: Surface area and size influence hydrolysis rates.
- Type of substrate: Rates of hydrolysis differ for the lipid, carbohydrate and protein fractions.
- Chemical binding: Various components may be intimately bound therefore hydrolysis rates decrease.

2.1.1.1 Hydrolysis of Carbohydrates

Most of the literature on the hydrolysis of carbohydrates comes from studies dealing with the hydrolysis of cellulose by pure cultures. The hydrolysis products of cellulose are cellobiose and glucose whereas hemi-cellulose hydrolyses to pentoses, hexoses and uronic acids (Colberg, 1988). Enzymes include cellulases.

2.1.1.2 Hydrolysis of Proteins

Proteins are hydrolyzed by extra-cellular enzymes, called proteases, into polypeptides and amino acids. The amino acids produced as a result of protein hydrolysis are further fermented to volatile fatty acids, carbon dioxide, hydrogen gas, ammonia and reduced sulphur. Generally, the hydrolysis of protein is slower than the hydrolysis rate of carbohydrates under anaerobic conditions (Heukelekian, 1958).

2.1.1.3 Hydrolysis of Lipids

The degradation of lipids in anaerobic environments proceeds through the initial breakdown of fats by lipases to their constituent long-chain fatty acids and the galactose and glycerol moieties. Upon complete hydrolysis, phospholipids yield one equivalent of glycerol, one equivalent of phosphoric acid and two equivalents of fatty acids.

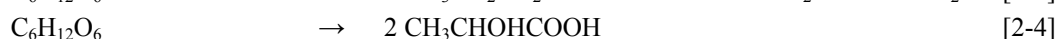
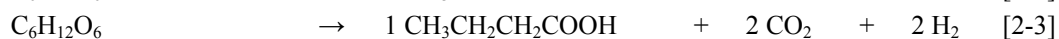
2.1.2 Stage Two: Acidogenesis

In the second stage, the products of the first stage are converted into acetic acid, propionic acid, hydrogen, carbon dioxide, and other low molecular weight organic acids by facultative and anaerobic bacteria (acid formers).

In the absence of methanogenic bacteria, the major products of soluble carbohydrate fermentation by anaerobic bacteria are ethanol, acetate, hydrogen and carbon dioxide gas (Wolin, 1979; Wolin, 1982). However, when hydrogen utilizing bacteria are present, a reduction in ethanol and an increase in acetate production are observed. The shift in the fermentation products is explained by the theory of interspecies hydrogen transfer, which efficiently reduces the hydrogen concentration and raises the redox potential of the H^+/H_2 couple (Thauer et al., 1977; Wolin, 1982). When hydrogen is effectively removed, the anaerobic, fermentative bacteria do not produce electron sink compounds e.g.: ethanol but rather produce hydrogen gas from NADH, which leads to an increase of the produced ATP energy.

2.1.2.1 Glucose Fermentation

Acid forming bacteria ferment glucose to produce a mixture of acetic, propionic, butyric and lactic acids, according to the following stoichiometry:

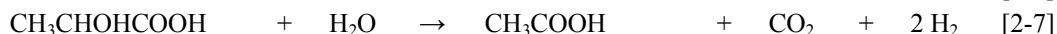
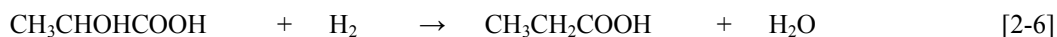


Assuming a cell formula of $C_5H_7O_2N$, the reaction describing the production of biomass from glucose is:

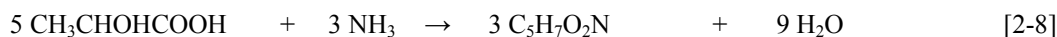


2.1.2.2 Lactic Acid Fermentation

Studies have indicated that lactic acid is a major intermediate in anaerobic digestion. Lactic acid, produced by glucose fermentation, is broken down into different ratios of acetic and propionic acids, depending on the hydrogen partial pressure, according to the reactions:



The reaction describing the production of biomass:



2.1.2.3 Amino Acid Fermentation

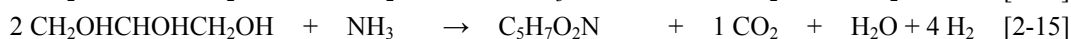
Results on the anaerobic degradation of amino acids produced by the hydrolysis of complex protein wastes indicate very low residual soluble nitrogenous organic matter. Studies have shown that the fermentation of amino acids produced during the anaerobic hydrolysis of proteins is fast and that the rate-limiting step is hydrolysis. Assuming that the formula $\text{C}_5\text{H}_9\text{O}_3\text{N}$ is a valid approximation for the average of all amino acids produced from hydrolysis, the reactions for the 4 main fatty acids are:



The biomass synthesis equation is:



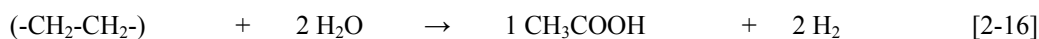
2.1.2.4 Glycerol Fermentation



2.1.2.5 Anaerobic Oxidation of Long-Chain Fatty Acids

During the anaerobic oxidation of long-chain fatty acids, molecular hydrogen is the main sink for electrons (Gujer and Zehnder, 1983). The breakdown of long chain fatty acids occurs by oxidation of the beta carbon atom, resulting in the formation of acetic acid and hydrogen. The major short-chain fatty acids produced as a result of fermentation of long-chain fatty acids are acetate, or acetate and propionate (McInerney and Bryant, 1981).

Studies have shown that the degradation rate of long-chain fatty acids was similar to the degradation rate of acetic and propionic acid (O'Rourke, 1968). The general stoichiometry for β -oxidation, as given by (Gujer and Zehnder, 1983) is:



For the specific case of palmitic acid the complete overall reaction is:



The cell synthesis reaction based on the breakdown of palmitic acid is:

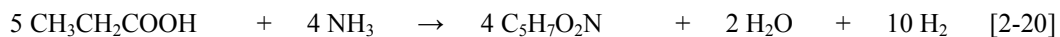
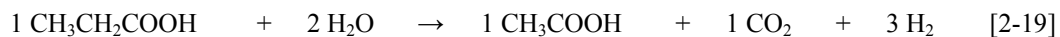


2.1.2.6 Acetogenesis

The main products of the anaerobic oxidation of short-chain fatty acids are acetate and hydrogen gas (McInerney and Bryant, 1981; Dolfig, 1988). These reactions are usually termed acetogenesis since acetate is the major carbon product. A number of bacteria capable of degrading butyrate and higher fatty acids have been identified, however only one acetogenic species capable of degrading propionate (and only propionate) has been identified, *Syntrophobacter wolinii* (McCarty and Mosey, 1991). Hence, for the purpose of modelling acetogenesis, the two groups should be kept separate.

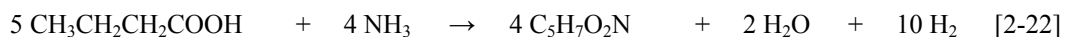
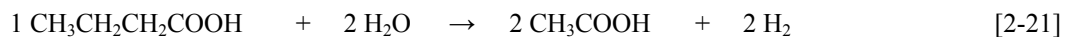
Propionate

The anaerobic oxidation reactions of propionate and biomass production by McCarty and Mosey, (1991) are:



Butyrate and Higher Fatty Acids

Butyrate oxidation and biomass production are represented by:

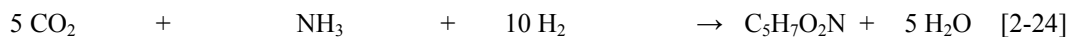


2.1.2.7 Homoacetogenesis

This refers to the production of acetic acid from carbon dioxide and hydrogen gas. The reaction provided by (McCarty and Mosey, 1991) is:



A suitable biomass synthesis reaction is:



Homoacetogenesis is only significant in relation to hydrogen consuming methanogenesis at temperatures below 20°C.

2.1.3 Stage Three: Methanogenesis

In the third stage, two groups of methanogenic bacteria are involved. One group converts hydrogen and carbon dioxide gas to methane. The other converts acetate to methane and bicarbonate. About 70 % of the methane produced in the anaerobic digestion process results from the degradation of acetic acid. This conclusion was drawn from studies on elective enrichment cultures and was based on the assumption that all methane not originating from the reduction of labelled carbon dioxide was formed from acetic acid (Jeris and McCarty, 1965).

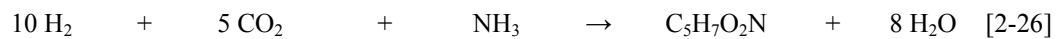
With few exceptions, most methanogenic bacteria use hydrogen and carbon dioxide gas for growth (Vogels et al., 1988). The efficient removal of hydrogen produced during the fermentation processes and the anaerobic oxidation of fatty acids by methanogens allows the aforementioned reactions to proceed under natural physiological conditions. Although about one third of the methane produced in a municipal digester comes from the reduction of carbon dioxide using hydrogen, the interspecies hydrogen transfer and utilization is far more important since it regulates the rate of hydrogen producing reactions by controlling the partial pressure of hydrogen. The dissimilation of acetic acid to methane requires the net transfer of one electron, and the free energy decrement of the conversion is small. The formation of a methane molecule by carbon dioxide reduction requires the net transfer of eight electrons. The free energy decrement of the conversion of hydrogen and carbon dioxide to methane is approximately three times the free energy decrement of the dissimilation of acetic acid to methane and carbon dioxide. From the assumption that one mole of methane is formed from one mole of acetic acid, the implication is that acetic acid would account for approximately 73 % of the methane produced by the sludge. If most of the remaining 27 % methane were formed from carbon dioxide and hydrogen gas, the substrates could provide more energy than acetic acid for growth of methanogenic bacteria. Therefore, it would be expected that the methanogenic bacteria, which utilize hydrogen gas, would be more numerous than the methanogens that utilize acetic acid. This was found to be true, the former outnumbering the latter in digesting domestic sludge (Smith, 1966).

2.1.3.1 Hydrogenotrophic Methanogenesis

Methanogenesis utilizing hydrogen and carbon dioxide can be described by the reaction:

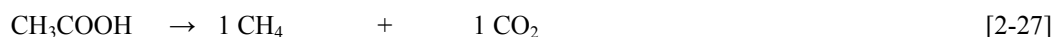


and using cell synthesis by,



2.1.3.2 Acetoclastic Methanogenesis

It is the generation of methane utilizing acetic acid as the substrate. The overall reaction for biological production of methane from acetate is given by:



2.2. Anaerobic Cometabolism

Many toxic organic compounds, such as nitro-aromatics and polychlorinated compounds, are recalcitrant to aerobic treatment (McCormick et al., 1978; Guthrie et al., 1984). They are transformed by anaerobic cometabolism with the utilization of a primary substrate that is usually an easily biodegradable organic compound (Cheng et al., 1996). The term cometabolism is defined as transformation of a non-growth substrate by growing micro-organisms in the presence of a growth substrate or primary substrate, or by resting micro-organisms in the absence of a growth substrate (Criddle, 1993). Some recalcitrant compounds can be considered growth substrates while others are not. A growth substrate is defined as carbon and energy sources for microbial growth and maintenance. Many cometabolic enzymes and cofactors are induced by utilization of a growth substrate. A non-growth substrate is biotransformed by these enzymes, but it cannot be utilized by the micro-organisms to support their growth. However, it must be considered that the biodegradation of a recalcitrant compound may be synergistically or antagonistically affected by the presence of other compounds (Evans and Ahlert, 1987; Kim and Maier, 1986; Schmidt et al., 1987). The presence of the easily biodegradable substrate may stimulate the growth of micro-organisms that then accelerate the biodegradation of recalcitrant compounds (Lu and Speitel, 1988). However, the increased microbial population may adversely affect the biodegradation of the recalcitrant compounds, if the micro-organisms shift the major carbon source from the recalcitrant compounds to the relatively easily biodegradable compounds.

2.2.1 Factors Limiting Microbial Degradation of Recalcitrant Compounds

Complex compounds can be incorrectly assumed to be unbiodegradable substances. It may be that the complex compound is biodegradable; however the biodegradation process is being limited by unfavourable conditions. The following section describes some of the factors that could limit the degradation of recalcitrant compounds.

2.2.1.1 Environmental Parameters

Proper environmental conditions are fundamentally important to microbial growth and survival. Unless the pH, temperature, water activity and redox potential are suitable for anaerobic degradation; microbial growth and consequently biodegradation will be limited (Providenti, 1993).

2.2.1.2 Low Aqueous Solubility

Limited availability in the aqueous phase of many environmental pollutants to micro-organisms is a major factor that affects biodegradation. Even if the capacity to degrade is present and environmental conditions are adequate, inability of microbes to acquire target compounds limits degradation.

2.2.1.3 Lack of Functionality

The lack of any functional groups has been a characteristic associated with the recalcitrance of hydrocarbons in anaerobic environments (Schink, 1985). Aerobic organisms can introduce functionality into unsubstituted hydrocarbons by inserting elemental oxygen with oxygenases. These enzymes activate the oxygen by partially reducing it, allowing for the incorporation of a hydroxy group. Anaerobes have a much more difficult task as they must introduce functional groups with H_2O , HCO_3^- or organic acids (Field, 2002).

2.2.1.4 Electron Donating Functional Groups

The presence of electron donating functional groups, such as amines can form a stumbling block, for the nucleophilic attack of the molecule by anaerobes (Knackmuss, 1996). Aromatic amines are important biotransformation products of azo dyes and nitroaromatics. Most aromatic amines are persistent to anaerobic degradation. The simplest aromatic amine, aniline, is extremely recalcitrant to degradation under methanogenic conditions (Field, 1987).

2.2.1.5 Uptake Limitations

It is assumed that recalcitrant hydrocarbons enter cells through passive diffusion, facilitated diffusion, and/or active transport mechanisms. Depending on the arrangement of atoms, a large molecule may not easily traverse cellular membranes (Providenti, 1993). During anaerobic treatment of pulping wastewater, molecular weight distribution studies demonstrated that the high molecular weight fractions are inert; while the low molecular weight fractions corresponding to monomers and oligomers are metabolized (Sierra et al., 1990). Large non-hydrolysable polymers cannot be taken-up by micro-organisms to be attacked intracellularly and are also not susceptible to extracellular hydrolytic enzymes of anaerobes (Field, 2002).

2.2.1.6 Metabolic Limitations

A metabolic barrier to microbial degradation is the lack of catabolic enzyme induction. Insufficient induction may result from a cometabolic requirement by some micro-organisms. Cometabolites are believed to supply energy and reducing equivalents, which support growth and allow degradation of non-growth substrates (Janke, 1985). In addition, cometabolites induce the production of catabolic enzymes that recognize contaminants and catalyze their transformation.

Preferential metabolism of alternate carbon sources by micro-organisms may limit biodegradation of some contaminants (Providenti, 1993). However, alternate carbon sources do not necessarily inhibit contaminant degradation. They may have no effect or may improve biodegradation depending on the culture conditions (Kim, 1986).

Another metabolic barrier to contaminant biodegradation is inhibition of mineralization. This can be caused by some chemicals or toxins already present in the environment or produced by micro-organisms. Some toxic compounds are degradation products produced from incomplete metabolism i.e.: by-products that accumulate and are more toxic than the parent or target compounds.

2.2.1.7 Inhibition of Metabolism

Contaminant biodegradation may also be inhibited by the presence of toxic metals. The mechanisms of metal toxicity may include interactions with electron transport chains, inhibition of enzymes, binding to nucleic acids and membranes, and inhibition to cell division (Hughes and Poole, 1989). This form of inhibition is especially relevant for heavy metals as they are often present in toxic waste sites or industrial sewage (Wild, 1991).

Many industrial wastes and polluted sites contain mixtures of different organic and inorganic chemicals. Different contaminants, when present together, can interact and affect biodegradation. The simultaneous presence of different toxic organic compounds may inhibit biodegradation even though, individually, each compound can be degraded (Providenti, 1993).

2.2.1.8 Unfavourable Thermodynamic Reaction

The thermodynamic favourability or negative Gibbs free energy change of a reaction will depend to a large extent on the redox potential of the electron acceptor available for the reaction. Elemental O_2/H_2O has the highest potential (0.82 V), however HCO_3^-/CH_4 (-0.24 V) has by comparison a much lower redox potential (Field, 2002). Metabolising hydrocarbons to acetate and hydrogen by fermentative bacteria is a highly endergonic reaction. Consequently, these bacteria would need to depend largely on syntrophic partners e.g.: methanogens to remove intermediates in order to make the reaction thermodynamically feasible.

2.3. Kinetic Models for Anaerobic Digestion

Biodegradation and biotransformation have been extensively studied for the treatment of organic compounds in environmental wastes. The establishment of kinetic models to describe the biodegradation processes and the estimation of the kinetic parameters can help us understand the intrinsic characteristics of the processes, and predict the fate of the organic compounds in certain systems (Suidan et al., 1988; Grady et al., 1989). Biological growth kinetics is based on two fundamental relationships: growth rate and substrate utilization rate. The effect of the growth limiting substrate i.e.: the essential nutrient concentration on the rate of microbial growth has been described by various mathematical models (Monod, 1949; Mosey, 1958; Contois, 1959; Grau et al., 1975). The most widely used model for biodegradation kinetics is the Monod equation (refer: **Table [2-1]**).

Table 2-1: Kinetic Models

Monod	$\mu = \frac{\mu s}{K_s + s} - b$	$-\frac{ds}{dt} = \frac{\mu X s}{Y (K_s + s)}$
Contois	$\mu = \frac{\mu_m + s}{BX + s} - b$	$-\frac{dS}{dt} = \frac{\mu_m X s}{Y (BX + s)}$
Grau et al.	$\mu = \frac{\mu s}{s_o} - b$	$-\frac{ds}{dt} = \frac{\mu X s}{Y s_o}$
Symbol	Definition	
μ	specific growth rate	
s	substrate concentration	
s_o	initial substrate concentration	
Y	yield co-efficient	
X	biomass concentration	
t	time	

2.3.1 The Rate Limiting Step

As previously described, the anaerobic treatment of complex compounds is a multi-step process. When a process is composed of a sequence of reactions, one step is usually much slower than the other steps. The last slow step in a sequence of reactions has been called the rate controlling or rate-limiting step (Hill, 1977). In anaerobic digestion, the rate-limiting step is related to the nature of the substrate, process configuration, temperature and loading rate (Speece, 1983). The rate-limiting step in anaerobic treatment is generally considered to be the methane fermentation step because methane-forming bacteria grow slowly and are relatively sensitive to environmental factors. However, hydrolysis of particulate substrates can become rate limiting (Sanders, 2001).

2.3.2 Monod Kinetics

According to McCarty and Mosey (1991), the Monod rate equation applies to a single strain of bacteria growing on a single ‘rate-limiting’ substrate and relates the rate of uptake of that substrate to its concentration in the growth medium. It assumes that all other substrates and nutrients are present in excess, and it further assumes that the products of the reaction do not accumulate sufficiently to inhibit fermentation. It describes a form of ‘saturation kinetics’ in which the rate of reaction, initially proportional to the concentration of the substrate, gradually approaches a maximum value that cannot be exceeded no matter how high a concentration of substrate is applied. By analogy with enzyme kinetics, this is believed to occur when the bacteria’s rate limiting enzyme system is saturated and the substrate is present in excess. Monod proposed in equation [2-28] a functional relationship between the specific growth rate (μ), of a microbial consortia per day, and an essential compound’s concentration (s). The Monod equation states that (μ_{\max}) is the maximum growth rate achievable when (s) is much greater than (K_s) and when the concentrations of all other essential nutrients are unchanged. K_s is the value of the limiting nutrient concentration at which the specific growth rate is half its maximum value and is commonly known as the Monod Half Saturation Constant reported as mgCOD.L⁻¹ when (s) is in the same units.

$$\mu = \frac{\mu_{\max} \cdot s}{K_s + s} \quad [2-28]$$

2.3.3 The Haldane Model

The Haldane substrate inhibition model (Haldane, 1930) has been frequently used to describe the biodegradation of inhibitory compounds. Haldane presented a relationship between the concentration of an inhibitory substrate and an enzymatic degradation rate equation [2-29], where (K_i) was the Inhibition Constant reported as mgCOD.L⁻¹.

$$\frac{ds}{dt} = -k \frac{s}{K_s + s + \frac{s^2}{K_i}} \cdot X \quad [2-29]$$

2.4. Instrumentation

Growing general concern about environmental protection and increasingly stringent regulations mean there is a need for improved process control efficiency in municipal and industrial wastewater treatment plants, to ensure the level of pollutants in final effluents is consistently low. Instruments used in preliminary screening surveys of contaminated groundwater and related risk assessment should be simple, give rapid responses and require a reduced analytical load (Rozzi and Ficara, 2000). Titration biosensors were recently developed to measure the activity of bacterial populations, adequately fulfil these needs.

A biosensor is distinct from a straight forward biological probe. A biosensor is more refined in that the sensor itself contains an integral biochemical component and is essentially a tool for converting a biochemical activity into a quantifiable electrical signal. The biologically sensitive component may be an enzyme, multi-enzyme system, antibody, antigen, whole cell or organelle from any source, suitably immobilised onto the transducer. The component while allowing incredible specificity and sensitivity is often unstable and subject to interference. According to the most rigorous definition of biosensors (Roger and Gerlach, 1996), devices such as respirometers or titration instruments should be named bioassays, but in practice they are commonly named as biosensors by environmental engineers.

Biosensors have found use in clinical analysis, general health care monitoring and environmental and pollution control. Their advantages are likely to include: low cost, small size, rapid and easy use, as well as a sensitivity and selectivity greater than in current instruments. Since they can be miniaturised and automated, biosensors are extremely useful in the environmental and pollution fields. Environmental water monitoring is an area in which cell biosensors may have substantial advantages for combating the increasing number of pollutants finding their way into the groundwater systems.

2.4.1 pH-Stat Biosensors

Principle of Operation

The principle of operation of these biosensors exploits the ability of some micro-organisms to convert a neutral substrate into an acid or alkaline product or to consume an acid or alkaline substrate to make a neutral product. The instrument consists of a thermostatic reaction vessel, a titration unit and a computer for pH control, data logging and data processing. In a typical titration biosensor, the probe is a pH electrode and the titrant is either an alkaline or acid solution. A sample of a microbial population, whose metabolism affects the pH, is transferred to the reaction vessel, and an aliquot of its substrate is added. The biomass starts producing acidity or alkalinity, which is immediately neutralised by the titrant dosed by the titration unit to maintain the pH at a constant pre-set value. The biological activity of the sample is determined by measuring the flow rate of the titrant required to neutralise the produced acidity or alkalinity in the reaction vessel while taking into account the stoichiometry of the reaction. Obviously, any appreciable interfering acidifying or alkalisng reaction must be avoided or carefully controlled into the reaction vessel during the titration test; otherwise accurate determinations cannot be obtained. In particular, attention should be paid to the production of carbon dioxide, which affects the pH of the mixed liquor in accordance with the well-known carbon dioxide/hydrogen carbonate equilibrium (Ficara, 2000).

The ANITA pH-Stat

The pH-stat concept was first developed to evaluate the activity of micro-organisms responsible for the first step of nitrification (Ramadori et al, 1980). Advances to the procedure led to a new instrument, ANITA (Ammonia and Nitrification Analyser), to measure ammonia concentration at the same time as nitrification activity. This biosensor may also be used to measure the inhibiting effects on nitrifiers, either to check the potential toxicity of the influent to a plant or to perform eco-toxicological assessments on chemicals to be released into the environment.

The MAIA Titration Biosensor

Following from the development of the ANITA pH-Stat biosensor, the MAIA (Methanogenic Activity and Inhibition Analyser) titration biosensor has been designed to measure the activity of the acetoclastic methanogens (Rozzi et al., 2001). This instrument is presently used to monitor anaerobic digesters and to test the potential inhibition of industrial effluents to anaerobic treatment. Further, MAIA can effectively measure toxicity effects on anaerobic micro-organisms exposed to landfill leachates (Rozzi et al., 2001).

Advantages

- pH-stat titration systems have the potential to work in anaerobic, sulphate reducing and methanogenic systems.
- This system is based on reliable methods that are well established for mixed liquor samples.

2.4.2 Respirometry

It is based on measuring the rate of substrate consumption or product generation from cellular respiration reactions, thus indicating catabolic activity levels. Anaerobic respirometry typically involves monitoring anaerobic electron acceptor uptake rates or product gas generation rates (Remigi, 2001).

2.4.3 Whole-Cell Sensors

They consist of viable micro-organisms immobilized onto a surface or within a polymeric matrix and located immediately adjacent to a transducer. The whole cells used for these sensors may be natural or genetically modified (Remigi, 2001).

Advantages

- Redox and carbon dioxide based sensors can be used in an anaerobic environment.

CHAPTER THREE

SCREENING METHODS

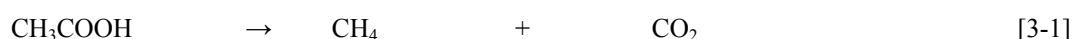
This study involves a comparison of the serum bottle method with the MAIA screening test and assesses the effectiveness of the latter as an anaerobic pre-screening tool. This chapter describes both methods in detail. It outlines experimental conditions, procedures and methods of data acquisition and analysis.

3.1. Methanogenic Activity and Inhibition Analyser

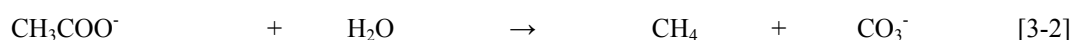
MAIA is a sophisticated titration device. The following section describes how MAIA works and the manner in which it was used to achieve the specific objectives of this study.

3.1.1 The Principle of MAIA

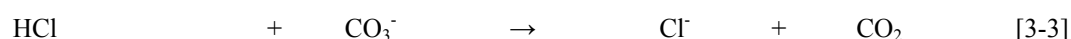
The titration biosensor is a pH-stat, where the alkalinity produced by acetoclastic methanogens is neutralized by an acid solution i.e.: hydrochloric acid (D'Ambrosio, 2000). Specifically MAIA is focused on the acetoclastic methanogens because they are responsible for more than 70 % of methane production (McCarty, 1965). The principle operational chemistry behind MAIA can be explained by considering the single substrate biodegradation of acetic acid. As the methanogenic activity starts, it converts the acetic acid into methane and bicarbonate according to the reaction:



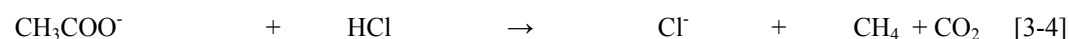
In an aqueous environment, the acetic acid is present as acetate and the carbon dioxide as bicarbonate. Therefore the actual reaction is:



This shows that the metabolism of the bacteria induces a pH increase i.e.: the production of carbonate ions. Therefore, titrating with an acid solution e.g.: hydrochloric or acetic acid, can keep the pH within a narrow range of variability. Equations [3-2] and [3-3] describe titration by hydrochloric acid while equations [3-5] and [3-6] describe the chemical reactions associated with acetic acid titration. In this study, **acetic acid** was used as the acidic titrant.

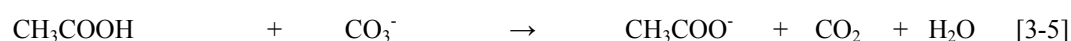


The overall hydrochloric acid reaction, adding equations [3-2] and [3-3], gives:

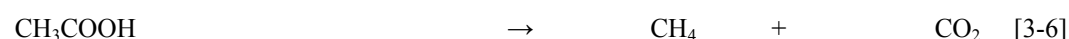


Any excess alkalinity produced is consumed by the hydrochloric acid while the substrate concentration decreases due to acetoclastic activity.

However titration with acetic acid neutralises the excess alkalinity and replaces the acetate that has been consumed such that the substrate concentration remains constant throughout the test (provided the dilution effect is neglected).



The overall acetic acid reaction, adding equations [3-2] and [3-5], gives:



3.1.2 Structure of MAIA

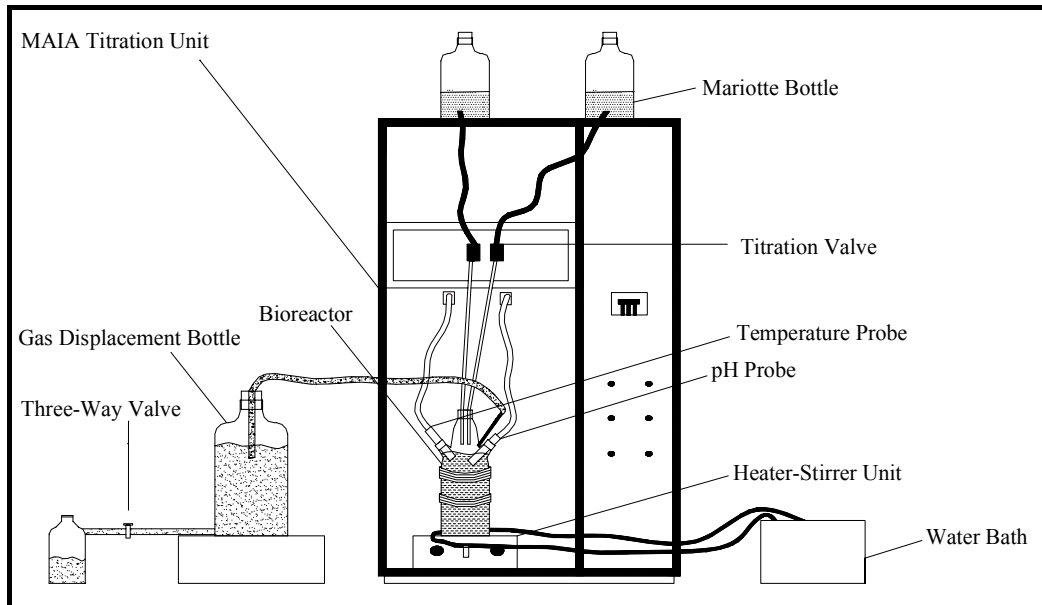


Diagram 1: Methanogenic Activity and Inhibition Analyser

MAIA consists of four components:

- The Bioreactor
- The Displacement System
- The Titration System
- The Control System

3.1.2.1 The Bioreactor

The reactor is a 1L, airtight, thermostatic glass vessel. It has six ports that accommodate the pH and temperature probes; a liquid and gas sampling points; an inlet for titrant dosing and an exit port for gas discharge. A magnetic stirrer continuously stirs the mixed liquor within the reactor. The reactor unit was encircled by soft plastic tubing that conducts warm water (37°C) from a water bath around the reactor.

3.1.2.2 Gas Displacement System

The displacement bottle is a 2 L, airtight Mariotte bottle. It is filled with a solution of acidified water i.e.: pH = 2, that does not permit the dissolution of any of the gases exiting from the reactor. The pressure within the headspace of the displacement bottle is monitored using a pressure gauge (250 kPa).

3.1.2.3 Titration System

The titration unit comprises of three micro-electrovalves (SIRAI, Model 301) and the corresponding reservoir tanks. However, for this study only two of the valves were operated. Mariotte bottles were used as reservoir tanks, which kept a constant head over the electrovalves regardless of the consumption of the titrant. Hence, the flowrate of the titrant is simply a function of temperature since temperature affects the viscosity of liquids. Both reservoir tanks contained 0.5 M solutions of acetic acid and sodium hydroxide respectively.

3.1.2.4 The Control System

The control unit consists of a software package (Denicon.exe), which translates pH and temperature signals from the respective electrodes and actuates the appropriate valve when the pH deviates from the set-point by a margin of 0.02 units. The software interface allows efficient control experimental parameters through different screens. It is possible to set the:

- frequency of measurements;
- pH set-point and the range of pH;
- opening and minimum closing time of the valves;
- initial sample volume and the optimal temperature.

During each test the software records the pH value, cumulative titrant volume dosed, the number of pulses of the valves, and the temperature as a function of time. This information is automatically recorded in a '*.dat' file format and then imported to a spreadsheet to be processed.

3.1.3 Experimental Conditions

Experiments with MAIA were conducted after defining certain experimental parameters. The following section explains why these parameters were considered important. However, detailed descriptions of the experimental conditions to be tested are presented in **Chapter Four**.

3.1.3.1 Liquid-Gas Equilibrium

For the correct operation of the titration biosensor, only the biological reaction under investigation should be the acidifying or alkalisng reaction taking place in the system. This implies that any other weak acid-base systems in the mixed liquor, especially the carbon dioxide/hydrogen carbonate system, must be kept as close as possible to equilibrium in order to avoid interference. Therefore, dissolved carbon dioxide in the mixed liquor must be in equilibrium with the carbon dioxide in the gas. Finally, the molar fraction of carbon dioxide in the headspace gas phase at the start of every test must be 0.50 because the biogas released by acetoclastic methanogens is made of a 50 % carbon dioxide/methane mixture. If this is not the case then activity determinations during the first part of the tests are affected by an error (Rozzi et al., 2001).

3.1.3.2 pH and Buffer Equilibrium

The pH plays a major role in anaerobic biodegradation. It influences the activity of micro-organisms which are active within certain, narrow pH ranges. Anaerobic digestion processes occur in the pH range of 6.0 to 8.3, however methanogens have a pH optimum value between 7 and 8 while the acidogens have a lower optimum value. If the pH of the waste is outside the optimal range and if the buffering capacity of the system is not sufficient, the anaerobic process will be inhibited. This will lead to under-estimation of the methane potential (Angelidaki, 2002). For example, if the pH were increased from the set-point value with the addition of a test substance, the system will titrate acid to correct this change. However, the titration of the acid in this scenario is not related to the activity of the micro-organisms, but to a change in the physical-chemical equilibrium (Rozzi et al., 2000).

The pH value of the mixed liquor depends on the concentration of bicarbonate ions. It is worth noting that in a system where the molar fraction of carbon dioxide is constant in the gas phase, the bicarbonate buffer capacity does not have a maximum for $\text{pH} = \text{pK} (\text{CO}_2/\text{HCO}_3)$ as in closed systems, but increases continuously with pH (Stumm, 1996). It follows that, the higher the pH, the higher the concentration of bicarbonate and the related buffer intensity

even for pH greater than 6.40, which is the value of $pK (CO_2/HCO_3)$. It is obvious that the buffer capacity in a pH-stat titration system has to be maintained to the lowest value compatible with the requirements of the biomass. From the above considerations, it is necessary that a test is started only after the physical-chemical conditions reach equilibrium conditions. If the pH at the beginning of a test does not correspond to the equilibrium value, then a transient phase occurs.

For the case where the starting pH is greater than the pH at equilibrium (pH_{eq}), an appreciable volume of titrant is dosed very rapidly, independent of the actual biomass activity. Where the start pH is less than pH_{eq} , the biomass consumes the substrate, while the pH control system is not actuated and therefore no activity is measured. These transients should be avoided, especially if it is important to run a test at a constant, controlled substrate concentration (Rozzi et al., 2001).

3.1.3.3 Importance of a Gas-tight Headspace

If the reactor is not completely gas-tight, there is gas exchange between the atmosphere and the headspace in the flask and some carbon dioxide escapes while nitrogen enters the headspace. Consequently, the mole fraction of carbon dioxide in the headspace decreases which also induces a decrease of dissolved carbon dioxide. This effect is detected by the instrument as an additional methanogenic activity. The relative interference (as a % error) increases at decreasing methanogenic activities. Hence, careful sealing of the reactor is essential for a low activity biomass while the error induced by leaks is less important when high activity biomass from high rate industrial digesters is assayed.

3.1.3.4 Importance of Efficient Mixing

Efficient mixing of the mixed liquor during activity tests is vital. If the titrant is not homogeneously mixed within the biomass, the pH probe does not detect the decrease of the pH during a time interval longer (typically minutes) than it would happen for a continuously stirred reactor (CSTR) system. Therefore more titrant than required would be added (Rozzi et al., 2001).

3.1.3.5 Stability of Biomass Activity

Anaerobic biodegradability studies have typically used biomass from full-scale anaerobic digesters treating either primary sewage sludge or a mixture of primary and secondary sewage sludge. However, standard protocols for evaluation of the anaerobic biodegradability of organic pollutants specify sewage sludge as the test biomass (Colleran and Pender, 2002).

3.1.3.6 Incubation Considerations

While biomass from domestic sewage digesters is typically exposed to a wide variety of organic compounds, it cannot be assumed that competent hydrolytic/fermentative micro-organisms capable of metabolizing the full range of potential test organic compounds exist, or are present in sufficient numbers in the biomass to be utilised in individual tests (Colleran and Pender, 2002). Therefore, acclimation of existing competent populations to a test compound may require a significant time period to synthesize the enzymes necessary for degradation. Acclimation may also be necessary to ensure growth of the population(s) involved (Colleran and Pender, 2002).

3.1.3.7 Storage Considerations

Anaerobic sludges are potentially active for long periods of storage because of their low decay rates. However, they can be slow to recover their maximum residual activity

(Colleran et al., 1992). Although this can be an advantage since it allows for biomass to be stored for a longer period of time, it may induce a longer lag period before the biomass activity is recovered. In a test conducted for a sludge that had been stored in a sealed bottle at 4°C for 30 d it was observed, that after a lag period of 6 to 7 h, the increase of activity was incompatible with methanogenic bacterial growth (Rozzi et al., 2000). After 12 h, the smoother increase of titrant volume indicated the full recovery of biomass activity that thereafter remained stable. Elimination of this recovery phase from inhibition tests is very important because, if methanogenic bacteria are still recovering when a toxicant is spiked at low concentration, an increase in activity is observed, this indicates a stimulating effect rather than an inhibiting one. In order to avoid such interference, biomass that has been removed from storage should be acclimated for about 2 d at the operating temperature and supplemented by trace elements and substrate before being used for inhibition tests. It may also be important to acclimate high activity sludge samples when they are directly drawn from a digester because of possible interferences in the titration due to slowly biodegradable substances that are adsorbed to the bacterial cells (Rozzi et al., 2000).

3.1.3.8 Mineral Medium

The anaerobic biodegradation of certain test substances may require the growth of a specific group of bacteria that are present in low numbers; therefore the medium should provide all of the inorganic nutrients required for growth. By contrast, the determination of the specific activity of anaerobic digester sludge populations should utilise a non-growth anaerobic medium in order to evaluate the “actual” activity of the test population (Colleran and Pender, 2002). Hence, the mineral medium i.e.: mineral salt solution or nutrient medium is a solution that provides the nutrients that the bacteria need for functioning. It contains buffer substances, nutrients, trace elements (minerals and vitamins) and an indicator, resazurine. The influence of trace elements on the growth of anaerobic bacteria is well known (Shen et al., 1993; Takashima, Speece, 1989); nevertheless it is not clear if these are always available at a sufficient concentration in the sludge. The effect of the nutrients and trace elements on the biodegradation has been studied by many authors, among them Stotmann et al., (1993) and Speece, (1996). Resazurine is an indicator that changes to pink when oxygen is present in the medium: a small concentration (1 mg/L) helps in detecting non-anaerobic conditions, which may affect the validity of results.

3.1.4 Experimental Procedure

Tests conducted with MAIA using both Textile Size Effluent (refer: **section 4.3.1**) and Landfill Leachate (refer: **section 4.3.2**) was subject to a carefully planned experimental procedure to ensure that data acquired would reflect a consistent experimental methodology.

3.1.4.1 Preparation of the Sludge

The biomass was sampled from anaerobic (mesophilic) digesters located at the Waste Water Treatment Works (WWTW) in Umbilo, South Africa. The sludge was sieved and then centrifuged at 3 000 rpm for 30 min. After centrifugation, most of the supernatant is decanted and the solid biomass retained. The concentrated biomass was stored at 4°C until use. Colleran et al., (1992) stated that an anaerobic sludge, after a long period of storage, can be slow to recover its maximum activity. Therefore, before each test, the sludge was acclimatized at 37°C i.e.: optimal mesophilic temperature, in a 50 % carbon dioxide/nitrogen gas atmosphere for a minimum of 18 h. This operation was fundamental to recover the maximum specific activity and to reduce the lag and recovery phases. Between two consecutive experiments, the biomass necessary for the second one was acclimatized before the end of the first one.

The sludge was characterised by measuring the solids content i.e.: total and volatile solids. Volatile solids are essential to express the activity of the sludge as a specific rate. A Chemical Oxygen Demand (COD) determination was conducted at the start and the end of each experimental run.

3.1.4.2 Test Procedure

Each test follows a procedure that can be separated into three parts:

- Calibration of MAIA
- Reactor Equilibration
- Test

Calibration of MAIA

The calibration MAIA is an essential operation for a successful test. This involves calibrating the pH and temperature probes in addition to the valve calibrations. The calibration of MAIA must be done each time a new test is started and is performed following the instructions of the interface of the control software (anita_31.exe).

Reactor Equilibration

Once the instrument calibration is complete, the reactor is ready to be equilibrated. Equilibration is conducted in order to remove the oxygen and to obtain the correct atmosphere of 50 % carbon dioxide, necessary for the operation of the biosensor (refer: **section 3.1.3.1**). This can be achieved by sparging the reactor mixed liquor with a 50 % carbon dioxide and nitrogen gas mixture until the pH is stable i.e.: until the pH set-point is obtained. Previous experimental work performed by D'Ambrosio (2000) utilised separate carbon dioxide and nitrogen gas cylinders to provide the equilibrium gas mixture. However, this method was prone to problems because adequate mixing equipment was not available (Remigi, 2003).

Although the optimal pH for methanogenesis ranges between a value of 7.0 and 8.0 pH units, 6.88 was selected as the pH set-point. It is understood that with an increase in pH, there is a related increase in the buffering capacity of the mixed liquor. However, any increase in buffering capacity decreases the sensitivity of titrimetric device to detect pH changes. The pH set-point of 6.88 satisfied these two concerns i.e.: methanogenic conditions and buffering capacity (Rozzi et al., 2001). The flushing of the gas mixture is followed by checking the function of the control software that scans and records the pH as a function of time. Plotting this data (refer: **Figure [3-1]**) it can be seen that the pH decreased to a constant level where the physical-chemical system was at equilibrium. However, this is only possible if the system is completely air-tight. Usually, equilibration lasts between 25 and 45 min. If the pH settles below the set-point value of 6.88, then a spike of sodium bicarbonate is added. The bicarbonate spike increases the pH without interfering with the sensitive carbon dioxide/hydrogen carbonate equilibrium. The alkalinity of the system i.e.: $[\text{HCO}_3^-]$ can be determined using the McCarty diagram (refer: **Figure [3-2]**) calculated at 35°C. The equilibrium point A is the intercept between the set-point pH line and the 0,50 carbon dioxide partial pressure line. Using the alkalinity value of the equilibrium point A and knowing the initial alkalinity of the mixed liquor, the amount of bicarbonate required for the pH correction can be calculated.

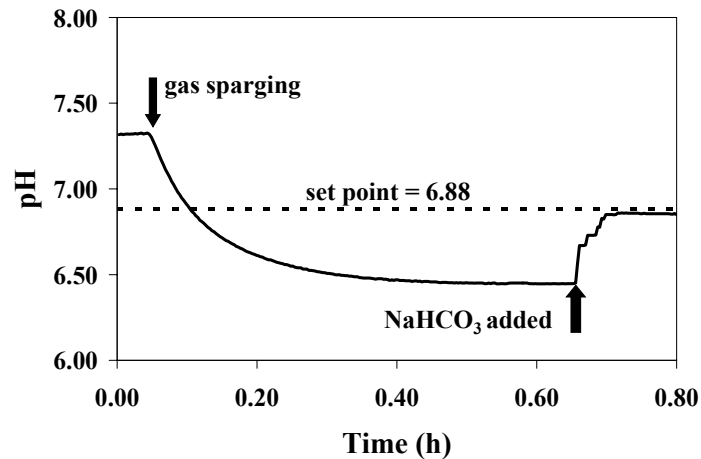


Figure [3-1]: Equilibration. The figure shows the pH profile during the equilibration process. During gas sparging, the pH decreases to a minimum when the liquor is saturated with carbon dioxide gas. Thereafter, sodium bicarbonate is added to raise the pH to the set point value.

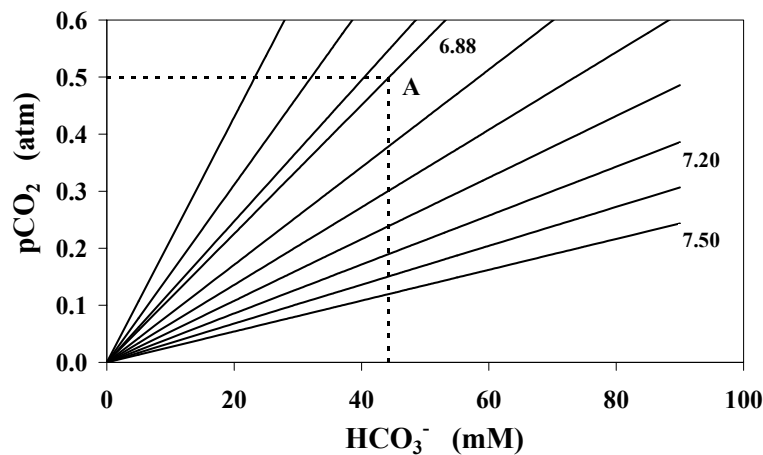


Figure [3-2]: The McCarty Plot. This plot was used to determine the correct pH-set point for a headspace composition of 0.50 atm carbon dioxide at an operating temperature of 35 °C.

The Test

The duration of a typical test was approximately 1 d. During a test, biogas production was monitored by measuring the liquid displaced from the Mariotte bottle connected to the reactor and the reactor headspace composition was analysed using gas chromatography.

3.1.5 Experimental Data and Interpretation

MAIA operates through a computer interface. This section details the type of data that MAIA provides and the manner in which that information is interpreted.

3.1.5.1 Data Record

The system is capable of recording pH, cumulative acid or alkaline titration volumes and the temperature within the reactor for a given period. **Figure [3-3]** presents graphically some of the outputs from MAIA.

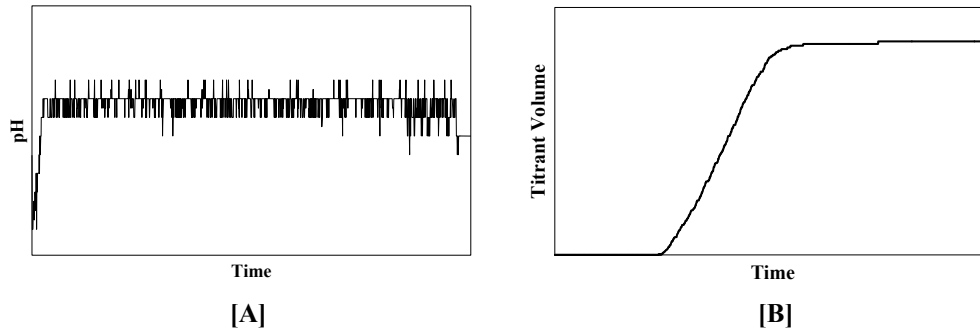


Figure [3-3]: Typical System Outputs. These include pH and cumulative titration volume data.

3.1.5.2 Interpretation of Data

Probably the most significant piece of information provided by MAIA is a record of the cumulative titration history i.e.: either acid or alkaline. Using the cumulative titration curve it is possible to assess activity of the micro-organisms. Activity can be either acidogenic or methanogenic. Acidogenic activity is related to hydrolytic and fermentative processes while methanogenic activity is based on the consumption of acetate (refer: **Activity Assessment**). Acidogenesis includes enzymatic degradation of complex substances in the extracellular environment (refer: **Figure [3-4], REALISTIC ACTIVITY**). It involves acidification and consequently a decrease in the pH of the system. Thus, the process initiates the titration of the alkaline titrant in order to maintain the pH at the set-point value (refer: **section 3.1.3.2**). However, the system is difficult to monitor because acetate conversion by the methanogens can occur simultaneously with hydrolytic and fermentative processes.

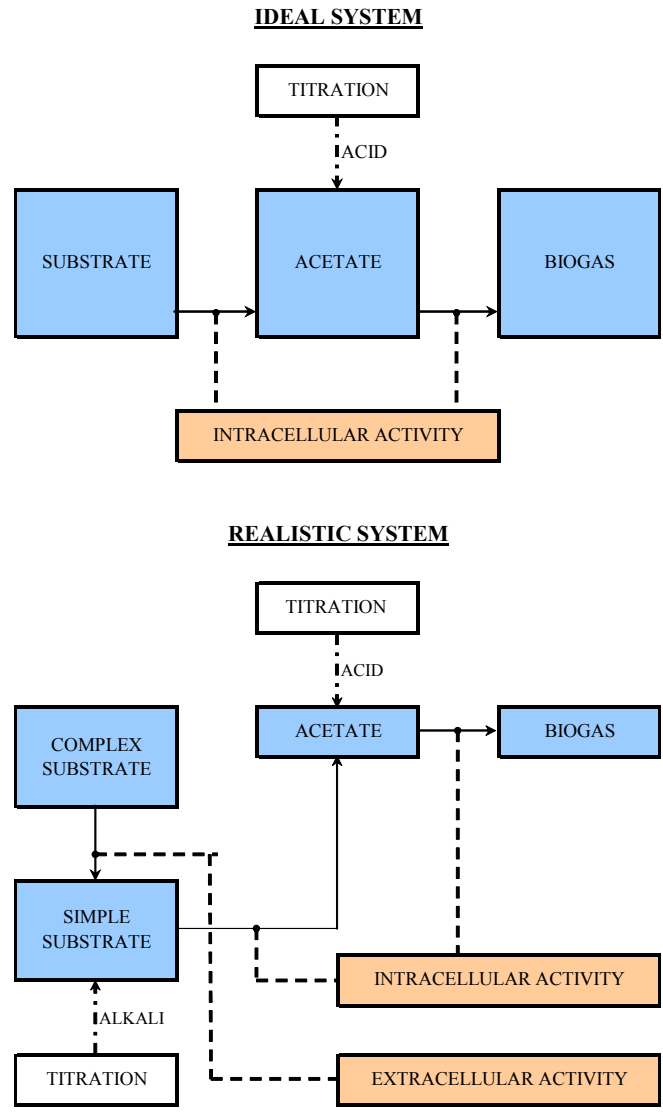


Figure [3-4]: Detection of Activity. The response of the titration system to pH changes is brought about by microbial activity and can involve both acid and alkaline titration depending on the type of biochemical process. In an Ideal System, all microbial activity is considered to be intracellular and results in the removal of acetate i.e.: H^+ , from the extracellular environment. This results in an increase in pH in the extracellular environment and consequent titration of acid by MAIA. However, in a Realistic System the substrate is considered complex and has to be acidified i.e.: production of H^+ , before it can be intracellularly consumed.

The conversion of acetate to biogas i.e.: carbon dioxide and methane gas is an intracellular process. The micro-organisms remove acetate and other substrates from the extracellular environment and metabolize it intracellularly. The removal of acetate by methanogenic activity in the extracellular environment i.e.: the bulk liquid is detected by the system as a pH increase and necessitates acid titration (refer: **Figure [3-4], IDEAL SYSTEM**). In an ideal system, the degradation of a simple substrate to acetate or the removal of acetate already present in the extracellular environment can be easily tracked by the titrimetric system because pH changes are related to only one biochemical process i.e.: acetoclastic methanogenesis.

Activity Assessment

By using the gradient of the cumulative titration curve and the stoichiometry of the system it is possible to evaluate activity. Methanogenic and acidogenic activity is defined in equation [3-7]. It is not strictly correct to use the same equation for both acidogenic and methanogenic activity because acidogenic reactions are performed by micro-organisms that are very different from those that undertake methanogenic reactions. However, it was assumed that the final product of all hydrolytic processes will be acetate. It may be possible to relate all alkaline titration or acidogenic activity to methanogenic activity, but this needs to be verified empirically. In the absence of any empirical data, it was assumed that the acid and the alkali react in the same stoichiometry ratio i.e.: equimolar.

$$A = B \cdot \left[\frac{C \cdot Q}{X} \right] \quad [3-7]$$

C = Molarity of the titrant [mol·L⁻¹]

Q = Slope of the titrant curve [L·d⁻¹]

X = Mass of active biomass [g VS]

B = COD equivalent of acetate [g COD.mol⁻¹]

A = Activity [g COD.d⁻¹.g VS⁻¹]

Biodegradability Assessment

Another aspect assessed during this study was effluent biodegradability. The biodegradability of the test substance may be determined by establishing the amount of organic content that is converted to biogas i.e.: methane. A COD (refer: **APPENDIX F**) mass balance was employed to graphically represent these results.

3.1.6 Protocol for Toxicity Tests

The methodology of the tests was developed to determine the toxic effect (if any) of an industrial effluent on the activity of acetoclastic methanogens.

There were three distinct types of tests:

- Control
- Standard
- Sample

3.1.6.1 Control

Since the biomass was not cultured in the laboratory, but sampled from an anaerobic digester, the sludge sample may contain residual substrates. It was important to determine the level of the methanogenic activity related to the biodegradation of this residual substrate (refer: **section 4-2**).

3.1.6.2 Standard

The standard test, unlike the control test, included a spike of sodium acetate. Acetate is a very labile substrate and was used to stimulate methanogenic activity. Therefore, this test was used to assess the effectiveness of the acetate spike to promote methanogenic activity as well as provide a basis from which toxicity could be evaluated (refer: **section 4-2**).

3.1.6.3 Sample

This test assessed the toxicity and potential anaerobic biodegradability of the wastewater and industrial effluents used.

3.1.7 Potential Experimental Scenarios

MAIA titrates either an acid or a base depending on the pH condition within the reactor environment. The response of MAIA to biochemical activities that induce pH changes needs to be understood if any meaningful interpretation can be made from the titration data. This section explains the titrating behaviour of MAIA using potential experimental scenarios as examples.

3.1.7.1 Titration of Alkali and Acid

Complex and/or particulate substrates have to be converted to acetate before they can be utilized by the methanogens. Consequently, it is anticipated that alkaline titration will precede any acid titration. It is possible that during the period of hydrolytic and fermentative action, no methanogenic activity occurs. Thus there could be a long lag period before acid titration or methanogenic activity occurs. This sequential use of the substrate can be explained simplistically using **Figure [3-5]**. Initially, fermentative bacteria i.e.: Bacteria [A] hydrolyse the substrate to acetate. Once all of the substrate has been completely degraded to acetate, then only do the methanogens i.e.: Bacteria [B] utilize it. The system detects both phases of microbial activity independently and doses either an alkaline or acid titrant to correct for the subsequent pH changes.

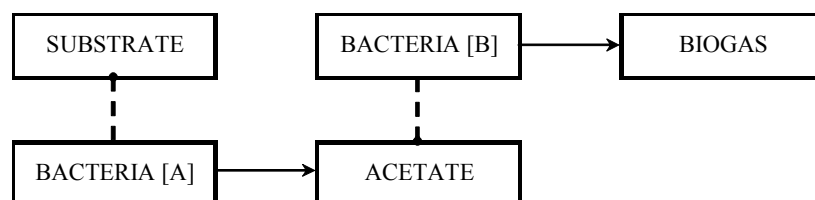


Figure [3-5]: Role of Different Microbial Species. The sequential use of substrate can depend on the type of micro-organisms present in the biomass.

3.1.7.2 Alkaline Titration Masks Methanogenic Activity

Complex substrates could be comprised of varying degradable fractions (refer: **Figure [3-6]**). These fractions e.g.: readily biodegradable (RBCOD), slowly biodegradable (SBCOD) and unbiodegradable (UBCOD), could be reduced all at once i.e.: simultaneous degradation or in stages i.e.: sequential degradation. In **Figure [3-6]**, the sequential strategy is illustrated with the **red** pathway (it is labelled 1) assumed to be a priority step for the micro-organisms. Rapid use of available simple substrates occurs prior to the use of any substrate provided by the fermentative bacteria.

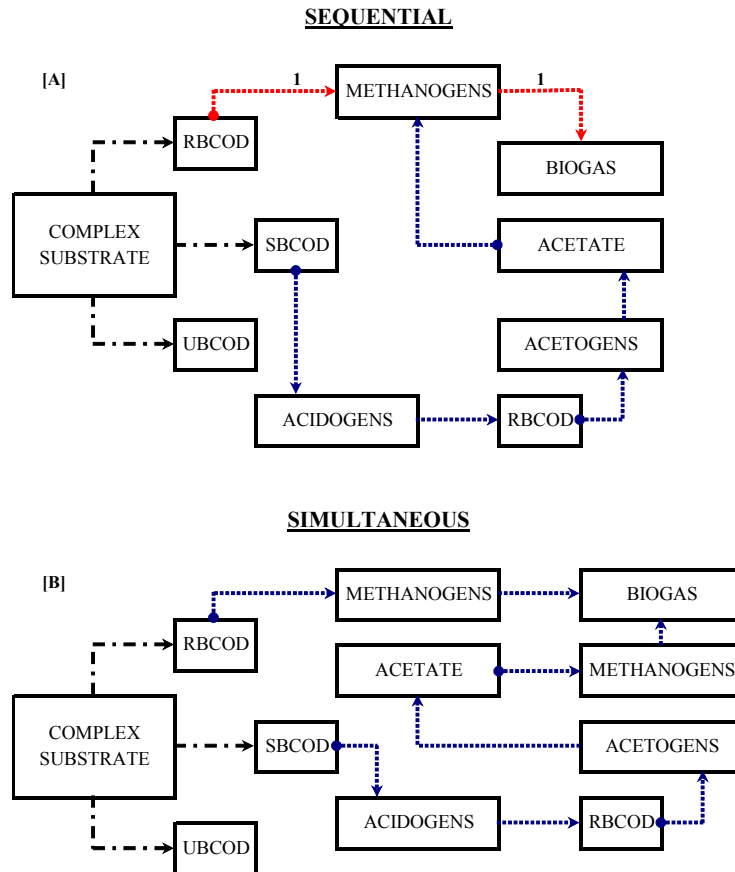


Figure [3-6]: Biochemical Pathways. The figure depicts possible pathways to biogas production. In Plot [A], the path labelled 1 (red) is considered a priority pathway and occurs before every other process occurs.

It is also plausible that methanogenic and acidogenic activity occur simultaneously. In **Figure [3-6]**, simultaneous biochemical processes are depicted using a **blue** line. Acidogenic activity could mask methanogenic activity if the former rate is greater than the latter (refer: **Figure [3-7]**). This could result in alkaline titration occurring even though methane gas emissions are being detected. Hence, this situation could prove to be a limitation of the titrimetric method because alkaline titration could suppress the titration of acid making activity determinations extremely inaccurate. Nevertheless, it is still possible to assess methanogenic activity during this period, if it is assumed that all degradable substrate will eventually form acetate. This means that the parameters (C) and (Q) in equation [3-7] may be based on the alkaline titrant rather than the acid titrant. Further, it is possible to estimate the fraction of the substrate already degraded in this interval (refer: **APPENDIX F**).

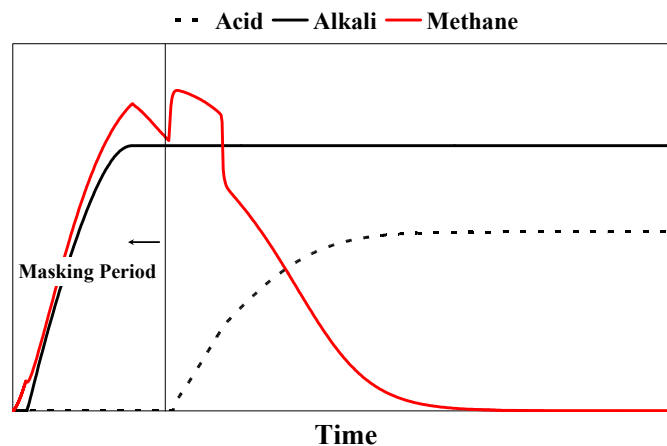


Figure [3-7]: Alkaline Titration. The figure shows acid titration lagging methane production as a result of alkaline titration masking methanogenic activity. Masking of methanogenic activity can occur if the rate of hydrolysis or fermentation exceeds the rate of methanogenesis. During hydrolysis, acidification of the substrate warrants the titration of an alkaline titrant to control the pH of the system. Consequently, excessive acidification would prevent MAIA detecting the production of alkalinity from methanogenic activities and delay the titration of the acid titrant.

3.1.7.3 Repetitive Titration Trends

Micro-organisms sometimes display the same behaviour separated by time intervals. The micro-organisms could begin by using the substrate rapidly, enter into a lag phase and may even lapse into a phase of decreasing activity. All microbial species possess constitutive enzymes that are capable of degrading a variety of complex substrates. Constitutive enzymes are those formed at constant rates and in constant amounts, regardless of the metabolic state of the micro-organism. An inducible enzyme is normally present only in trace amounts in a given species of bacterial cell, but its concentration can increase rapidly when its substrate is present in the medium, particularly when its substrate is the only carbon source of the cell (Lehninger, 1970).

In situations where constitutive enzyme activity fails e.g.: degradation of a recalcitrant substrate, the micro-organisms induce enzymes that can either degrade or biotransform the recalcitrant substrate. The period during constitutive enzyme production could be characterized by a lag period. Multiple lag phases may occur when the medium contains multiple carbon sources (refer: **Figure [3-7]**). This phenomenon is commonly known as diauxic growth. It is caused by a shift in metabolic patterns in the midst of growth. After one carbon source is exhausted, the cell must divert its energies from growth to prepare for the new carbon supply (Bailey and Ollis, 1986).

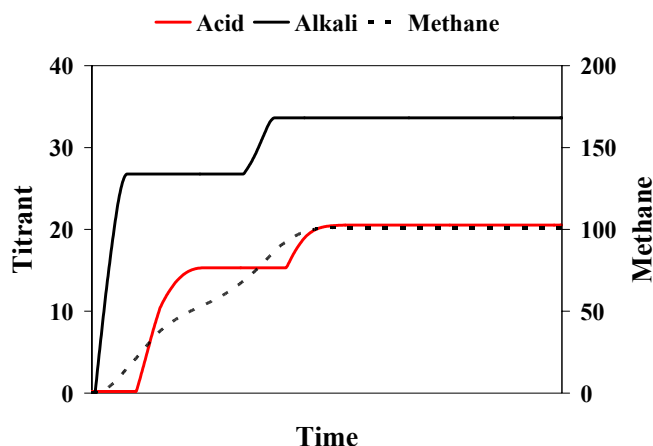


Figure [3-8]: Diauxic Growth. A diauxic growth pattern is characterised by multiple lag phases.

3.2. Serum Bottle Test

This section describes the preparation of the serum bottles and the manner in which the serum bottle study was conducted.

3.2.1 Sludge Preparation

The preparation and characterisation of the biomass is identical to that used for the MAIA tests.

3.2.2 Bottle Preparation

The assay bottles were gassed with oxygen free nitrogen at a flow rate of 0.50 mL/min for 15 min according to (Owen, 1979). A 30 % (v/v) inoculum was added to each bottle that is equivalent to 30 mL of biomass in a total working volume of 100 mL. To this was added a 40 mL sample of substrate i.e.: industrial effluent, 30 mL of a mineral medium and a 2 mL spike of sodium acetate. The bottles were then gassed for 5 min with nitrogen gas at 0.50 mL/min before being sealed with butyl rubber septa and aluminium crimp seals. The sealed bottles were equilibrated at 35°C for 1 h in a constant temperature room. After equilibration of 1 h, the gas volumes were zeroed to ambient pressure using a glass syringe. The bottles were manually shaken to facilitate contact between the micro-organisms and the substrate, once a day.

3.2.3 Experimental Procedure

Gas volume sampling and measurement during incubation were performed with a graduated 20 mL glass syringe fitted with a 22-gauge disposable needle. The syringe plunger was lubricated with distilled water prior to sampling. The syringe needle was inserted through the rubber septum into the headspace. All readings were taken at the equilibration temperature and the syringe was held vertical during measurement. All volume determinations were made by allowing the syringe plunger to move and equilibrate between the bottle and atmospheric pressure (Sacks, 1997). Readings were verified by pushing the plunger past the equilibrium point and releasing to ensure that the plunger returned to the original equilibration volume (Owen, 1979). To continue the assay, the gas was re-injected into the bottles without contamination or loss otherwise the gas was wasted (Sacks, 1997). Gas was wasted when the difference between the internal and atmospheric pressures was greater than 0.50 atm. This was equivalent to about 12 mL of the syringe volume. If gas production was less than this, the measured gas was re-injected into the serum bottle. At the end of the test i.e.: 7 d, the mixed liquor was analysed for Total Solids (TS), Volatile Solids (VS) and organic content (refer: **section 3-3**).

3.3. Analytical Procedures

For the tests conducted with MAIA and the serum bottles, four analyses were conducted:

- Gas Composition
- Organic Content
- Total Solid
- Volatile Solid

3.3.1 Gas Composition

Gas chromatography was used to measure the content of methane and carbon dioxide of the biogas produced. Liquid gas displacement systems were compared to gas chromatographic methods and it was concluded that the latter was more accurate for low methane productions (Soto et al., 1993). The compositional analysis was conducted using a GOWMAC 350 gas chromatograph equipped with a thermal conductivity detector (TCD), which could detect methane, carbon dioxide and nitrogen gas. A packed column was used for the separation operated at the conditions outlined in **APPENDIX B**.

A biogas sample was drawn from the reactor and serum bottles using a 100 μL precision syringe. A sample volume of 30 μL was immediately injected into the gas chromatograph. Using a calibration curve of peak area versus moles sampled (refer: **APPENDIX B**), it was possible to quantify the gas composition in both the reactor and serum bottles.

3.3.2 Organic Content Measurement

The chemical oxygen demand (COD) was used as a measure of the oxygen equivalent of the organic matter content of samples that were susceptible to oxidation by a strong chemical oxidant.

3.3.2.1 Open Reflux Method

The Standard Method was used (APHA, 1985). This method is suitable for wastes where a larger, more concentrated sample is preferred. The test was used to evaluate the COD of the solid fraction of both the sludge and effluent mixtures.

A 1 mL sample of the test substance was diluted to 500 mL in a volumetric flask. The dilution is necessary because the sample COD could be greater than 900 mg O_2/L . A 50 mL aliquot of this was placed into a 250 mL refluxing flask. To this was added 1 g of mercuric sulphate, several glass beads and 5 mL of sulphuric acid reagent. A 25 mL aliquot of potassium dichromate solution (0.0417 M) was added. The solution was mixed and allowed to cool. The flask was attached to the condenser and cooling water turned on. The remaining 70 mL of sulphuric acid reagent was added and the mixture was refluxed for 2 h. A blank consisting of 50 mL distilled water, instead of the substrate, was refluxed in the same way. The samples were cooled and diluted to about twice its volume with distilled water. Thereafter, they were titrated with ferrous ammonium sulphate solution (FAS) using ferroin indicator. The COD of the sample was evaluated using equation [3-8].

$$\text{COD} = \frac{(A - B) \times C \times 8000}{V_s} \quad [\text{mgO}_2\cdot\text{L}^{-1}] \quad [3-8]$$

A = FAS (Blank) [mL]

B = FAS (Sample) [mL]

C = Molarity of FAS [M]

V_s = Volume of Sample [mL]

Angelidaki (2002), identified problems associated with COD measurements. These were:

- halogens can be oxidised;
- aromatic carbohydrates and some aromatic heterocyclic compounds are not oxidised;
- volatile straight-chain aliphatic compounds are not oxidised to any appreciable degree;
- reduced inorganic compounds e.g.: ferrous iron, are oxidised quantitatively under the species.

Nevertheless, the open reflux method for organic carbon measurement is still considered reliable and accurate.

3.3.3 Total Solid

The Standard Method was applied (APHA, 1985) for the determination of total solids in a sample. A 20 mL well mixed sample was transferred to a previously weighed crucible and placed into a drying oven (105°C) to be evaporated to dryness (usually overnight). The crucible was cooled in a desiccator and then re-weighed. The difference in weight represented the total residue. The total solid in the sample was calculated using equation [3-9]:

$$\text{Total Solid} = \frac{(A - B)}{V_s} \quad [\text{g}\cdot\text{mL}^{-1}] \quad [3-9]$$

A = Weight of sample and crucible [g]

B = Weight of the crucible [g]

V_s = Volume of sample [mL]

3.3.4 Volatile Solids

This measurement of volatile solids is an approximation to the amount of organic matter present in the solid fraction. The Standard Method was applied (APHA, 1985). The method for total solid was followed. The solid residue was then ignited in a muffle furnace ($550 \pm 50^\circ\text{C}$) for 2 h. The crucible was cooled in a desiccator and then weighed. The loss of weight on ignition was reported as the total volatile solid.

$$\text{Volatile Solid} = \frac{(A - B)}{V_s} \quad [3-10]$$

A = Weight of the crucible and residue before ignition [g]

B = Weight of the crucible and residue after ignition [g]

V_s = Volume of sample [mL]

CHAPTER FOUR

RESULTS AND DISCUSSION

The following chapter is divided into seven sub-sections. Sections 4.1 to 4.4 describe the experiments conducted with MAIA. Sections 4.5, 4.6 and 4.7 present the results from the serum bottle, component and mathematical simulation studies respectively.

4.1. SYSTEM ASSESSMENT

Two assumptions are implicit when conducting experiments with MAIA. Firstly, it is assumed that the sophisticated titration process effectively tracks the activity of the anaerobes. However, this is only true if the activity of the micro-organisms remains within the limits of the titrating range of MAIA (refer: **Titration Ability**). Secondly, the reliability of the experimental data depends to a large extent on the nature of the biomass used in each experimental series. The quality of the biomass with regard to volatile solid content and residual organics should remain relatively constant throughout the course of the study (refer: **Biomass Assessment**). This section investigates both these parameters and discusses their implications to the scope of this study.

4.1.1 Titration Ability

The aim of this experiment was to define the limits of the titrating range of MAIA and consequently establish the range of microbial activity in gCOD/gVS.d that MAIA can effectively track through its pH detection system.

Materials and Methods

The calibration tests were performed using 500 mL Erlenmeyer conical flasks as reactor vessels. A buffer solution was prepared according to the recipe presented in **APPENDIX B**. Two solutions of sodium hydroxide (0.50 M and 0.25 M) were prepared to simulate biological alkaline production while acetic acid (0.50 M) was used as titrant. A peristaltic pump was used to pump the alkaline solution into the reactor at pre-determined flowrates. The operating range of the pump is presented in **APPENDIX C**. The tests were performed in triplicate with each test being defined by either a unique flowrate, concentration of base or both.

Results and Discussion

The calibration curve for MAIA is defined as Error versus Activity. The error is the difference (positive or negative) between the activity measured by titration (A_m , in mol/s) and the activity simulated by the peristaltic pump (A_s , in mol/s). It is calculated according to equation [4-1].

$$E = \frac{A_m - A_s}{A_s} \cdot 100 \% \quad [4-1]$$

The error also describes the tendency of the pH to increase, when the titration unit is not able to keep the system pH constant i.e.: close to the upper limit of sensitivity. **Figure [4-1]** depicts how the titration system progressively reduces the error between measured activity and simulated activity as the simulated activity is lowered to within the titrating range of the instrument. It can be seen that the pH shifts from a runaway situation to one where the pH is effectively controlled i.e.: the pH curve finally flattens out suggesting that the pH set-point condition is being maintained. From these tests it was possible to determine a narrow operating range over which the titration system was effective.

This range, based on standardised units i.e.: gCOD/gVS.d (refer: APPENDIX F), was found to exist between an activity of 5.0 and 10.0 gCOD/gVS.d. If methanogenic activity lies within these limits then the error in the titrating ability of the instrument is restricted to less than 10 % (refer: Figure [4-2], A). The implication of this finding is important since should the specific activity of the biomass be less than the 5.0 gCOD/gVS.d⁻¹, then the biomass needs to be concentrated in order for a strong enough signal to be detected by MAIA. Similarly, if the activity of the methanogens proves to be too rapid for the system to follow then the concentration of the acid titrant must be increased or the biomass must be diluted. Dilution of the biomass is probably the simpler alternative. The dosage of titrant is characterised by the pulsing of the dosing valve.

It is useful for an operator to quickly assess the rate of activity within the reactor. From the calibration tests it is evident that there exists an optimal valve dosing range where the titrating error is minimised. This range lies between 14.0 and 25.0 pulses/min and is depicted by the dashed region in Plot B, Figure [4-2].

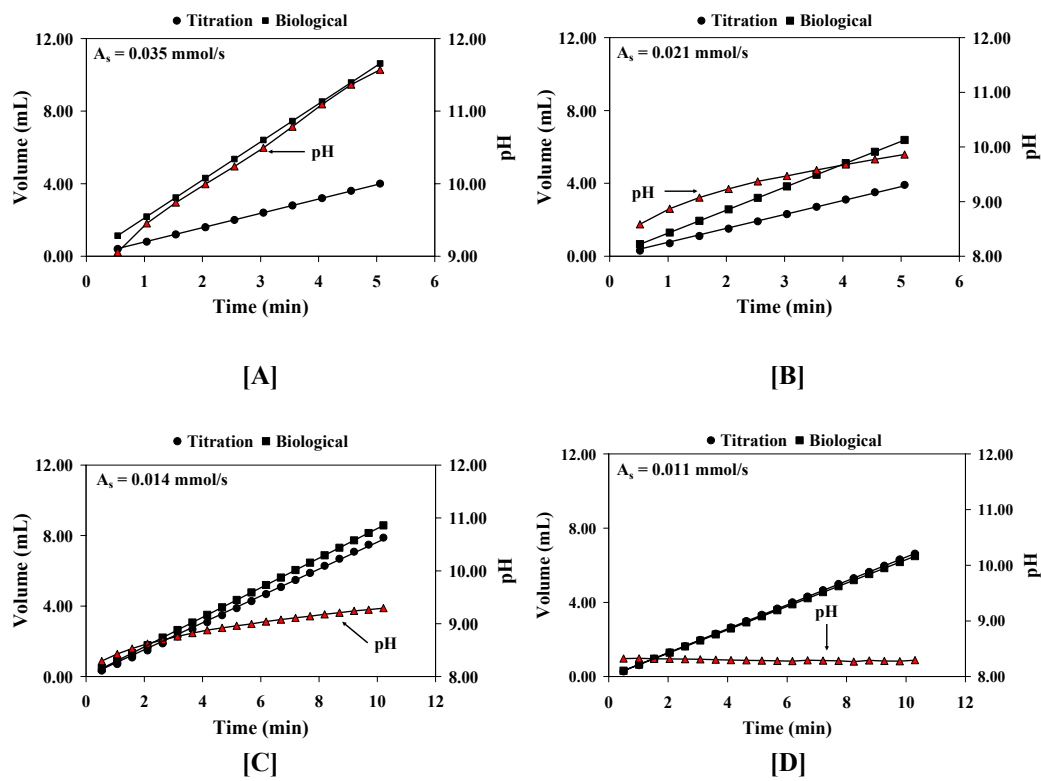


Figure [4-1]: Assessing the Titration System. The figure shows how effective the titration system of MAIA is in tracking simulated activity. The triangular data points indicate pH and the biological curve represents the dosage of alkaline solution by the pump.

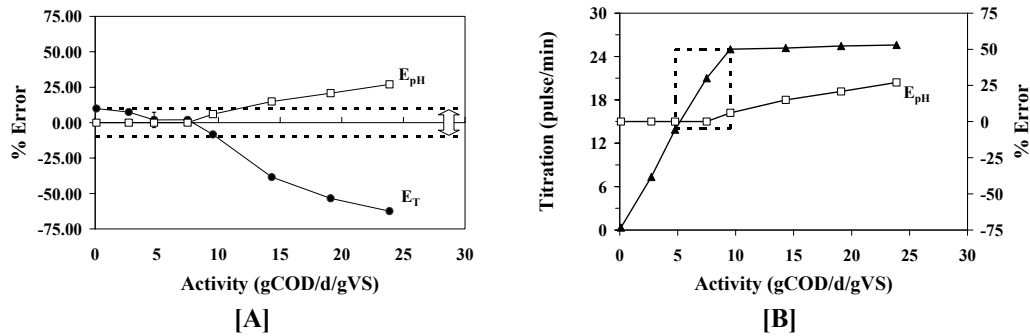


Figure [4-2]: Limits of the Titration System. There is a range of acceptable biological activity where the titration error is minimised. The dashed lines show the acceptable operating range of MAIA i.e.: the range in which the system can effectively track biological activity. E_T is the percentage error in the titration while E_{pH} is the percentage error in the pH control of the system.

4.1.2 Biomass Assessment

The aim of this study was to monitor the specific characteristics of the biomass i.e.: volatile solids and COD content, sampled from the anaerobic digester over the experimental period of the study and to establish that each batch did not vary significantly from each other.

Materials and Methods

The biomass was sampled from an anaerobic digester at a wastewater treatment plant in Durban, South Africa. It was prepared according to the method outlined in **APPENDIX D**. The volatile solid content and residual organic concentration of the biomass were determined by the methods described in **CHAPTER THREE**.

Results and Discussion

The study with MAIA was conducted over a period of six months (June to December 2002). Biomass was sampled in monthly batches which were used to conduct a specific set of experiments. It was expected that the biomass from the same digester across the period of sampling would be comparable. However, changing climatic conditions e.g.: rainfall and changing influent characteristics e.g.: increased industrial and municipal waste disposal operations could mean that batches can differ from each other. **Figure [4-3]** summarises the results from the characterisation tests performed on the sludge over the duration of this study. It can be seen that the quality of the biomass with regard to these two parameters was fairly constant. However, use of the average result makes the comparison of all the experiments conducted over the experimental period more reliable.

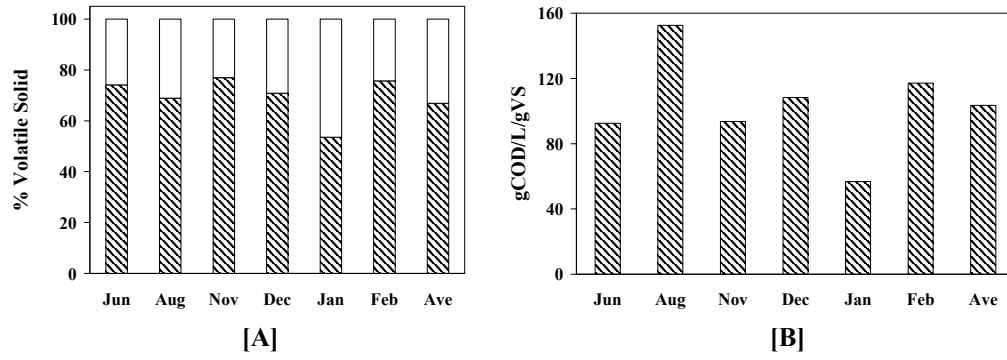


Figure [4-3]: The Anaerobic Biomass. The characteristics of the biomass remained reasonably constant over the project sampling period.

4.2. GENERAL STUDY

Experiments performed with MAIA were planned to closely resemble the serum bottle method without compromising the unique screening ability of the former. Like a typical serum bottle study, experiments conducted with MAIA included control, standard and nutrient tests.

Hypothesis

It was proposed that the labile acetate substrate does stimulate methanogenic activity; that cold storage adversely affects the activity of the biomass; that the nutrient medium is not an essential element to the screening tests performed with MAIA and that the residual substrate does contribute to the overall methanogenic activity. Therefore, the detailed objectives of this investigation were to:

- evaluate the lowest effective concentration of acetate to use;
- quantify the maximum activity of the biomass using the acetate spike;
- determine the impact of the residual substrate.
- determine if the biomass recovers its previous maximum activity after storage;
- evaluate the effect of using a nutrient medium.

In pursuit of the above mentioned objectives, three different types of tests were considered:

- Control-Standard
- Storage
- Nutrient

The Control-Standard Test

The aim of this study was to establish the base-line behaviour of the biomass in the absence of the any substrate e.g.: Control Test and then to establish the response of the biomass to the presence of a readily biodegradable substrate i.e.: acetate, at varying concentrations e.g.: Standard Test.

Materials and Methods

The Control-Standard Test was a 24 h test constituting two different parts i.e.: the first 15 h being regarded as the Standard Test while the remaining 9 h was considered to be the Control Test. For the Standard Test, 240 mL of biomass was placed into the reactor and diluted with 560 mL of distilled water. After equilibration, five 16 mL spikes of varying concentrations of sodium acetate i.e.: 0.1; 0.25; 0.5; 1.0 and 2.5 M, were added to the reactor approximately every 3 h. After 15 h had elapsed, the activity of the biomass was related to the utilisation of a constant acetate substrate concentration of 2.5 M. During this period i.e.: the last 9 h, no additional spikes of acetate were added. Three identical tests were planned to run over three consecutive days. The same batch of biomass was used for each test. The biomass for Test 2 and Test 3 had spent 6 h and 30 h respectively in storage at 4°C prior to acclimation and use. The biomass for each test was acclimated for 18 h at 37°C in a carbon dioxide rich atmosphere.

Results and Discussion

The following discussion summarises the results of the Standard Test and Control Test respectively.

The Standard Test

The purpose of the standard test was to determine the impact of the acetate spike; to evaluate the lowest effective concentration of spike to use and finally to quantify the activity of the acetoclastic methanogens. The test had been performed in triplicate and the trend observed with regard to the production of methane was consistent across all three tests. The titration of acetic acid is a direct response to the production of alkalinity which is indicative of methanogenic activity (refer: **section 3.1.5.2**). It is noticeable from **Figure 4-4, [D]** that activity increases in response to the addition of the acetate spike. Further, the steady, increasing titration addition observed in **Figure [4-4]** confirmed that the sodium acetate spike does promote methanogenic activity. **Figure [4-4]**, also compares the theoretical estimation of methane gas production (refer: **APPENDIX C**), based on gas displacement, to the titrimetric method. Ideally, both curves should plot upon each other. This was not the case, but the comparison did imply a reasonable congruence in both methods. It was decided that only the initial two tests could be accepted since they showed a strong quantitative similarity i.e.: with respect to activity, to each other (refer: **Figure [4-4]**). Consequently, the result from the third test was discarded when quantifying the activity of the acetoclastic methanogens.

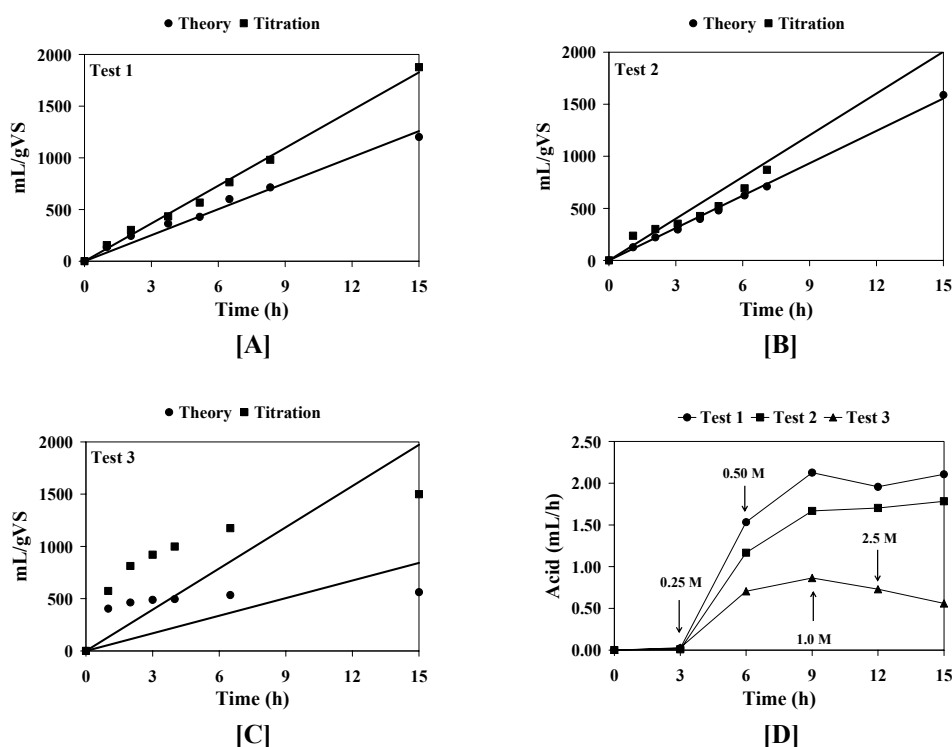


Figure [4-4]: Accuracy of the Titrimetric Method. Each plot compares the titrimetric method of assessing methanogenic activity to the theoretical methane gas yield. The theoretical gas curve is based on the assumption that 50 % of the biogas produced is methane. Plot [D] displays the timing of the acetate spikes every three hours for all three tests.

Table [4-1] shows the effective concentration of the acetate spikes after considering the dilution effect in the reactor.

Table 4-1: The Standard Test

Sodium Acetate Spike (16 mL)	
Standard Solution	Effective Concentration
M	M
0.10	0.0020
0.25	0.0050
0.50	0.0100
1.00	0.0200
2.50	0.0500

It can be seen from **Figure [4-5], Plot [B]** that the average maximum methanogenic activity achieved in the 15 h period was 0.875 gCOD/gVS.d. This compared well with a literature estimate of 1 gCOD/gVS.d⁻¹ (Remigi, 2003). This result confirmed the suitability of the 2.5 M sodium acetate spike, more than the other spike concentrations, to foster an active methanogenic population. The test also highlighted the influence of storage on methane activity, which seemed to affect the result of Test 3. The impact of storage on methanogenic activity is discussed further (refer: **section 4.2.3**).

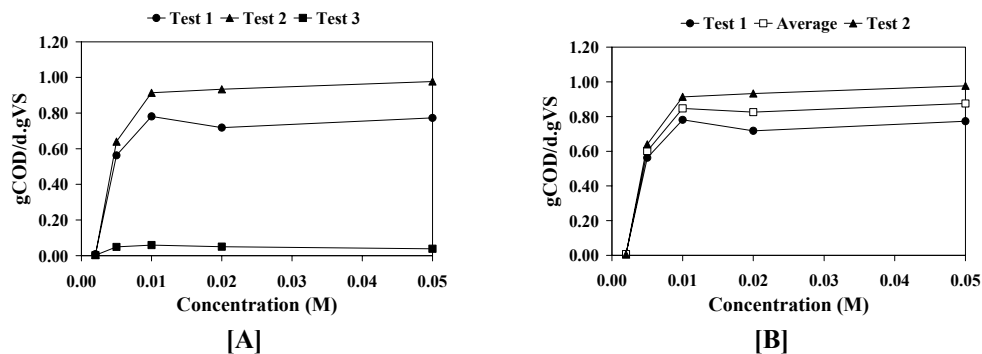


Figure [4-5]: Maximum Activity. Sodium acetate at varying concentrations were used to quantifying the maximum methanogenic activity. The tests were performed in triplicate (refer: Plot [A]), however only Test 1 and Test 2 were considered for the determination of activity.

The Control Test

It was expected that the titration of acid in the last 9 h would remain constant. This situation was anticipated since substrate utilisation in this period should be based on the acetate concentration of the last spike added i.e.: 2.5 M, because titration with acetic acid replenishes the acetate consumed (refer: **section 3.1.1**). However, the Control Test suggested that the residual substrate, present with the biomass, did impact upon the activity of the methanogens. **Plot B in Figure [4-6]** shows that the titration of acetic acid increased at a constant rate. The titration rate corresponded to an increase in methanogenic activity of 5.0 % every 3 h (refer: **Figure [4-6], A**). This result pointed to the fact that once all the acetate that was injected into the reactor had been depleted, there was still “excess” acetate to sustain a higher methanogenic rate. The excess acetate is possibly the hydrolysed remnants of biodegradable fractions within the residual substrate that had only become available after 15 h. It is plausible that while the micro-organisms were utilising the acetate spikes, the residual substrate was undergoing a process of hydrolysis and acidogenesis. The absence of any alkaline titration (suggestive of hydrolysis) after 15 h implied that all or most of the residual substrate had

already been fully degraded at this point. Thus the acid titration curve displayed an increasing trend rather than remain constant, which was expected for this period of the test (refer: **B**, **Figure [4-6]**). The implication for future study is that the biomass should be prepared in such a manner so as to minimise the effect of the residual substrate i.e.: possibly washing the biomass with distilled water. However, for the purposes of this study, the effect of the residual substrate would be considered low enough to ignore.

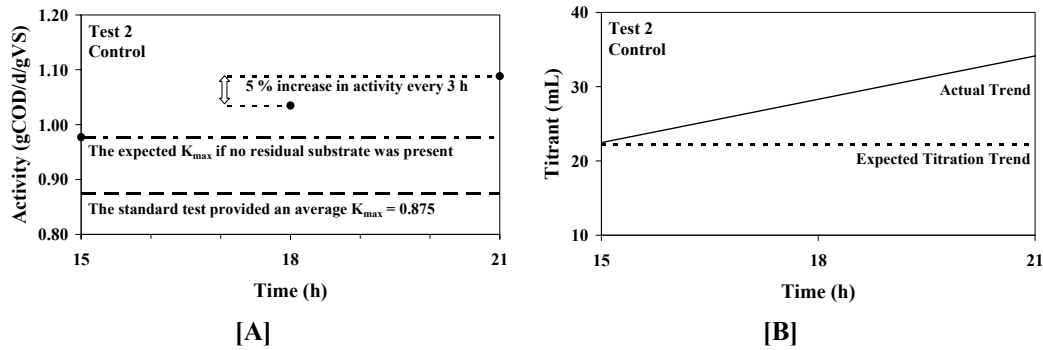


Figure [4-6]: Residual Substrate. It was important to determine the impact of the residual substrate on microbial activity. Plots [A] and [B] suggests that the residual substrate contributes to an increase in microbial activity. In Plot [B] the actual acid titration curve (cumulative volume) is increasing at a constant rate suggesting that methanogenic activity is increasing with time.

The Storage Test

The aim of this study was to assess the impact of cold storage on the activity of the biomass.

Materials and Methods

The contents of a Standard Test (**Test 2**) were stored at 4°C overnight. After equilibration, three 16 mL spikes of acetate (2.5 M) were added to the reactor every 3 h over a 9 h period. The following 6 h period was used to establish if methanogenic activity had recovered to the (maximum) level observed prior to storage. The duration of the entire test was 15 h.

Results and Discussion

Table [4-2] compares the activity results of the Average Standard Test (refer: **Figure [4-5]**) with the activity results of Standard Test 2 which had been stored at 4°C overnight and then spiked with acetate. From **Table [4-2]**, it can be seen that cold storage had a pronounced inhibitory effect on the activity of the biomass. It was thought that three doses of a high concentration of sodium acetate (2.5 M) would be sufficient to ensure the biomass recovered to its previous level of activity i.e.: $K_{max} = 0.98$ gCOD/gVS.d or to a value close to the average observed activity i.e.: $K_{max} = 0.875$ gCOD/gVS.d. Although the biomass did show signs of rapid recovery initially, the recovery did not persist. After 6 h, the rate of activity decreased and continued to decrease at a constant rate. This slow down in activity does not mean inhibition had occurred but could suggest that an accumulation phase had ensued. It is possible that the rapid early recovery was a consequence of the biomass being starved overnight and that once the threat of substrate deficiency had abated; the micro-organisms proceeded to build substrate reserves. Since no breakdown of acetate occurred during this accumulation phase, there was an observable decrease in the titration rate. The constant reduction in the titration rate suggested a gradual shift from acetate usage to acetate storage. The phenomenon of rapid uptake of easily biodegradable substrates is commonly observed in the anaerobic zones of activated sludge systems (Bailey and Ollis, 1986). However, recovery over the 15 h period after storage did advance to the initial level of activity observed in the first 3 h of the Standard Test (refer: **Table [4-2]**). This result is not unexpected since a similar

observation was made by Rozzi et al. (2000) where it was found that the lag phase was approximately 2 d. However, it was not established if there is a direct relationship between the time of storage and the duration of the lag phase because this phenomenon was deemed to be beyond the scope of this study.

The Storage Test was planned despite prior knowledge of this result. It was necessary to conduct such a test in order to estimate the time the biomass could be stored before storage became activity limiting. This interest was triggered by the activity results from the Standard Tests where Test 3 showed no correspondence to the initial two tests after having had its biomass stored for 30 h (4 °C) before use (refer: **Materials and Methods, Control-Standard Test**). However, the result from Test 2 i.e.: its comparability with Test 1 suggested that storing the biomass for 6 h did not significantly affect activity. Therefore, as a rough estimate for future work, the biomass should not be stored for more than 6 h. Thereafter, acclimation is essential to ensure the results from activity and inhibition tests are reliable.

Table 4-2: Determining the Impact of Storage on Biomass Activity

Time	Standard Test	Time	Storage Test
h	gCOD.d ⁻¹ .gVS ⁻¹	h	gCOD.d ⁻¹ .gVS ⁻¹
3	6.58 x 10 ⁻³	3	3.98 x 10 ⁻⁵
6	6.01 x 10 ⁻¹	6	1.92 x 10 ⁻²
9	8.47 x 10 ⁻¹	9	1.27 x 10 ⁻²
12	8.26 x 10 ⁻¹	12	8.12 x 10 ⁻³
15	8.75 x 10 ⁻¹	15	5.80 x 10 ⁻³

The Nutrient Test

The aim of this study was to determine if nutrient addition can recover the activity of biomass that had been previously stored at 4 °C.

Materials and Methods

In the Nutrient Test, 240 mL of biomass was mixed with 560 mL of a nutrient medium and equilibrated in the reactor. Thereafter, sodium acetate i.e.: 0.1, 0.25, 0.5, 1.0 and 2.5 M were spiked into the reactor every 3 h. The test lasted for 15 h. The medium was prepared according to the recipe described in (**APPENDIX A**). The biomass had been in storage at 4°C for a period of 30 h prior to acclimation and use. The biomass was acclimated for 18 h at 37°C in a carbon dioxide rich atmosphere.

Results and Discussion

The intention of this experiment was to assess if the nutrient medium remedies the effect of storage and if it enhances methanogenic activity i.e.: if there is a marked improvement in the use of the acetate spikes in the presence of the medium. Although, the Nutrient Test displayed signs of accelerated activity in the initial 6 h, this seemed to slow down and then stop after 9 h. The biomass for the Nutrient Test had been stored prior to use so it was expected that the activity after the first 3 h would be lower than that observed in the Standard Test (refer: **Table [4-3]**). However, it seems that nutrient addition had **little** impact on the biomass recovery and the utilisation of the acetate spikes.

Table 4-3: Determining the Impact of Nutrient Addition to Microbial Activity

Time	Sodium Acetate Spike	Methanogenic Activity	
		Standard Test	Nutrient Test
h	M	gCOD.d ⁻¹ .gVS ⁻¹	gCOD.d ⁻¹ .gVS ⁻¹
3	0.10	6.58 x 10 ⁻³	7.12 x 10 ⁻⁵
6	0.25	6.01 x 10 ⁻¹	2.96 x 10 ⁻³
9	0.50	8.47 x 10 ⁻¹	7.87 x 10 ⁻³
12	1.00	8.26 x 10 ⁻¹	3.19 x 10 ⁻³
15	2.50	8.75 x 10 ⁻¹	3.53 x 10 ⁻³

4.3. EFFLUENT STUDY

The potential for MAIA to be used as a screening procedure depends on its ability to quickly assess the toxicity and biodegradability of effluents and wastewaters. This chapter explores this possibility by focusing on the two studies conducted with textile size effluent and landfill leachate. The chapter is divided into two sections that discuss each study independently.

Hypothesis

It is proposed that both the textile size effluent and landfill leachate are biodegradable. Further, it is proposed that MAIA is capable of pre-screening both effluents for use in anaerobic digesters. The detailed objects of this study are to:

- characterise each effluent;
- relate MAIA titration data to methane gas production data;
- perform mass balances with regard to organic content;
- use titration data to assess activity i.e.: acidogenic and/or methanogenic;
- assess the practicality of the titrimetric method.

4.3.1 Textile Size

Size effluents represent the main component of the organic load of the effluents from textile finishing mills (Schluter, 1991). During the sizing process, individual yarns are coated with a protective film of size to resist abrasion during weaving. Hence, the size strengthens the yarn (Water Research Commission, 1983). The traditional sizing agent was starch which resulted in an effluent that was a high-strength organic wastewater. The sizing agents comprise substances which are pre-dominantly polar in nature. These polar organic pollutants pose problems because they are non-biodegradable and their elimination is incomplete (Marttinen, 2002). Consequently, when mixed with the remainder of the mill effluent, they increase the COD of the final effluent.

With the growing demand for synthetic fibres, synthetic sizes have become the material of choice, but their use has not eliminated the textile industries' disposal problems. The wide variety of synthetic sizing recipes coupled with their extensive consumption rates further complicates the issue by making treatment costly. Typical processing effluents contain some or all of the following materials:

- oils, fats and waxes inherent or added to fibres during processing
- vegetable or protein impurities associated with natural fibres
- monomers or oligomers associated with man-made fibres
- residual agricultural chemicals
- natural pigments, salts and metals
- alkaline salts from dyeing operations

Sizing recipes can be very diverse. **Table [4-4]** lists some of the chemical substances that constitute a textile size effluent while **Table [4-5]** presents four different recipes used in the manufacturing process of a typical textile company.

Table 4-4: Constituents of the Textile Size Effluent

Chemical	Comment
Kollotex	Starch Ether
Elvanol	Polyvinyl Alcohol
Duralube	Lubricant
CMC-30	-
Aqua Defoamer	-
Atebin A1 Micro	-
Sico Wax GR	Wax
Kuraray 1100	Polyvinyl Alcohol
Solvitose	Starch
Stayco C	Starch
Stysol 60	Starch
Stytex 60	Starch
Styclor 60	Starch
Techem L127	-
Superlube	Lubricant

Table 4-5: Textile Size Effluent Recipes

*Recipe	*Components					
	Kollotex	Wax	Maize Starch	PVA	Elvanol	CMC-30
	g/L	g/L	g/L	g/L	g/L	g/L
A	8.0	3.3	-	-	-	-
B	-	1.7	42.0	25.0	-	-
C	33.0	1.7	-	-	16.7	-
D	-	1.7	-	-	-	25.0

*Source: Frame Denim Mills (2000)

Carboxymethyl Cellulose

Carboxymethyl cellulose (CMC) is usually a sodium salt and is formed by treating cellulose with sodium hydroxide and mono-chloroacetic acid (Water Research Commission, 1983). It tends to absorb and hold moisture, while its usefulness depends on its water-binding ability because it reduces the need for high humidity conditions in the weaving shed (Sacks, 1997).

Polyvinyl Alcohol

This is a synthetic polymer resin produced by acid or alkaline hydrolysis of polyvinyl acetate (Water Research Commission, 1983). Polyvinyl alcohol (PVA) is an excellent textile warp size because of its strength, adhesion, flexibility and film-forming properties (Kirk-Othmer, 1982).

Wax

The term wax includes most lubricants of a solid nature. The chemical composition varies widely but is generally based on a long chain hydrocarbon molecule or a derivative (Seydel, 1972).

Starch

Starch granules consist of α and β amylase with the former being insoluble in water. The qualities that give starch its usefulness as a sizing agent are its ability to form a pliable film and the ability to provide a good coating without excess penetration into the yarn (Seydel, 1972).

Whatever the make-up of a typical effluent, all aqueous discharges are subject to certain standards for disposal. Conventional biological systems have concentrated on the use of activated sludge systems to reduce the BOD and COD of textile trade effluent. However, anaerobic systems can achieve a large reduction in BOD of high-strength wastes and has the advantage of producing relatively small amounts of sludge. It does not however, reduce BOD levels to those achieved by aerobic processes.

Materials and Methods

The textile size effluent used in this study was sampled from a Textile Mill in Durban, South Africa. The constituents of the sample batch were not known, however it could be one or a combination of the recipes depicted in **Table [4-5]**. The sample batch was characterized with regard to suspended solid and total organic content (refer: **section 3-3**). The results are presented in **Table [4-6]**.

Table 4-6: Textile Size Effluent – Solid Content

Solids	1	2	3	Average
	g/L	g/L	g/L	g/L
Total	60	60	63	61
Volatile	62	44	60	55

The size effluent, consisted of both solid and liquid fractions. Therefore, it was preferable to use a test batch where both these fractions were known exactly. The sample batch was used to prepare a homogenous test batch. A typical test batch of size effluent contained 50 % (v/v) of liquid and solid respectively. The organic content of the liquid and solid fractions were 83 gCOD/L and 210 gCOD/L respectively. Therefore, a 1 L test batch has a combined value of 146 gCOD/L.

Similarly, the characterisation results for the biomass used in this experimental series were:

Table 4-7: Textile Size Effluent Study – Biomass Content

Suspended Solids	1	2	3	Average
	g/L	g/L	g/L	g/L
Total	25	27	26	26
Volatile	19	20	19	19

The total organic content of this batch of biomass was determined to be 35.77 gCOD/L. For each sample test, 240 mL or 30 % (v/v) of biomass was placed into the reactor along with an aliquot of effluent. The aliquot sizes were: 32; 80; 160; 240 and 320 mL which corresponded to effluent concentrations in the reactor of 5.9; 14.7; 29.4; 44.1 and 58.7 gCOD/L respectively. A 16 mL spike of sodium acetate (2.5 M) was added to promote acetoclastic methanogenesis. Finally, the solution was made up to 800 mL with distilled water. The reactor was gassed with a 50 % mixture of carbon dioxide gas (refer: **section 3.1.4.2**). The reactor was then sealed except for one exit port that was connected to the gas displacement bottle. During each test gas production and composition were measured (refer: **section 3.3**). The duration of each test was 1 d.

Results and Discussion

At the start of any sample test, it is imperative that the initial conditions be as close as possible to the set-point values. Specifically the pH, temperature and headspace atmosphere should be 6.88, 37°C and 50% carbon dioxide respectively. However, this is not always easy to achieve. **Figure [4-7]** summarizes these conditions for all the textile size sample tests.

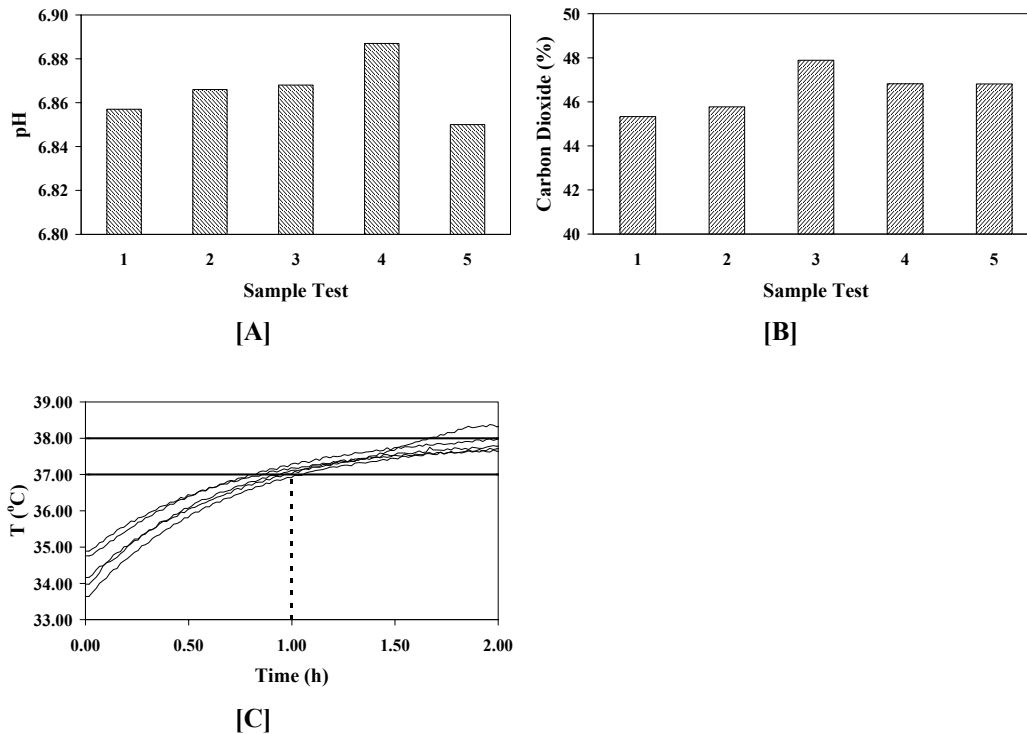


Figure [4-7]: Initial Test Conditions. Plots A, B and C present a summary of the initial conditions i.e.: pH, headspace composition and temperature for the experiments with the textile size effluent. It can be seen from Plot C, that the temperature curves from each sample test i.e.: Test 1 to Test 5, follow a similar trend.

It is clear from **Figure [4-7]** that the initial conditions of the tests do not always coincide, however it can be said that they lie within acceptable limits i.e.: within 10 % of the set-point, except in the case of the temperature. Further, it seems to take 1 h for the reactor to heat to 37°C (refer: **Figure [4-7], C**). This is an important result because it is not possible to accurately assess activity within this period. Changes in temperature affect the delicate equilibrium established at the start of each test (refer: **APPENDIX E**), therefore titration during this period will be associated with physical changes rather than with methanogenic activity. Consequently, the implication for an operator would be to start the test only after the reactor contents have reached the set-point temperature. Nevertheless, this period of temperature adjustment is comparable to the 1 h equilibration period associated with the serum bottle method (refer: **section 3.2**). Therefore, provided the titration rate is not excessive in this period, the impact of the temperature adjustment will be considered negligible (refer: **APPENDIX E**).

All the sample tests in the textile study displayed the same general titration pattern i.e.: only base titration, except Test 1 which had both acid and base titration. Based on the composition of the substrate i.e.: mostly starch, the titration of base was anticipated (refer: **Figure [4-8]**). Starch is a complex substrate and some hydrolysis was expected prior to methanogenesis. This period of hydrolysis is characterised by acidification which necessitates the titration of base. Following hydrolysis, it is expected that methanogenesis will begin and

acid titration will ensue. However, the duration of a test could prevent an operator observing both base and acid titration periods. It is possible that at high effluent concentrations, the period of hydrolysis may be incomplete after 24 h while at lower concentrations hydrolysis may take a few hours (refer: **section 3.1.7**).

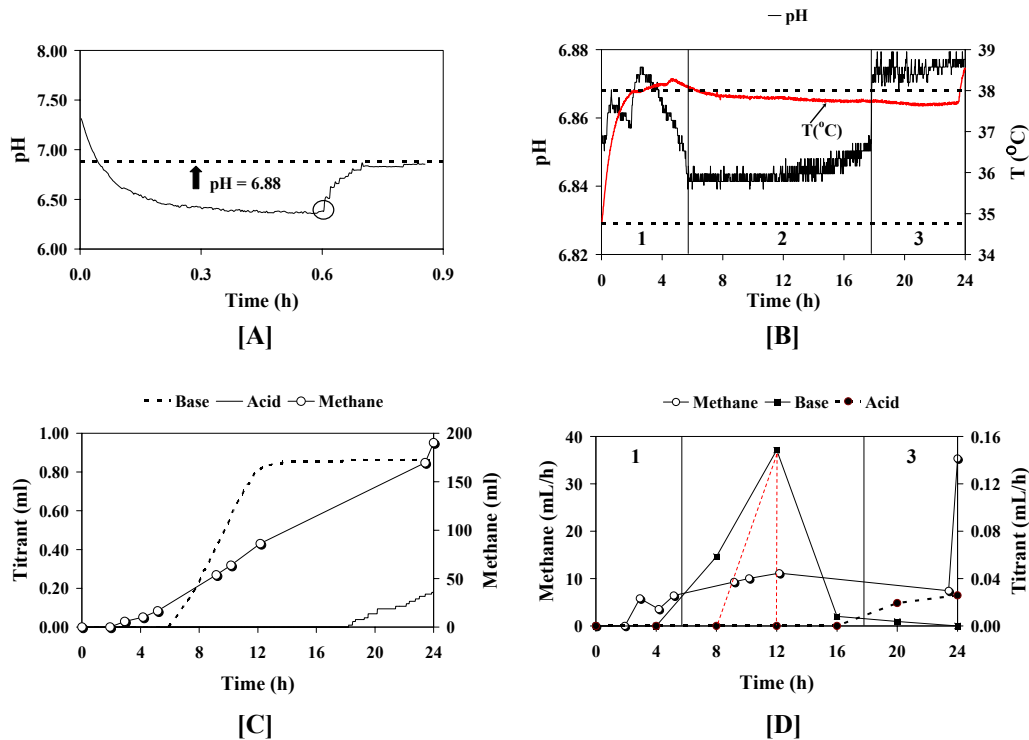


Figure [4-8]: A Typical Textile Test. Plot [A] describes the change in pH during the equilibration process; Plot [B] gives the essential parameter history; Plot [C] depicts titrant dosage and the gas production trend while Plot [D] is used to estimate substrate consumption during the period of alkaline titration masking methanogenic activity.

The titrimetric trend observed in Test 1 corresponded strongly with the expected trends for this substrate i.e.: base and acid titration within 24 h. Therefore, it was appropriate to consider this test as representative of all the tests conducted in the textile study. The pH realised after equilibration (for 0.60 h) was 6.40 and after the addition of an aliquot of sodium bicarbonate i.e.: the ringed region (refer: **Figure [4-8], A**), the set-point value of 6.88 was achieved. The rapid increase in temperature i.e.: from 35°C to 37°C (refer: **Figure [4-8], B, Region 1**) did impact upon the pH. However, since no titration occurred during this period i.e.: first 1 h, (refer: **Figure [4-8], D**) the disturbance to the system can be considered minimal (refer: **APPENDIX E**). Base titration occurred after 4 h suggesting that hydrolysis began at this time (refer: **Figure [4-8], C**). However, methanogenesis began much sooner i.e.: approximately 2 h earlier as Plot D suggests. Methanogenic activity in this period is probably the result of the utilisation of the acetate spike added at the start of the test. It was determined from the Standard Test that the spike is used within three hours of its introduction (refer: **section 4.2**). Further methanogenic activity i.e.: during the period 4 to 16 h, is difficult to quantify since it is masked by the hydrolytic activity. However, if it is assumed that all the methane gas produced and all the base titrated within this period is the result of already degraded and partially degraded substrate respectively, then it is possible to use the available data to make a reasonable estimate of the fraction of substrate utilised. It was determined that 84 % (refer: **APPENDIX F**) of the effluent substrate had been biodegraded within this period.

One drawback associated with the estimation process was the fact that no gas measurements were performed for the period $t = 12$ h to $t = 16$ h (refer: **Figure [4-8], D**). Based on the area under the titrant curve between 8 h and 16 h, the triangular region was used to predict the

fraction of the substrate consumed in the period 12 h to 16 h. Another remarkable feature, observed in these tests, was the resemblance of the shape of the alkaline titration curves to the batch growth curve (refer: **Figure [4-8], C**). The titration curve has a distinct lag; growth and stationary phase, typical of the batch growth curve. In addition, with an increase in the effluent concentration, the growth cycle seems to repeat itself (refer: **Figure [4-9]**). This diauxic behaviour exhibited by the anaerobes is not uncommon. Hongwei et al. (2002) measured the activity of dehydrogenase as a means to determine biodegradability of organic compounds. They reported that some organic compounds are partially degraded to intermediate products that require a period of adaptation before the biomass can effectively utilise them. This behaviour is characterised by an activity plot that has two distinct periods separated by a lag period.

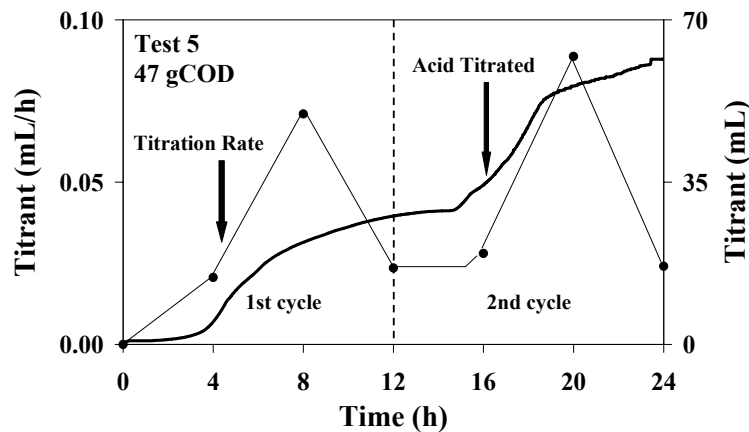


Figure [4-9]: Diauxic Growth Pattern. The titration of the alkali tracks the activity of the micro-organisms. The shape of the curve is characteristic of a diauxic growth curve.

Two major objectives of this study were to quantify: methanogenic activity and to relate titration data to gas production data. MAIA was designed to titrate acid in response to methanogenic activity. The chemistry of the system, therefore does not allow for methanogenic activity assessments to be based on alkaline titration. Hence, it was not possible to accurately evaluate methanogenic activity from the titration data under the described conditions i.e.: during the acidogenic phase. Nevertheless, the activity determination was performed using the alkaline titration data (refer: **APPENDIX F**). It is highly likely that if the test was run for a longer period i.e.: greater than 24 h, then methanogenic activity could have been measured i.e.: during the acid titration phase.

Further, **Figure [4-10]** compares the titration data to the measured gas production data across all the tests. Work performed by Buswell, (1939) introduced a relationship between an organic substance and the stoichiometric amount of methane and carbon dioxide gas produced when that organic was anaerobically degraded (refer: **APPENDIX C**). Specifically, it related the mean oxidation state of the carbon in the substrate to the gas composition. Using his result, it was estimated that 50 % of all biogas produced i.e.: from the degradation of the textile size effluent which is comprised of mostly starch, should be methane. This was the basis for the theoretical methane gas production plot in **Figure [4-10]**. The measured methane gas curve was generated using gas chromatographic data. Further, the measured curves have been adjusted to compensate for the error associated with the titration system. Ideally, all three curves i.e.: theory; titration and measured should plot upon each other. Clearly, this is not the case. The theoretical curves plot higher than the measured curves which in turn plot above the titration curves for the 5.9 to 29.4 gCOD.L⁻¹ tests. The plots become more variable from Test 4 onwards. These results are not conclusive, especially if MAIA is to be used to quantify methanogenic activity.

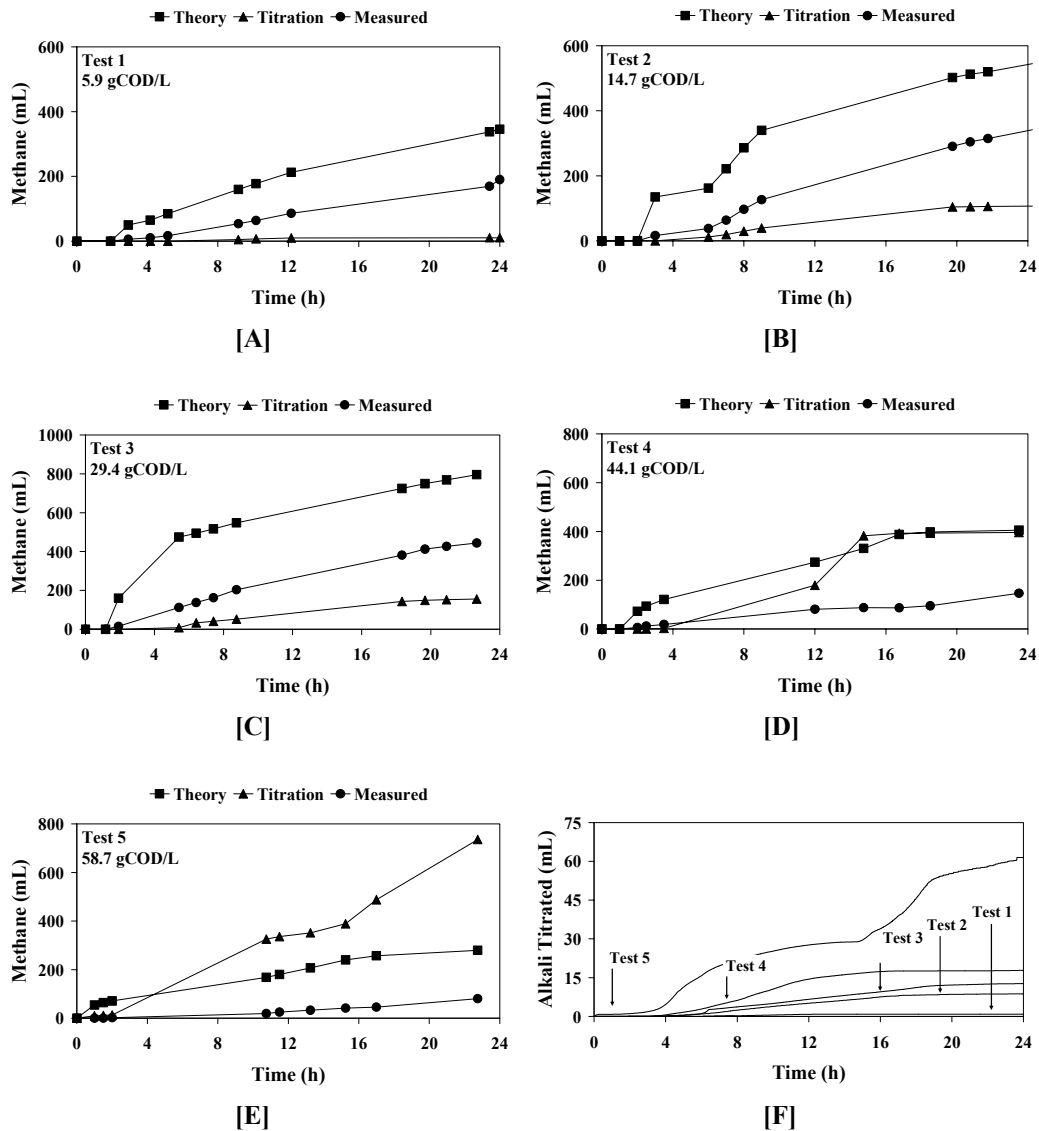


Figure [4-10]: Evaluating Methane Production. The figure compares different methods of evaluating methane production. The theoretical curves assume that 50 % of the biogas produced is methane gas while the measured curves are the actual methane gas estimates taken from the tests. Plot [F] plots the results of the titration data obtained from MAIA.

The disparity between the theoretical curve and the measured curve has a possible explanation. Gas samples were drawn from the reactor headspace and analyzed. However, the current system does not provide for a stirrer in the headspace. Consequently, when the rate of gas production is low, the headspace is not uniformly mixed and this affects the composition of the sample. This may explain the disparity between the relevant curves of Test 4 and Test 5 (refer: **Figure [4-10]**). It is feasible that an innovation to the existing system i.e.: an automated sampling system in conjunction with a headspace mixing unit, could improve the quality of these results. Nevertheless, the quantitative trend seems to be consistent across all the tests. This, at least, supports the idea that the effluent is biodegradable.

Another concern is the quantitative dissimilarity between the titration and measured curves. MAIA's theoretical stoichiometry, based on acid titration, applies to certain organic substrates only e.g.: acetate, but stoichiometry based on alkaline titration is not clearly understood. In an attempt to relate methanogenic activity using alkaline titration data and acid stoichiometry, errors are introduced into the calculation process.

A mass balance i.e.: on the organic content, was performed to assess biodegradability and the effluent concentration at which methanogenesis was maximized i.e.: where COD removal was the highest. The results are presented in **Figure [4-11]**. The balance confirmed that the textile size effluent is biodegradable and that the best effluent concentration to use in the reactor is 14.7 gCOD.L^{-1} i.e.: Test 2. The balance also highlighted the fact that biodegradability assessments using MAIA are susceptible to poor sampling conditions (refer: **APPENDIX E**).

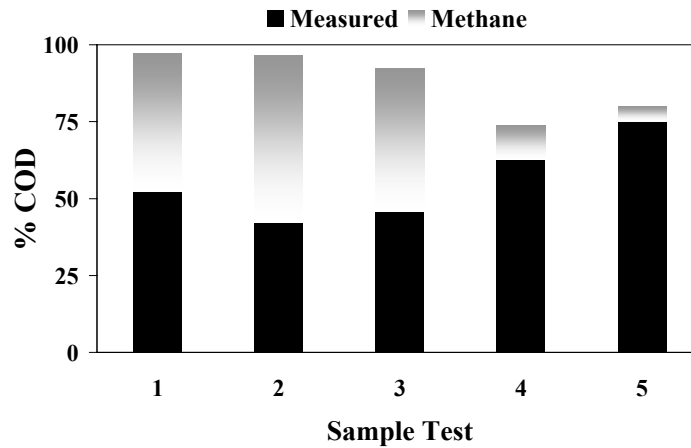


Figure [4-11]: Assessing Biodegradability. The organic carbon mass balance shows that the textile size effluent is biodegradable. The light region of the bar graph shows the percent COD removed as a result of methane production. The dark region represents the percent COD that remained at the end of the test.

The textile size effluent was considered to be a biodegradable substrate because this effluent is 50 % biodegradable under the prescribed experimental conditions, and the results confirmed this. However, it was also important to conduct experiments with a more recalcitrant i.e.: a difficult to degrade substrate. Therefore, it was decided to conduct similar experiments using a semi-hazardous landfill leachate.

4.3.2 Landfill Leachate

Sanitary landfilling is the most common way to eliminate solid urban wastes (municipal and industrial). Comparative studies of the various means of eliminating solid urban waste e.g.: landfilling and incineration have been carried out in several countries (Lema, 1988). These studies have shown that landfilling is the cheapest method. Besides its economic advantages, landfilling minimizes adverse environmental effects and other risks while allowing waste to decompose under controlled conditions until its eventual transformation into relatively inert, stabilized material (Robinson, 1983). However, little attention has been paid to the collection and treatment of landfill leachates which has now been recognized as a significant problem associated with landfills (Lema, 1988).

Leachates are formed when water i.e.: rainwater percolates through the dumped waste and transport the organic and inorganic products from both physical extraction and hydrolytic and fermentative processes. Leachates generally contain high concentrations of soluble organic matter and inorganic ions (Wong, 1982).

The pH of leachates lie in the range of 5.5 to 8.0. A large proportion of suspended solids are usually volatile (at 550 °C), though this is greatly influenced by the sampling technique; the great majority of measured Total Kjeldahl Nitrogen is ammoniacal nitrogen while phosphate levels are low and there are usually high concentrations of zinc and manganese (Lema, 1988). Analysis of the organic fraction of leachates shows that volatile fatty acids contribute the majority for the high COD levels. Other organic fractions present include proteins, carbohydrates and hydroxylated aromatics. The presence of these aromatic hydroxyl compounds have been found by some authors (Field, 1987) to be possible sources of toxicity and inhibition when biological treatment is applied. However, studies have shown that the incorporation of anaerobic digested sludge in landfills assists landfill management by greatly reducing the COD of leachates (Lema, 1988).

Leachate treatment can be very difficult. They can have COD values up to 200 times greater than those of urban sewage and their composition can vary considerably both seasonally and from year to year (Lema, 1988). Strategies for the treatment of leachates are hindered by their great diversity, which results in techniques successfully developed for one site not necessarily being applicable elsewhere (Keenan et al., 1984). One common means of leachate disposal is combined treatment with domestic sewage at conventional sewage plants. An argument in favour of such combined treatment of leachate and sewage is that since the former contains an excess of nitrogen and the latter an excess of phosphorous, neither of these nutrients need to be supplied at the treatment plant (Lema, 1988). The main difficulties are posed by high concentrations of organic and inorganic components. Only when leachates make up less than 5 % of the total sewage plant input and leachate COD is less than 10 g O₂/L is joint treatment acceptable. Otherwise, it should be diluted before being discharged into the sewer system and the hydraulic retention time of the plant should be increased (Boyle, 1974).

Table [4-8] summarizes some general characteristics of a leachate sampled from a typical semi-hazardous landfill site in September 2000.

Table 4-8: Characteristics of the Landfill Leachate Sampled from Shongweni

Component	Units	Quantity*
pH	-	7.66
Acetate	mg/L	2 596
Alkalinity	mg/L	2 903
COD	mg/L	5831
Mn	mg/L	4.8
NO ₃	mg/L	<0.05
NH ₄	mg/L	282
Cl ⁻	mg/L	1 073
SO ₄	mg/L	124
PO ₄	mg/L	3 773
Phenol	mg/L	15 400
*Source: Berry (2001)		

Materials and Methods

The leachate used in this study was sampled from the Shongweni Landfill site. It is located in Durban, South Africa and is classified as a semi-hazardous site (Berry, 2001). Samples were taken during July 2002. The sample batch was characterized with regard to suspended solid and total organic content. No suspended solids were present. The organic content was determined to be 13.82 g O₂.L⁻¹.

Table [4-9] shows the characterisation results for the biomass used in this experimental series. The organic content of the biomass was 70.45 g O₂.L⁻¹. The Sample Test conditions and analysis were identical to those for the textile size effluent study. The effluent concentrations used in the reactor were: 0.6; 1.4; 2.8; 4.1 and 5.5 gCOD/L respectively.

Table 4-9: Landfill Leachate Study – Biomass Content

Solids	1	2	3	Average
	g/L	g/L	g/L	g/L
Total	34	32	35	34
Volatile	23	22	24	23

Results and Discussion

Figure [4-12] summarizes the initial conditions for the leachate study. The results indicate poor reproducibility of the initial temperature conditions. Despite this, the set-point of 37°C was achieved after 1 h. However, the pH and headspace gas composition set-points of 6.88 and 50% carbon dioxide gas respectively were achieved within reasonable limits.

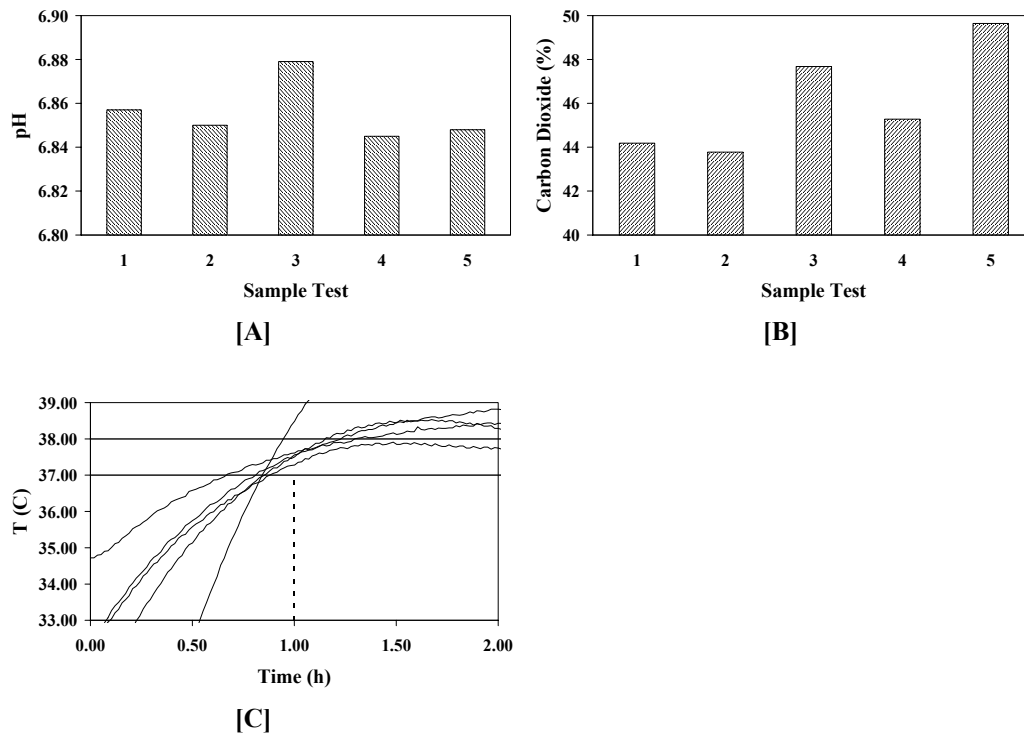


Figure [4-12]: Initial Test Conditions. The initial conditions for the experiments with landfill leachate.

All the sample tests in this study displayed the same general titrimetric trend. Acid titration and methanogenic activity was observed in each test. It was expected that the leachate will undergo little or no biodegradation because of its inherent toxicity. Despite this, it was necessary to highlight the potential of MAIA to be used as a pre-screening tool. MAIA had previously shown that it can produce reliable toxicity data when using simple, easy to degrade substrates. However, the question still remained: could it still work with recalcitrant substrates?

Figure [4-13] shows the results for Test 1, which was considered to be representative of this experimental series. It is evident that the system conditions i.e.: pH and temperature were satisfactory. The pH set-point was reached after the addition of the bicarbonate spike and the temperature remained relatively constant throughout the test (refer: **Figure [4-13], A**). In addition, MAIA seemed to have effectively maintained the pH at the set-point value for the duration of the test, suggesting that the activity of the micro-organisms lay within the titrating range of the instrument. **Figure [4-13], Plot C** seems to suggest a batch growth trend for the acid titration curve (acetic acid); however **Plot B**, **Figure [4-13]** implied a different interpretation. The cumulative acid volume curve depicts a lag phase in the initial hour of the test, but **Plot B**, **Figure [4-13]** suggested the micro-organisms were active in this period because the pH increases. So instead of a lag phase, there seems to be a phase of low but steadily increasing activity. This is possible if it is considered that the acetate spike was utilised within the first 3 h of a Standard Test (refer: **section 4-2**). However, a more plausible explanation for the acid titration would be the change in temperature that occurs in this period. As the temperature increases from 34°C to 37°C, the pH also increases. This situation arises because as temperature increases, the concentration of carbon dioxide in solution decreases resulting in an increase in pH. This may have initiated the titration of acid.

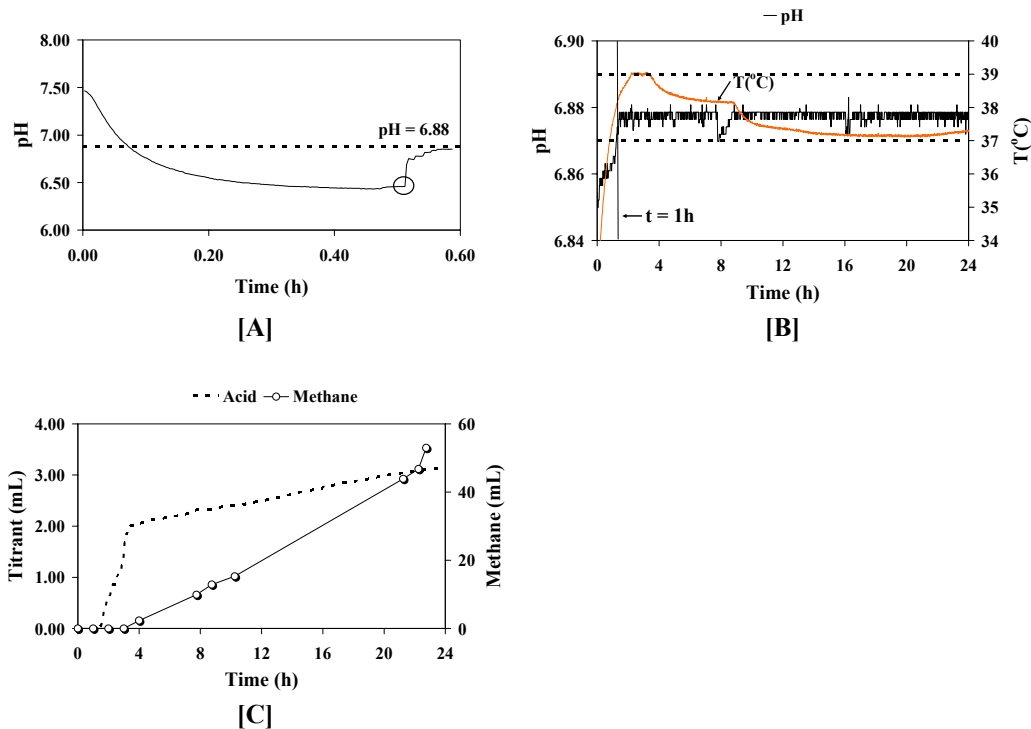


Figure [4-13]: A Typical Leachate Test. Plot [A] describes the change in pH during the equilibration process; Plot [B] gives the essential parameter history; Plot [C] depicts titrant dosage and the gas production trend while

Figure [4-14] compares the three curves used to assess methanogenic activity for all the tests. At first glance there seems to be no reliable trend associated with increasing the concentration of the leachate. However, closer inspection shows excellent correspondence between the titrimetric and measured curves of Test 1 and Test 2 respectively. These tests seem to suggest that an increase in concentration of the leachate leads to greater methanogenic inhibition. This type of trend was expected, since the leachate does contain many toxic substances e.g.: phenol. However, the remaining plots only serve to emphasize the difficulty in pre-screening a recalcitrant substrate like leachate.

Both studies i.e.: textile size and landfill leachate have shown that it is possible to use MAIA to assess toxicity. However, they have also shown that it is difficult to relate gas production (measured curves) to titration data (titration curves). This can be related to toxic effects, but it also suggests that there may be an optimal acetate concentration range for the system using a complex substrate. This means that the introduction of a toxic compound changes the system conditions in such a way that the stoichiometry of the system cannot be predicted. This probably explains why there can be good correspondence between measured and titration curves at low substrate concentrations but not at higher substrate concentrations. There is little else that can be concluded about biodegradability from these curves except that the leachate shows potential for anaerobic degradation. It is possible that if the tests were conducted over a longer period i.e.: greater than a day, then the micro-organisms would have had more time to acclimate to the effluent. Maybe then, biodegradability would have been better assessed.

The mass balance (refer: **Figure [4-15]**) suggests that 0.6 gCOD/L was the best effluent COD concentration to use in the reactor. This result can be seen from the fact that the methane gas production of **Test 1** is greater than the gas production of any of the other tests suggesting that this concentration did not negatively impact upon microbial activity. Therefore, it is possible that a lower concentration would have performed better. This result implies that the concentration range selected for pre-screening a recalcitrant effluent needs to be considered

very carefully. This can be a tedious task for an operator who does not know much about the effluent to begin with. This situation could have been avoided, had MAIA been used as a multi-channel system. The multi-channel system allows for numerous tests to be conducted simultaneously. This ensures that the optimal range can be assessed relatively quickly. Unfortunately, the system employed in this study could only perform a single test at a given time. Consequently, the result from this study i.e.: the optimal effluent concentration is inconclusive.

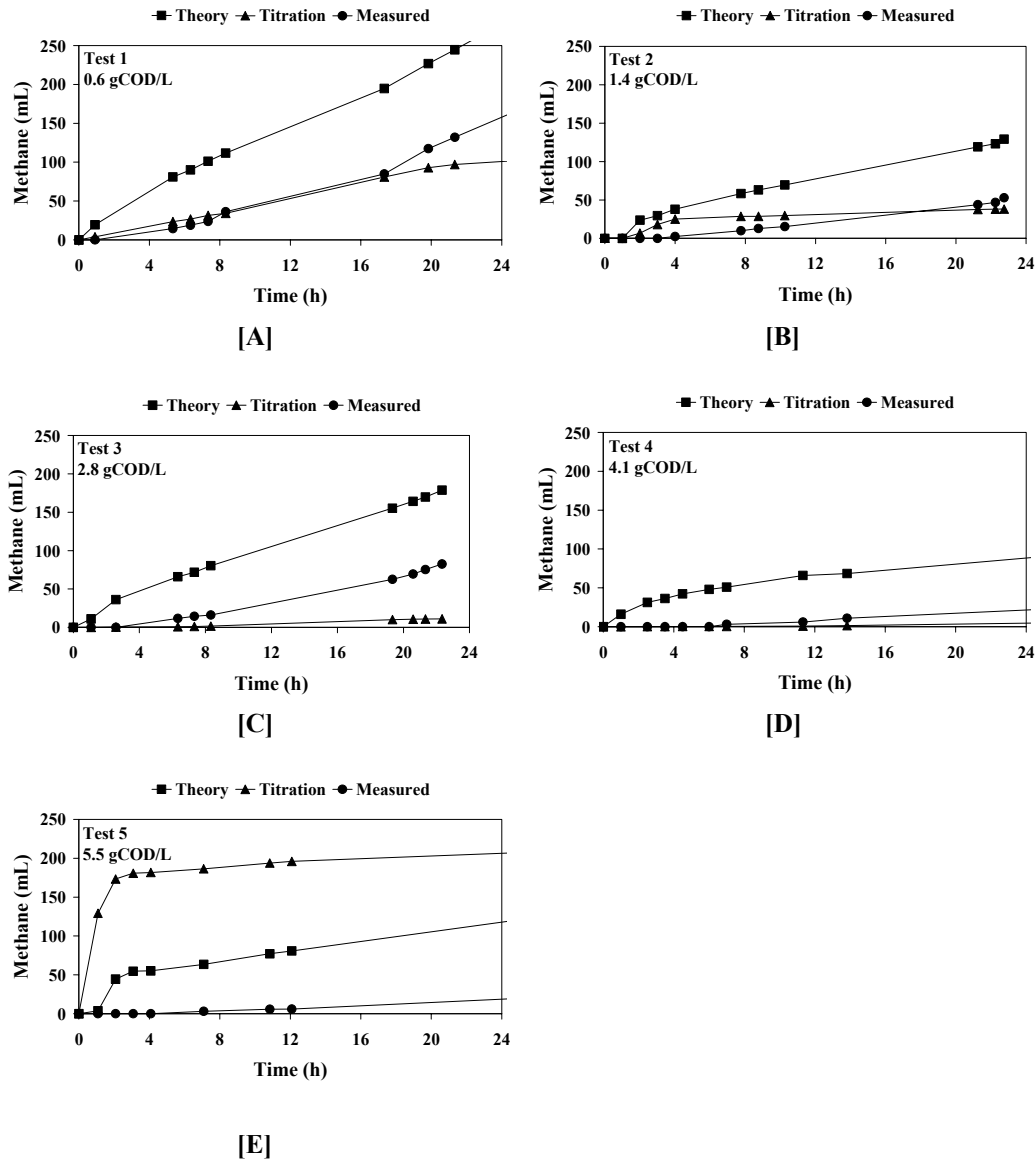


Figure [4-14]: Estimating Methane Production. Different methods were used to evaluate the production of methane. The theoretical curves are based on the assumption that 50 % of the biogas produced is methane gas. The tests were performed using the same batch of effluent at increasing COD concentrations.

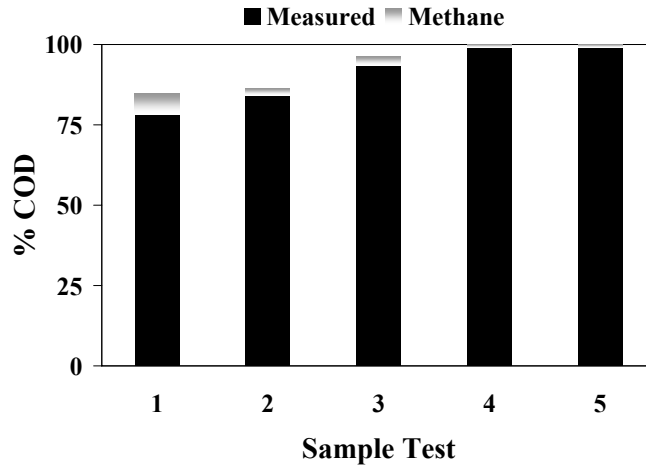


Figure [4-15]: Assessing Biodegradability. It is obvious from the organic carbon balance that the leachate is difficult to biodegrade at least in the short run i.e.: 24 h. The light region of the bar graph shows the percent COD removed in the form of methane. The dark region shows the percent COD that remained after the test.

4.4. NUTRIENT STUDY

Previous experimental work with MAIA had been conducted without the use of a nutrient medium. However, it was important to assess the benefit, if any, of utilising a nutrient medium primarily because the serum bottle method requires nutrient addition. This chapter presents the results of the study conducted with textile size effluent supplemented with a nutrient medium.

Hypothesis

It was proposed that the nutrient medium can enhance microbial activity when degrading an industrial effluent. However, it was also proposed that the use of the nutrient is not an essential component of the pre-screening protocol. The objectives of this study were:

- evaluate the impact of the nutrient medium on the system conditions;
- compare the nutrient enriched test with the nutrient deficient test;
- conclude on the importance of using a nutrient medium for pre-screening purposes.

Materials and Methods

Three identical nutrient tests were performed. In each test, 240 mL of biomass was placed into the reactor along with 80 mL of textile size effluent and a 16 mL spike of sodium acetate (2.5 M). Finally, the solution was made up to 800 mL with the nutrient medium (refer: **APPENDIX A**). The experimental test procedure thereafter was the same as previous sample tests. The duration of each test was 1 d.

Results and Discussion

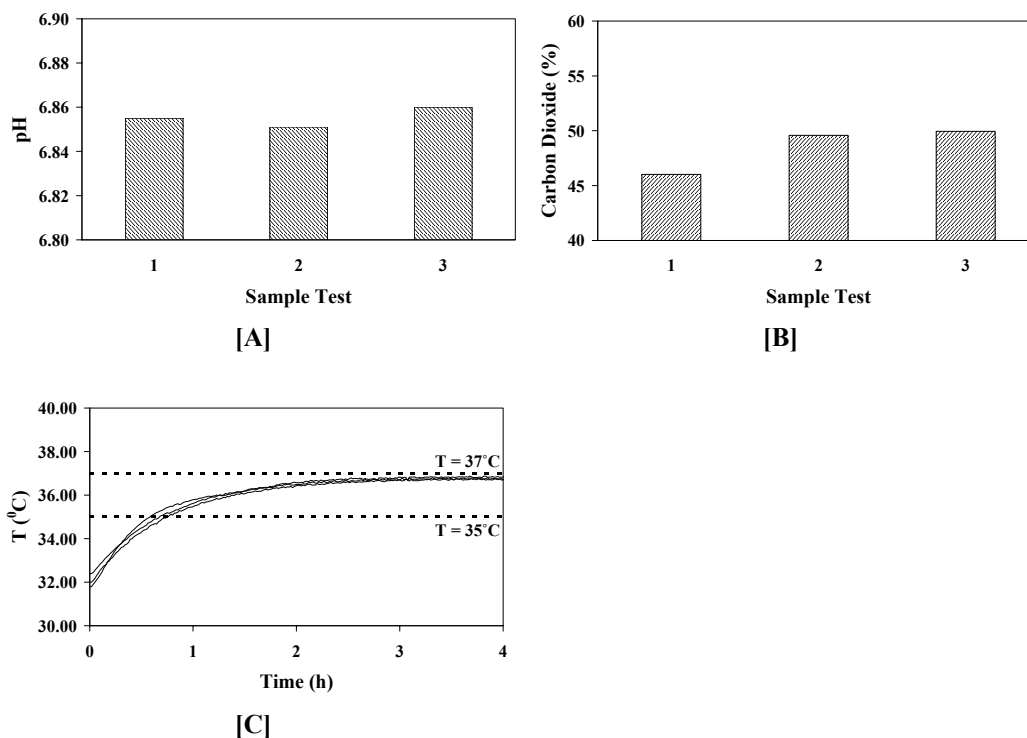


Figure [4-16]: Initial Test Conditions.

Figure [4-16] presents the initial conditions for the nutrient experiments. The result suggested that the presence of the nutrient medium may have a stabilising influence on the equilibration process. This was plausible since the nutrient medium contained a buffer component (pH = 7). Consequently, there was good reproducibility in the initial pH and gas composition condition for all the tests.

All the nutrient tests were qualitatively similar (refer: **Figure [4-17]**). **Plot A** shows the alkaline titration curves for all the Nutrient Tests while **Plot B** compares the average nutrient curve with the nutrient deficient curve from the Textile Study. Although, the experimental conditions for the nutrient tests were devised to be identical, differences in the activity plots are possibly due to differences in the characteristics of the sample biomass e.g.: active methanogenic population. Both sets of curves (refer: **Figure [4-17], B**) show the same batch growth type characteristics. A striking indication that the nutrient medium enhanced activity was the fact that the volume of alkali titrated in the same period was much higher for the nutrient enriched case i.e.: approximately six times more. Further, the nutrient enriched tests (on average) seem to start much sooner than the nutrient deficient test i.e.: approximately 4 h sooner. This result suggested that the nutrient improved the rate of degradation. This was expected because the nutrient medium contains many trace metals and compounds e.g.: salts that are required by the micro-organisms during biodegradative processes (refer: **section 3.1.3.8**).

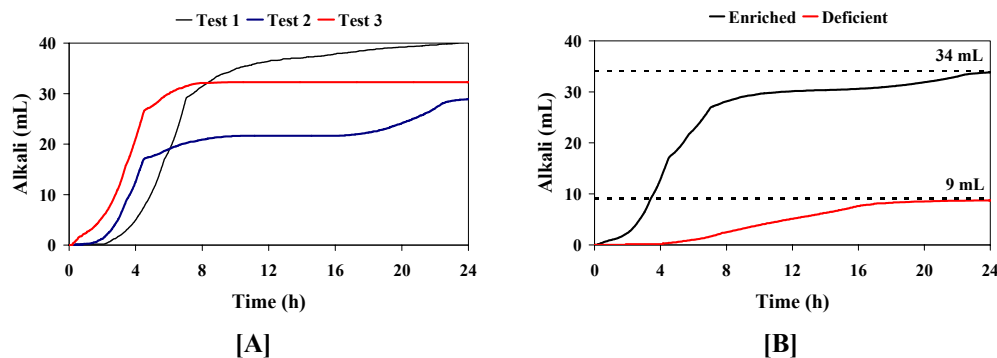


Figure [4-17]: Impact of Nutrient Use. Plot [A] presents the alkaline titration curves for the experimental series and Plot [B] depicts the difference in alkaline titration between the nutrient enriched (the average of all three tests) and nutrient deficient tests.

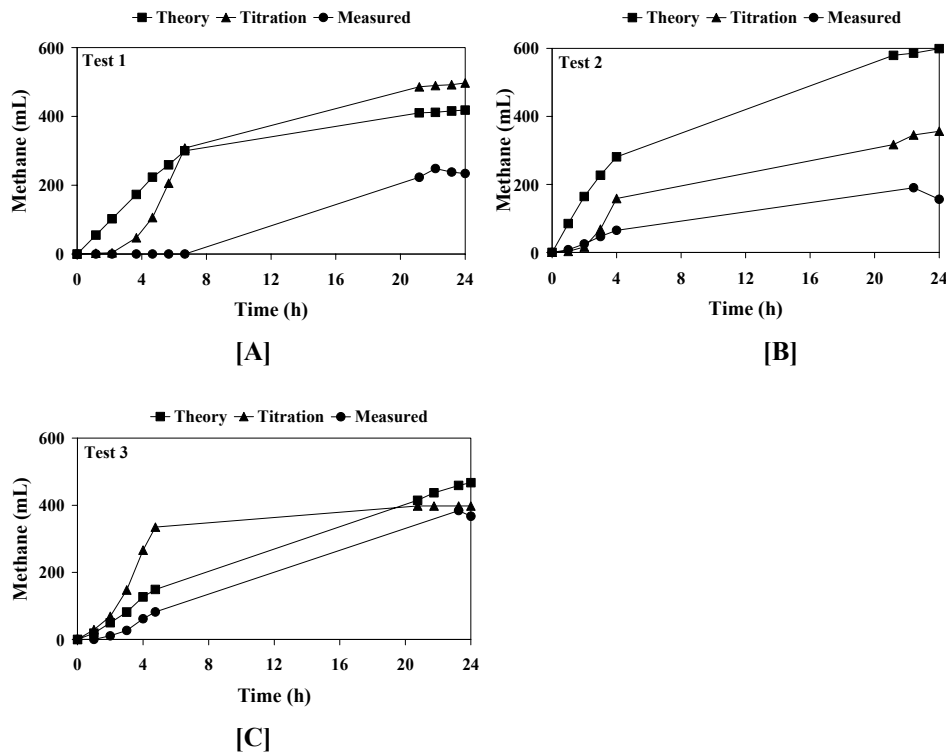


Figure [4-18]: Evaluation of Methane Production.

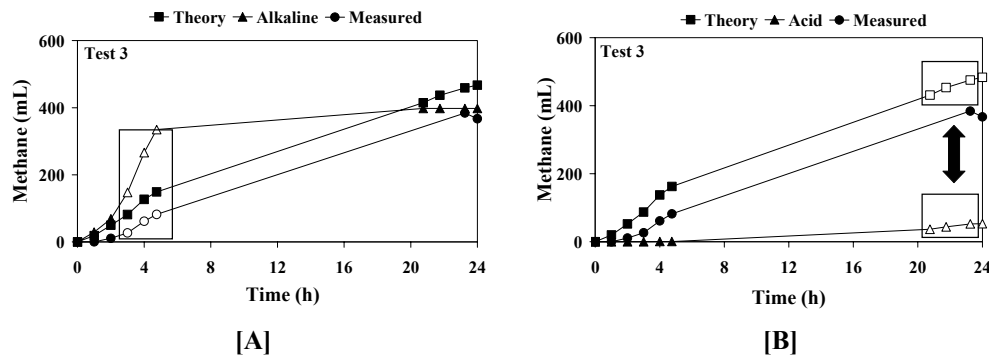


Figure [4-19]: Titration and Microbial Activity. The figure suggests that there is a close relationship between titrant addition and gas production in different time periods. Plot [A] relates the alkaline titration in the early stages of the test to methane gas production. Plot [B] shows the correspondence of acid titration to methane gas production in the final stages of the test.

The results of Nutrient Study are shown in **Figure 4-18**. Examining Plots A, B and C (refer: **Figure 4-18**), it was noticed that the methanogenic trend was consistent across the experimental series. However, it was determined that Nutrient Test 3 was the most interesting. Firstly, it displayed both alkali and acid titration periods. The same effluent concentration i.e.: 14.7 gCOD/L in the Textile Study did not show any acid titration. Further, comparing The plots in **Figure 4-19** it is noticeable that the alkali titration curve corresponds well with the theoretical gas production curve in first 4 h of the test (refer: **blocked region, A**) while the acid titration curve corresponded well with the theoretical gas production curve in the last 4 h of the test (refer: **blocked region, B**). This was a significant result because it reinforced the idea that alkaline titration may be related to methanogenic activity during the hydrolysis period of a test. Further, it seemed that the increased buffering capacity, offered by the addition of the nutrient medium, enhanced the titrating ability of the instrument. By stabilising the pH condition within the reactor, it was possible that the instrument received a

clearer pH signal i.e.: there is less noise associated with the signal. Consequently, the improved response of MAIA to adjust the pH made the estimation of activity more reliable.

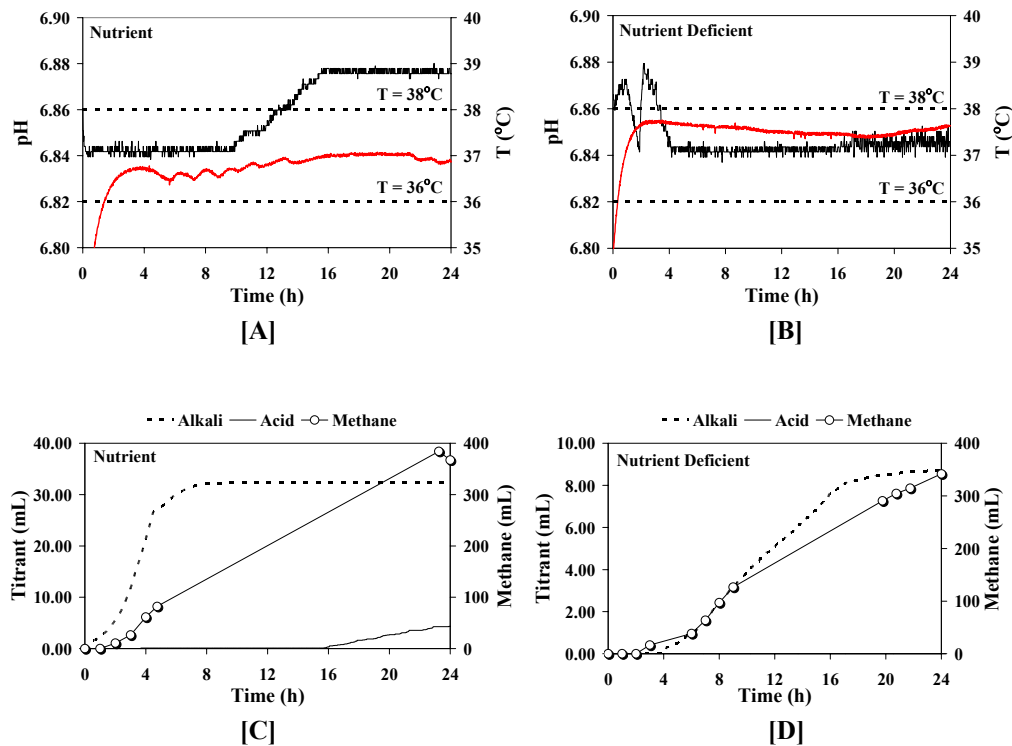


Figure [4-20]: The Effect of Nutrient Use on Titration. The presence of the nutrient medium seems to improve the quality of the titrimetric method results.

A major objective of this study was to compare the results of a nutrient enriched test with a nutrient deficient test. Therefore, **Figure [4-20]** compares the result of Nutrient Test 3 with the Textile Test 2 (refer: **section 4.3**). In both these tests, the effluent concentration was 14.7 gCOD/L. From a comparison of the pH curves; it was evident that the nutrient medium reduced the noise associated with a poor pH signal response. In addition, it seemed that buffering further reduced the impact of temperature interferences at the start of the test (refer: **Figure [4-20], A and B**). Contrastingly, the temperature interference was possibly responsible for the rapid titration of alkali at the start of the Nutrient Deficient Textile test (refer: **Figure [4-20], B**). The impact of using a nutrient medium to limit certain external interferences was investigated (refer: **APPENDIX E**). Further, no acid titration was detected in the 24 h period in the case of the Nutrient Deficient Textile test. It is possible that when the biomass experiences nutrient deficiency, the methanogens (in particular) require a greater period of time before they can utilise acetate.

The alkali plot for the Textile test (refer: **Figure [4-20], D**) suggested that there was little hydrolytic activity in the last 4 h of that test i.e.: the slope of the curve seemed to reach a constant level, however the pH trend in Plot B suggested that there was a build-up in activity. In the absence of any other interference e.g.: temperature, the disturbance in the pH curve was probably noise. Contrary to this, Plot A displayed a smooth pH trend in the last 4 h when methanogenic activity was occurring. The smooth pH pattern or the absence of any noise was probably because MAIA received a clear pH signal and was able to dose titrant more effectively. This implied that buffering increased the sensitivity of the instrument. Hence, it can be concluded that the nutrient medium should be considered as an integral part of the MAIA pre-screening protocol.

Examining Plots C and D in **Figure [4-20]** showed that while the nutrient medium increased the rate of activity i.e.: titration of alkali in the nutrient enriched case was almost four times greater than the nutrient deficient case, it did not improve the quality of biodegradation significantly i.e.: rate of methane production was determined to be 15 mL.h^{-1} for the last 16 h of each test. Nevertheless, it did reduce the duration of hydrolysis and consequently the biodegradation period by increasing the buffering capacity of the system. It is important to realise that methane gas measurement was not dependent on the buffering capacity of the system i.e.: the methane gas production curve reaches about the same level in both plots. However, the titration curves were sensitive to the buffering capacity. The buffered titration profile displayed a rapid initial titration phase that tended to a steady state. This trend was repeated in the unbuffered system but this system took much longer to reach equilibrium. Further, acid titration was noticed in the buffered system but not in the unbuffered system. This suggested that the buffer may help reduce the period of hydrolysis and ensure methanogenesis is detected earlier.

These experiments suggested that the biodegradability of the effluent was not enhanced by the presence of the nutrient. However, this may not always be true. The textile size effluent showed that it has a strong predisposition to be degraded. The nutrient medium may not have contributed significantly to its degradation, but it is possible that the addition of a nutrient medium may greatly improve the degradation of recalcitrant substrate i.e.: landfill leachate. Unfortunately, this possibility was not investigated further. In addition, the buffer improves the ability of MAIA to detect pH changes.

Therefore, this study suggests that a nutrient medium should always be used when conducting experiments with MAIA.

4.5. SERUM BOTTLE STUDY

Experiments conducted with MAIA have provided insights into the pre-screening potential of the instrument. There is little doubt that MAIA is a useful pre-screening tool, however whether it is an appropriate alternative to the serum bottle method still has to be investigated. This section compares both the titrimetric and serum bottle method using a textile size effluent.

Hypothesis

It was proposed that the titrimetric and serum bottle method were comparable. It was also proposed that the titrimetric method could be used as an alternative to the serum bottle method, to pre-screen an industrial effluent for treatment in anaerobic digesters. The objectives of this study were to:

- evaluate if the textile size effluent is inhibitory to the methanogens using the serum bottle method
- compare the results of the serum bottle method with the titrimetric method i.e.: with regard to the best effluent concentration to use in an anaerobic digester
- compare the specific total gas rates, based on the utilisation of textile size effluent, obtained from the titrimetric study with the serum bottle method
- conclude on the feasibility of MAIA as an alternative pre-screening tool

Materials and Methods

The serum bottle test sets included an Endogenous Control Test, Acetate Standard Test and six Textile Effluent Tests. Each test set was performed in triplicate i.e.: a total of 24 bottles. The methods of effluent preparation, biomass preparation, serum bottle set-up and analytical procedure were identical to those discussed in **section 3.2**.

The Control Test consisted of biomass (30 mL) and nutrient medium (70 mL). The Standard Test comprised of biomass (30 mL), a sodium acetate spike (2 mL, 2.5 M) and nutrient medium (68 mL).

The Textile Tests contained biomass (30 mL), nutrient medium (30 mL), a sodium acetate spike (2 mL, 2.5 M) and an aliquot of effluent appropriately diluted with distilled water. The effluent concentrations were: 5.9; 11.7; 14.7; 29.4; 44.1 and 58.7 gCOD/L respectively. These were identical to the effluent concentrations used in the Textile Study. The sodium acetate spike and nutrient medium were prepared according to recipes presented in **APPENDIX A**.

Results and Discussion

The type of serum bottle experiment conducted in this study was the Anaerobic Toxicity Assay (ATA). The purpose of this assay is to assess toxicity. This means the test evaluates how toxic a chemical substance e.g.: an industrial effluent is to an anaerobic biomass. The method relies on gas production as an indicator of activity. Specifically, gas production rates are used. **Figure [4-21]** presents the results for the Control and Standard Test conducted for this study. The Control Test is used to evaluate the level of activity not associated with the test substance i.e.: endogenous activity. It provides a basis from which inhibition can be determined. The Control Test indicates the level of biological activity based on the utilisation of the residual substrate. If it is observed that methane production based on the utilisation of the residual substrate is high, then the impact of the residual substrate on methanogenesis cannot be ignored. It was expected that this activity would be low suggesting a low residual content and therefore a reduced potential for interference when determining activity related to

the utilisation of the effluent. The residual substrate is not inhibiting, therefore a decreased level of biological activity in the presence of the test substrate i.e.: acetate or effluent, would constitute inhibition.

It can be seen that the endogenous methanogenic activity or activity related to utilisation of a residual substrate was low for this study (refer: **Figure [4-21], B**). This implied that either the impact of the residual substrate on methanogenic activity is negligible or that the biomass does not contain any methanogens. The Standard Test showed a high level of methanogenic activity suggesting that the biomass did contain a methanogen population. Therefore it was decided that the influence of the residual substrate on methanogenesis can be ignored for this study. Further, a comparison of the standard curves of **Plot [A] and [B]** in **Figure [4-21]** suggested that approximately 50 % of the total gas produced was the result of methanogenic activity. This result was useful because it confirmed that the acetate spike used in these tests did have a positive impact on methanogenic activity.

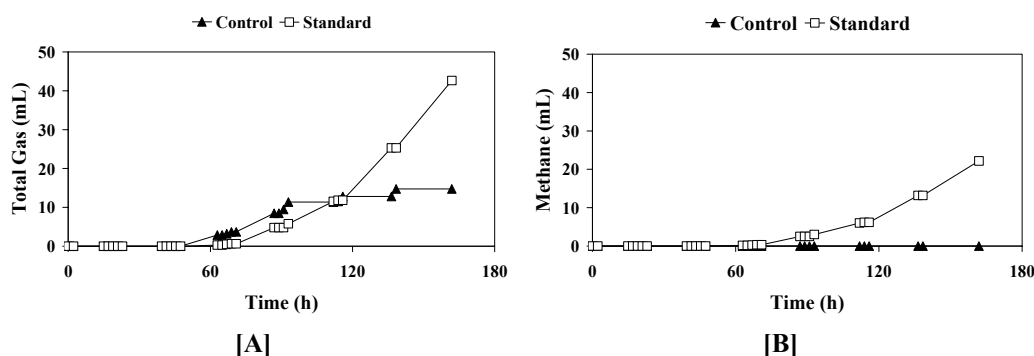


Figure [4-21]: The Control and Standard Test. The curves of Plot [A] and [B] are an average of three serum bottle results. Plot [A] compares the average total gas production of the Control Test with that for the Standard Test. Similarly, Plot [B] compares the average methane gas production of the Control and Standard Test respectively. The trend of the control curve in Plot [B] suggests that any residual substrate present with the biomass does not contribute to methanogenesis.

The results of the ATA are plotted in **Figure [4-22]**. Inhibition was inferred in cases where the Sample Test curve plotted below the Control curve. From the figure, it seemed that microbial activity in Test 1 started slowly but recovered sufficiently to exceed the control at the end of the 180 h (7 d) period. Further, there seemed to be a concentration effect associated with degrading the textile substrate. An increase in the concentration of the effluent resulted in a corresponding increase in gas production indicating no inhibition. It was noticed that the maximum level of total gas production was in excess of the maximum level associated with the utilisation of acetate (refer: **Standard Test, Figure [4-21]**). This result reinforced the idea that the test substrate supported methanogenesis.

The slow rise in the total gas production curve of Test 1 compared to the other gas production curves of **Figure [4-22]** suggested inhibition. It was decided that inhibition could be either a result of substrate limitation or substrate toxicity. If the substrate were toxic i.e.: lethal to the micro-organisms, then it would be expected that total gas production at higher effluent concentrations would also be low or negatively impacted upon. However, the remaining curves display increasing total gas production trends suggesting that the industrial size effluent was labile and consequently anaerobically biodegradable. Therefore, the inhibition noticed in Test 1 was attributed to substrate limitation rather than to a toxic effect.

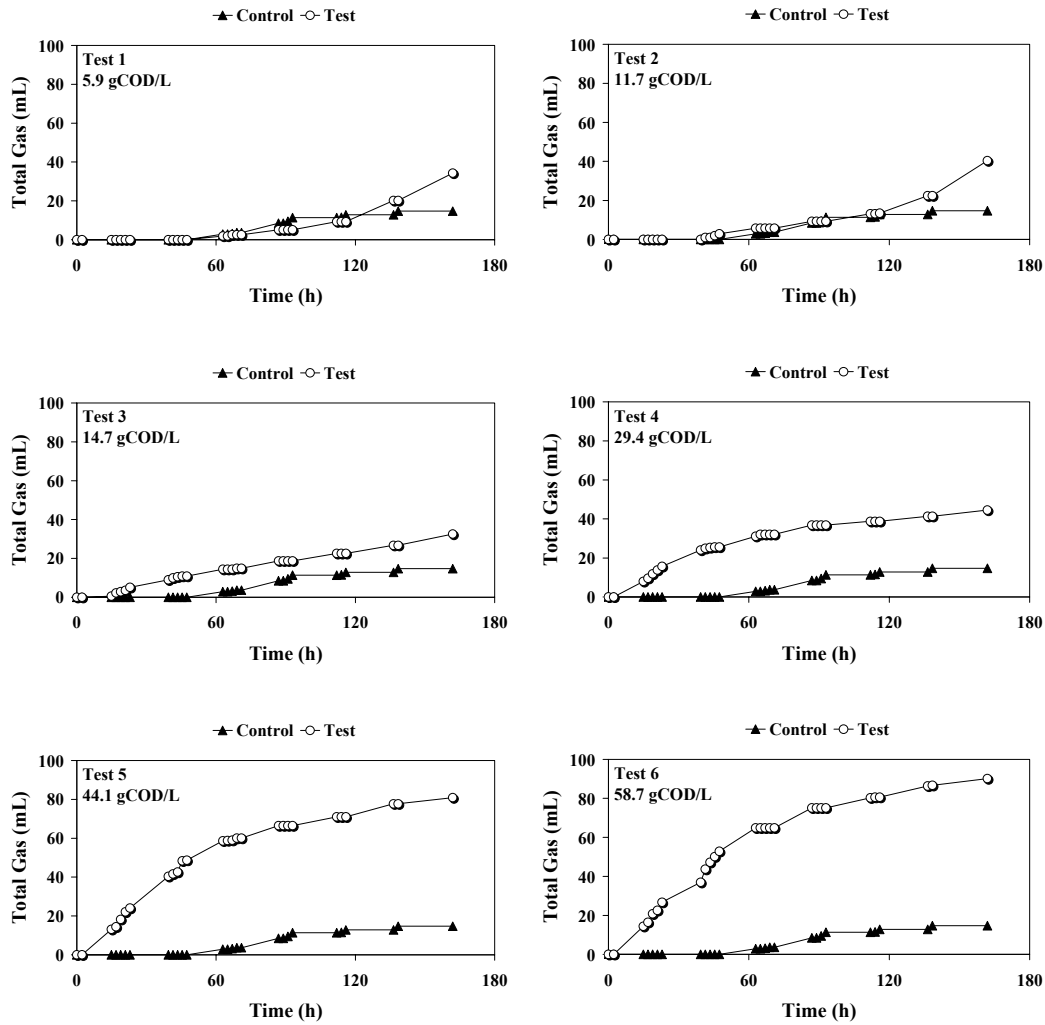


Figure [4-22]: The Serum Bottle Study. The anaerobic toxicity assay results for varying concentrations of textile size effluent. Each of the curves depicted in these plots are based on the average of three serum bottle results.

The dashed lines in **Figure [4-23]** indicate the value of the specific rates determined for the Standard Test and Control Test respectively. At an effluent concentration of 23 gCOD/L the specific gas rate curve for the Sample Tests drops below the value for the Standard Test. The value of the specific rate for the Standard Test indicates the maximum specific methanogenic activity level because it is based on the utilisation of the acetate substrate only. The shape of the specific gas production rate curve (refer: **Figure [4-23], A**) suggests that the effluent becomes inhibitory at high concentrations. However, it was decided from the shape of the gas curves in **Figure [4-22]** that the substrate was not toxic i.e.: not lethal to the micro-organisms. Therefore, it is plausible that the decrease in rate observed in Plot A (refer: **Figure [4-23]**) can be attributed to the degree of organic overloading within the serum bottle system. It seems that organic overloading determines the rate of biodegradation and consequently the specific rate of gas production.

The result does not suggest that the effluent is unbiodegradable. It does suggest that at effluent concentrations greater than 23 gCOD/L, organic loading becomes inhibitory toward methanogenic activity. Hence, only effluent concentrations less than 23 gCOD/L should be used in an anaerobic digester. Specifically, the study found that an effluent concentration of 14.7 gCOD/L worked the best for this experimental series (refer: **Figure [4-25]**). This result was identical to that provided by MAIA (refer: **section 4.3.1**). **Plot [B], Figure [4-23]** shows

that the trend observed for the serum bottle study was comparable to the textile study conducted with MAIA.

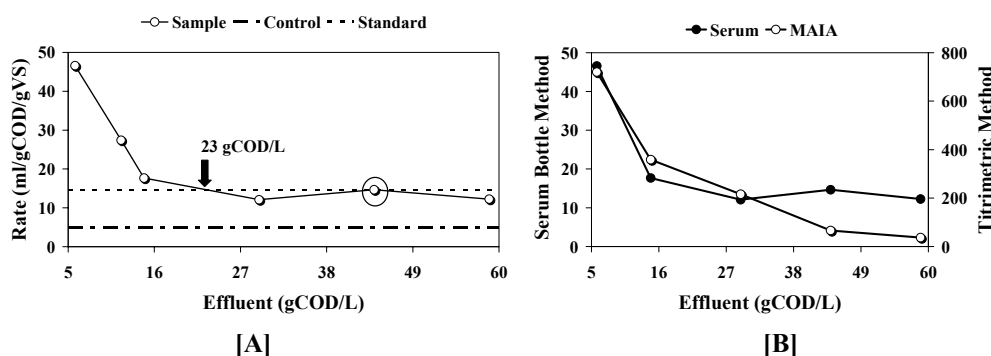


Figure [4-23]: The Serum Bottle and Titrimetric Methods. Plot [A] shows that although the specific rate of activity based on the degradation of the textile size effluent decreases with an increase in effluent concentration it does not decrease below the rate of activity based on acetate usage. This implies that the textile size effluent is easily biodegradable. Plot [B] suggests that the specific rates provided by both methods is not significantly dissimilar.

The serum bottle concentrations were chosen so that they could be identical to the textile size effluent study performed with MAIA (refer: **section 4.3**). It was proposed that the MAIA pre-screening method was comparable to the serum method. This was established with the ATA test above. Further, it was also suggested that MAIA could be used as an alternative to the serum bottle method. An argument in favour of this possibility was the time-saving benefit afforded by MAIA. MAIA can pre-screen an effluent with regard to methanogenic toxicity within 24 h (refer: **section 4.3.1**). However, it is evident that the serum bottle method takes 7 d to produce the same result (refer: **Figure [4-24]**). The figure shows that it takes approximately 16 h using the serum bottle method to detect methane gas production compared to the 4 h period in the MAIA experiments (refer: **section 4.3.1**).

Another limitation of the serum bottle method is the fact that the period of hydrolytic activity cannot be assessed from the available data. It is possible that the period of hydrolysis could exceed the duration of the ATA i.e.: greater than 7 d. In this intensive hydrolytic period, acidification may lower the pH to a level that cannot support methanogenesis. Further, it is plausible that the buffering capacity of the nutrient medium may be insufficient to prevent such a situation from occurring. This could explain the long lag periods observed in **Figure [4-24]**.

As a result, methanogenesis may not start before the test is terminated and it may be erroneously concluded that the test substrate is not biodegradable. However, the titrimetric method provides protection for the methanogenic consortium while not impeding hydrolytic processes. By maintaining the pH at a level that is suitable to both acidogenic and methanogenic consortia, MAIA ensures a more rapid toxicity assessment. Nevertheless, it is still possible that effluents that return a positive result for biodegradability using either the serum or titrimetric method may fail when degraded in an anaerobic digester. The absence of sufficient buffering in the digester could mean lower pH levels and uncertain methanogenic conditions that prevent effective biodegradation.

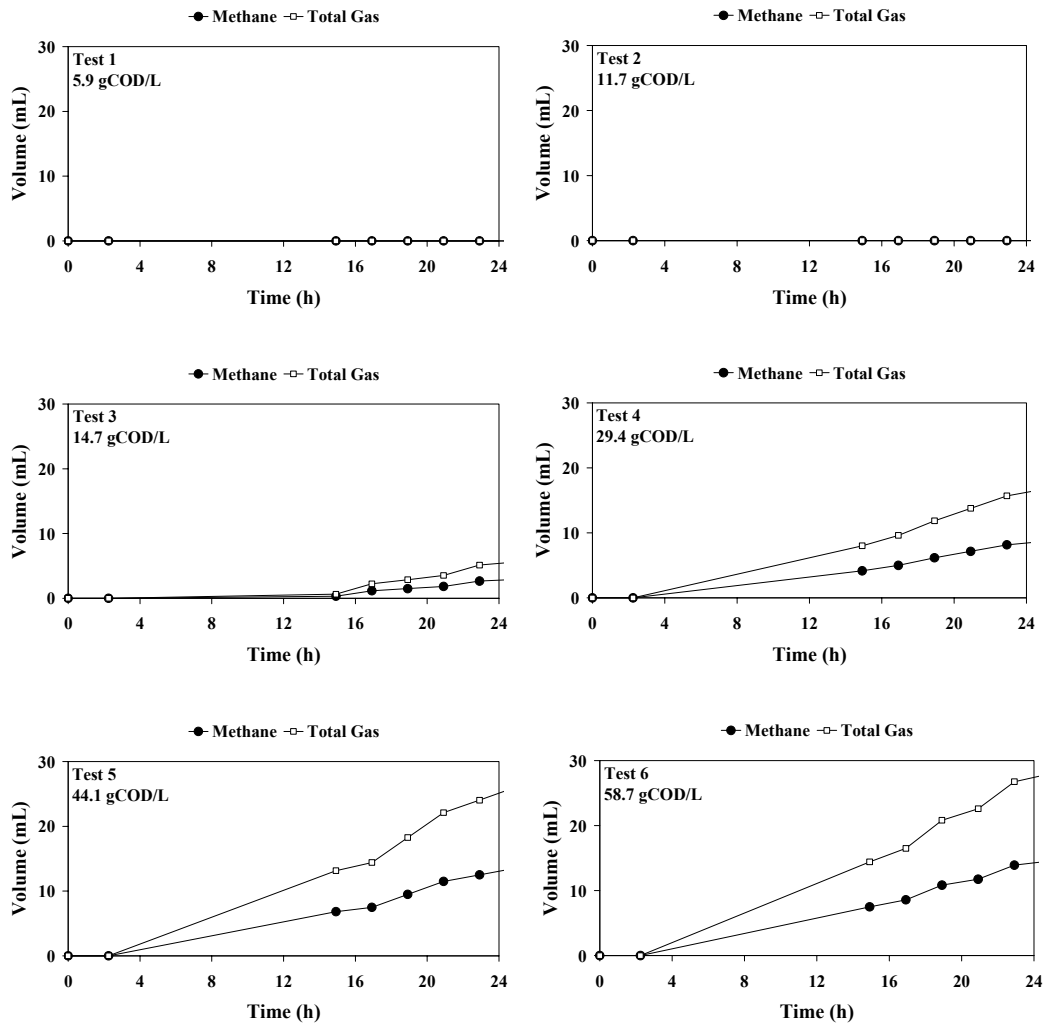


Figure [4-24]: Assessing Biodegradability. The gas production trends observed for varying organic concentrations suggests that the textile size effluent is anaerobically biodegradable. The results plotted in these curves are the average gas results of three serum bottles.

A plot of the average gas production rate observed for the different effluent concentrations is depicted in **Figure [4-25]**. The figure suggests that at the lower effluent concentrations, substrate limitation has a significant impact upon the rate of gas production. However, at the higher effluent concentrations, the trend is unclear. It is difficult to make any firm conclusions based on the gas production rate results; however if **Figure [4-25]** is assessed in conjunction with **Figure [4-24]**, it can be concluded that methanogenic activity could have increased significantly if the tests at the higher effluent concentrations were run for a longer period of time. This is plausible (refer: earlier discussion) since the higher effluent concentrations would require a longer period of time for hydrolysis and acidification, prior to methanogenesis becoming the dominant process.

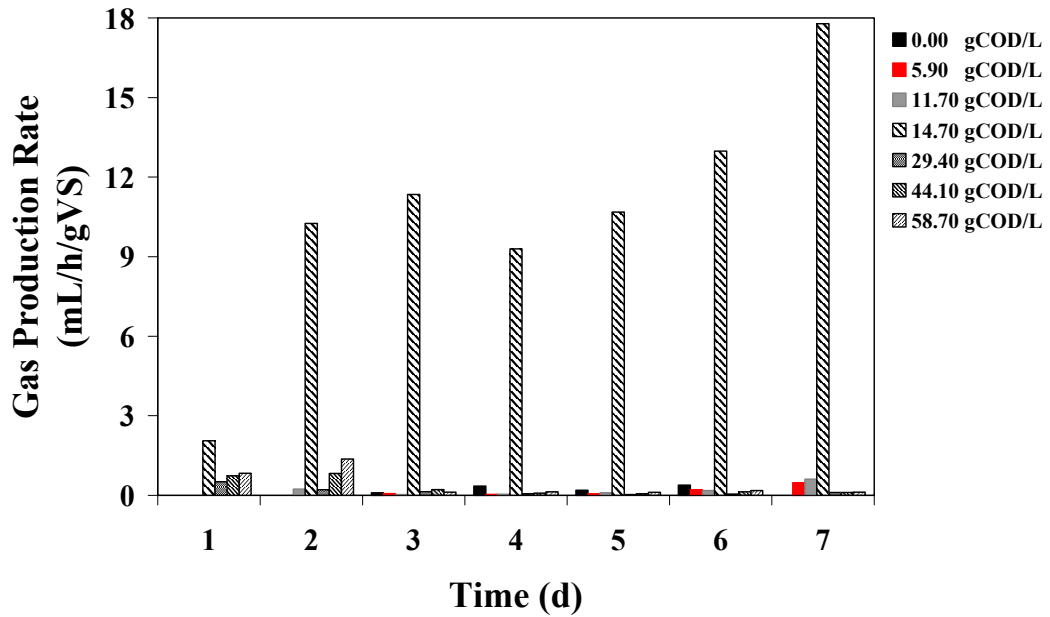


Figure [4-25]: Summary of Gas Production Rates. The results for the serum bottle study suggest substrate limitation at the lower effluent concentrations. However, the trend in the gas rate at the higher concentrations is inconclusive. Despite this, the best effluent concentration to use in the digester is 14.70 gCOD/L. The results depicted in the figure are based on an average gas production rate per day across three serum bottles per experimental set.

It can be concluded that the serum bottle method is identical to the titrimetric method if a qualitative assessment of toxicity and biodegradability is needed. Further, the titrimetric method i.e.: MAIA produces these results in a much shorter period of time i.e.: within 1 d compared to the serum bottle method that required 7 d. Therefore, when compared in this way the titrimetric method seems to be the better alternative. However, if a quantitative assessment is required, the titrimetric method is not as reliable as the serum bottle method.

4.6. COMPONENT STUDY

It is accepted that MAIA works well when assessing single substrate systems. However, the Textile Study suggested that the instrument was not sufficiently specific enough to effectively assess multi-substrate systems i.e.: that a complex substrate may be difficult to pre-screen unless the chemical interactions of its constituents and their stoichiometry are better understood. The presence of particulate fractions in the effluent posed a further complication to pre-screening of a multi-substrate system. It was not possible, within the scope of this study, to evaluate how the chemical interactions between the system constituents impacted upon MAIA's pre-screening ability, but it was possible to assess how the system reacted to the individual constituents. This section seeks to highlight the difference in the manner that the MAIA responds to single and multi-substrate systems respectively.

Hypothesis

It was proposed that the presence of a substrate different from acetate i.e.: more complex, would be difficult to assess, because the sequence of alkaline and acid titration could be complex. In addition, it is proposed that the particulate i.e.: insoluble material, in the textile effluent affected the way the titrimetric system behaved. It is further proposed that complex substrates can be pre-screened using the titrimetric method if they are individually assessed, however complex multi-substrate systems are pre-disposed to problems. Therefore, the objectives of this study were to:

- investigate the response of the system in the absence of any particulate material;
- show that it is possible to pre-screen a complex substrate;

Materials and Methods

The primary constituent of the textile size effluent was starch. Therefore, only this substrate was tested. The component was tested at three different concentrations. Standard solutions of soluble monosaccharide starch i.e.: 0.5, 1.0, and 2.0 gCOD/L were prepared. The organic content of these standard solutions i.e.: starch, were determined using the method presented in **APPENDIX C**. Each test consisted of: biomass (240 mL); sodium acetate (16 mL) and the substrate (544 mL). The concentration of the starch in the reactor was 0.34; 0.68 and 1.36 gCOD/L respectively. The experimental test procedure was the same as previous sample tests. However, the duration of each test was 15 h.

Results and Discussion

The degradation process is characterised by an initial period of hydrolysis and fermentation (refer: **Figure [2-1]**) and the period of hydrolysis can be intensive, especially if the substrate has a large particulate fraction. These large particulate i.e.: insoluble, fractions have to be extracellularly degraded and it was proposed that the titrimetric system responds to the hydrolytic process with alkaline titration (refer: **3.1.5.2**). Starch is considered to be a complex substrate because it is comprised of long hydrocarbon chains and its covalent bonds make it relatively insoluble. Despite this i.e.: its complex chemical structure, starch can be anaerobically degraded (Noike, 1985). Therefore, it was expected that in the absence of any particulates, hydrolysis would occur intracellularly and the titrimetric system will titrate mostly acid.

Figure [4-26] depicts the pH trends observed for the tests using soluble monosaccharide starch. In all the tests there was a strong, acid titration trend without any significant alkaline titration.

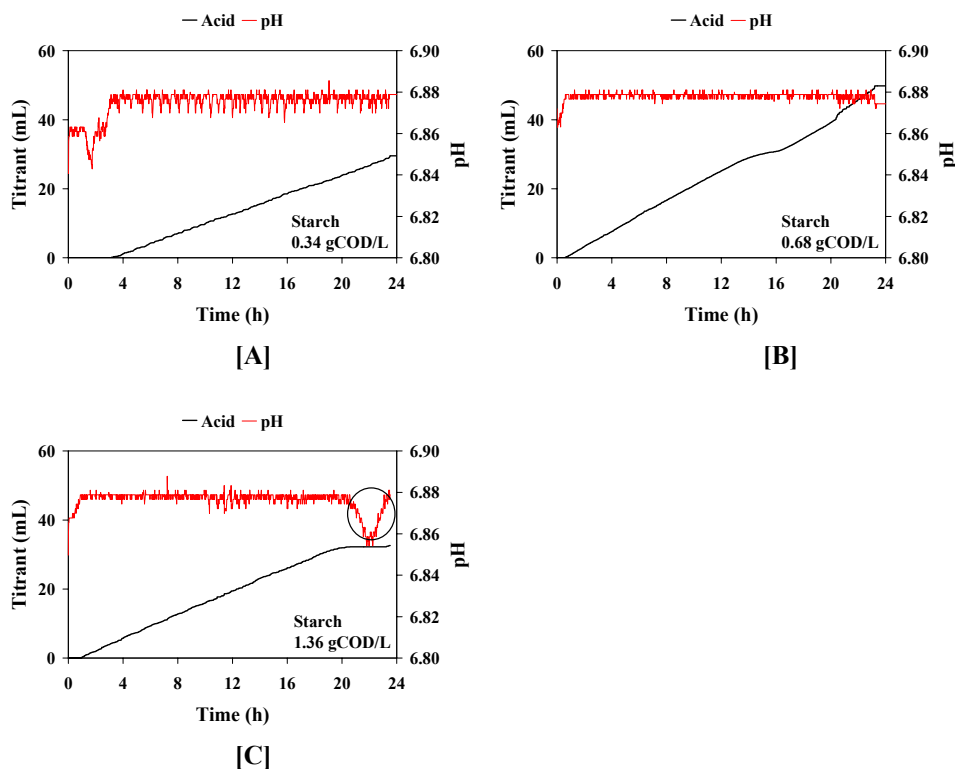


Figure [4-26]: Tracking Single Substrate Systems. The pH and titration trends with starch at varying concentrations suggest that MAIA can effectively follow the microbial activity.

The fluctuation seen in the pH (refer: **Figure [4-26], Plot C**) i.e.: circled area, was the result of acid contamination from the gas displacement system. However, had the accident not occurred and the test duration was extended, the available information seemed to suggest that the extent of biological activity was incomplete. Therefore, it was possible that a longer test would have revealed a longer period of acid titration. This was evident from the fact that, the pH continued to increase after the effect of the accident was corrected. This result seemed to suggest that alkaline titration observed in the Textile Study could be related to high particulate fractions there. However, this result was not conclusive because it ignored the possibility of multi-substrate chemical interactions that may have contributed to the alkaline titration observed in the Textile Study. It was determined that a detailed chemical analysis of the size effluent would have been necessary to accurately assess the chemical impact of the constituents during biodegradation. However, the inclusion of this would have undermined the broad aim of this study i.e.: to highlight the potential of MAIA to be a **rapid** pre-screening tool.

The composition of the biogas produced from the degradation of a chemical compound can be evaluated using the Buswell equation (refer: **APPENDIX C**). It was determined that the composition of the biogas produced from the degradation of starch should be 50 % methane gas. It was clear from **Figure [4-27]** that the biogas composition, in all the tests, approached a maximum level of 50 % methane gas.

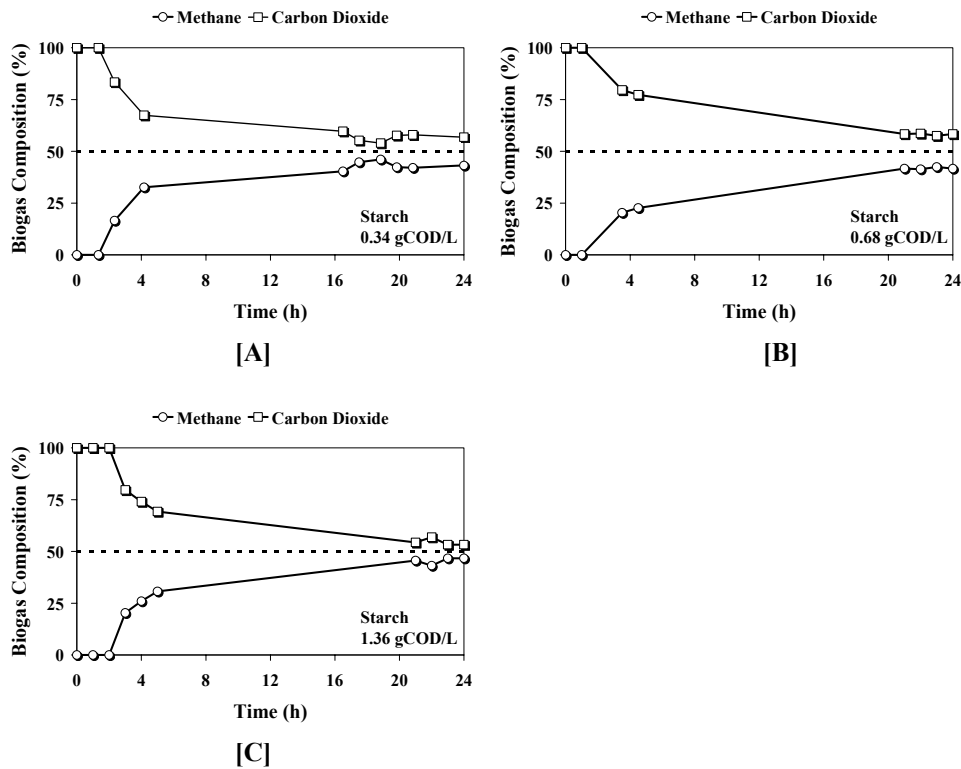


Figure [4-27]: Biogas Production. At the start of each test the headspace composition consists of an equimolar composition of nitrogen and carbon dioxide gas. As methane production increases nitrogen gas is displaced. Buswell predicted an equimolar biogas (CH_4 and CO_2) composition for a starch substrate at steady state. The graphs show a tendency for the biogas composition approaching 50 % methane gas after 24

Further, it is understood from theory (refer: equation [3-1]) that the conversion of acetate results in the production of an equimolar mixture of carbon dioxide and methane gas. This means that an increase in carbon dioxide concentrations should correspond to increases in methane gas concentrations. The gas production trends in **Figure [4-28]** confirmed this theoretical result i.e.: with an increase in the volume of methane gas produced there was an immediate increase in carbon dioxide gas production as well. The total volume of methane gas produced after 24 h was used to determine a specific methane gas rate which was then compared to the total acid titrated by MAIA for the same period. The results of this comparison are plotted in **Figure [4-29]** and tabulated in **Table [4-10]**.

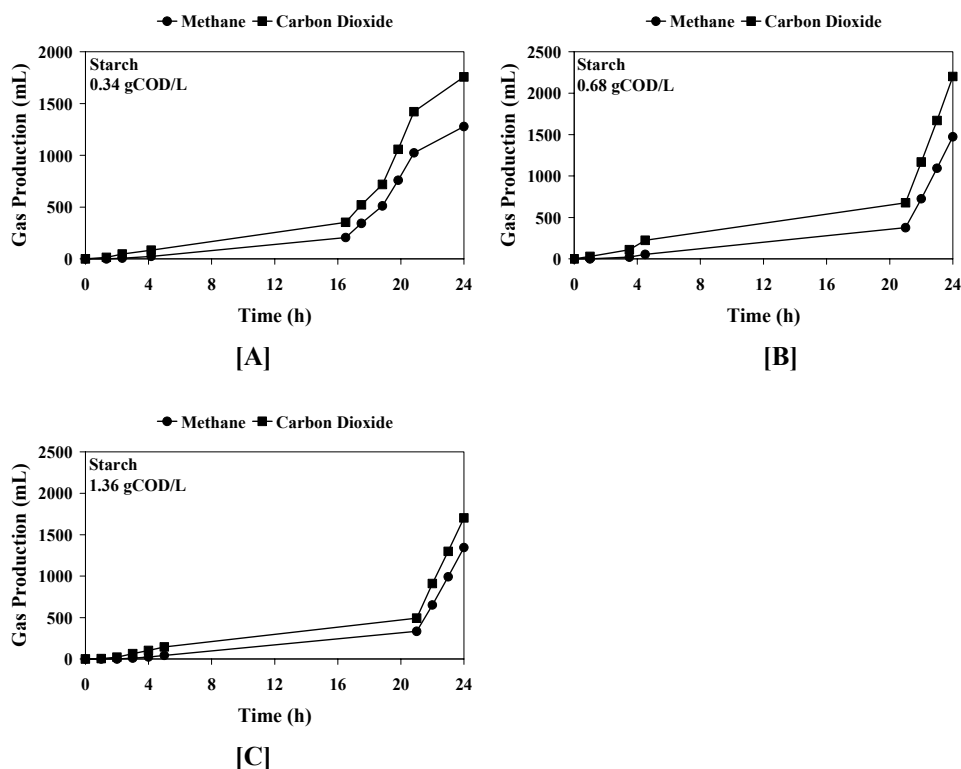


Figure [4-28]: Methanogenic Trends. For every mole of acetate consumed during methanogenesis of starch an equimolar mixture of methane and carbon dioxide gas should be produced. The gas production results i.e.: GC, at varying substrate concentrations confirm that this process is occurring.

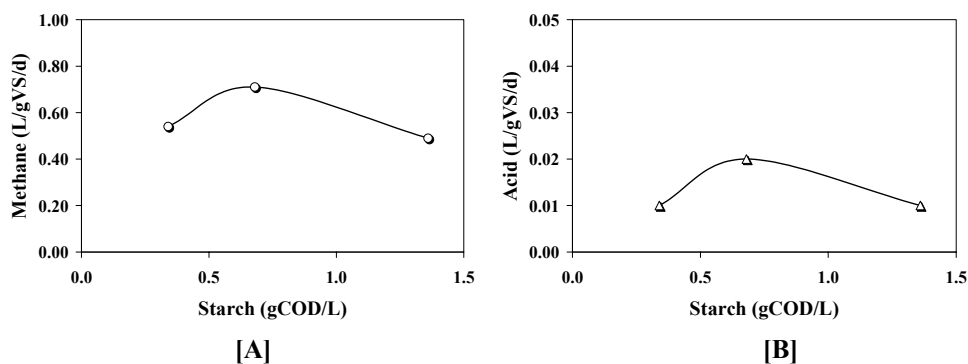


Figure [4-29]: The Pre-Screening Potential of MAIA. The similarity between the specific methane and titration rates at varying substrate concentrations suggests that MAIA is more suited to analysing single substrate systems.

Table 4-10: Specific Methane and Titration rates

Test	Concentration	Methane	Titration
	gCOD.L ⁻¹	L.gVS. ⁻¹ .d ⁻¹	L.gVS. ⁻¹ .d ⁻¹
1	0.34	0.54	0.01
2	0.68	0.71	0.02
3	1.36	0.49	0.01

The qualitative trend in both plots was similar (refer: **Figure [4-28]**). Further, it is evident from the tabulated results that all the tests were quantitatively comparable. The relatively constant acid titration rate seemed to suggest that the test concentrations of starch used were

higher than the half saturation constant value. Therefore, there seemed to be no perceptible relationship between the acid titration rate and the substrate concentration. The qualitative and quantitative similarities observed in this study were not present in the Effluent Study. Thus, this result seemed to confirm the idea that MAIA is more suited to assessing single substrate rather than multi-substrate systems.

In the absence of any alkaline titration or microbial acidification it was possible to quantify methanogenic activity and inhibition of increasing substrate concentration on that activity (unlike the multi-substrate system). **Figure [4-30]** presents these results. Inhibition was based on the measured difference between methanogenic activity utilising acetate (refer: **section 4.2**) and that observed for starch. A plot of activity based on the utilisation of starch at a rate lower than that observed for the simple substrate i.e.: acetate, within a 24 h period was considered to be an indication of inhibition. It is strange to think that starch can be inhibitory at any concentration level and therefore this statement needs to be clarified. Starch is not toxic to the methanogens. However, it is a complex substrate and requires time to be completely biodegraded. Therefore, the test duration of a single day becomes increasingly inappropriate as the concentration of starch is increased. This means that higher concentrations of starch usage may suggest methanogenic inhibition but in fact merely requires a longer test period to provide a more accurate result. However, the implication from the available information was that the starch can be best degraded at a concentration level less than 0.45 gCOD/L.

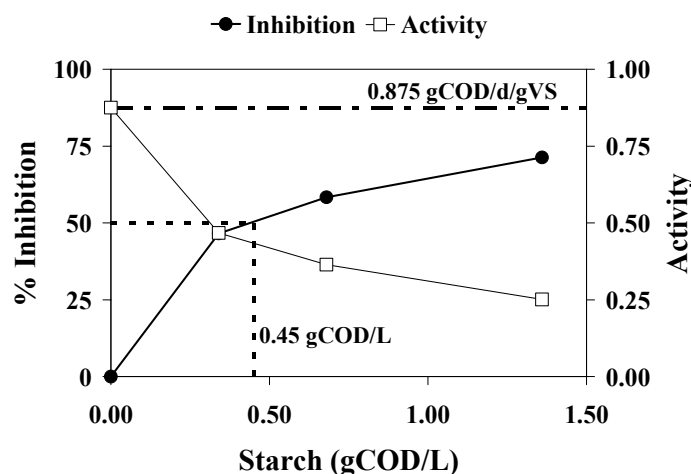


Figure [4-30]: Assessing Inhibition from Titration Data. Activity is in gCOD/d/gVS.

This study, when compared to the Textile Study, suggests that multi-substrate systems are inherently difficult to pre-screen using the titrimetric method. This difficulty is not related to the complexity of the substrate being tested, as this study indicated, but possibly to the chemical interactions between the constituents in a multi-substrate system. However, it is more plausible that the MAIA titration system is not sensitive enough to follow multiple, series or parallel reactions. Furthermore, the effort involved in chemically analysing for the constituents of the test substrate may be tedious and could cancel out the time benefit of using MAIA.

In addition the Component Study has shown that MAIA was effective in pre-screening a single substrate in the absence of particulates. Insoluble substrate fractions introduce alkaline titration which may prevent activity determinations from being accurately performed (refer: **section 3.1.7**). It is possible that soluble polymers could also introduce problems from similar acidification processes, however it was not possible to investigate this further.

4.7. MATHEMATICAL SIMULATION

Following the experimental work conducted with MAIA, it was evident that certain system parameters e.g.: temperature can significantly affect titration measurements (refer: **APPENDIX E**). It was decided that a mathematical model would help in better understanding the impact of such factors because mathematical modelling can be a powerful tool to verify the path and extent of biochemical reactions. A well developed model can significantly improve the way in which experiments are planned and conducted. This chapter explores the possibility of implementing a mathematical model to the anaerobic processes investigated in this study.

4.7.1 The Software Package

There are many computer packages that can be used to simulate the chemical processes occurring within an aquatic environment; however **AQUASIM** was selected for this study because this software package was specifically designed for the identification and simulation of aquatic systems in the laboratory, technical plants and in nature (Reichert, 1998). In order to implement a dynamic process, AQUASIM requires that a matrix of processes has to be defined. The International Water Association (IWA) approach (Henze et. al., 1987) was used. The advantage of this format is that it allows the user to appreciate the impact of all conversion processes in all compartments instantaneously.

A model in AQUASIM consists of a system of differential and/or algebraic equations, which deterministically describe the evolution of a set of state variables. It is structured in four subsystems (refer: **Figure [4-31]**). For each subsystem to be fully implemented all the subsystems upstream have to be completely defined.

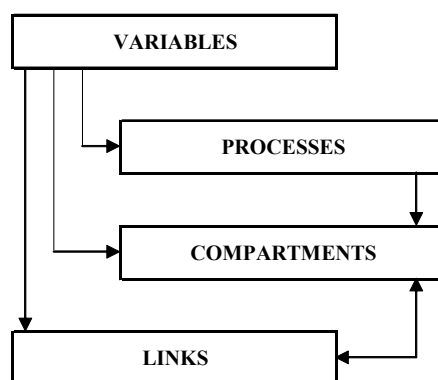


Figure [4-31]: The Structure of AQUASIM. Adapted from Remigi, (2001).

Variables

The variables are objects that are assigned a numerical value. Variables implemented in the pH-stat model include:

- Dynamic and Equilibrium state variables. Dynamic variables are solutions of a system of differential equations obtained from a matrix of dynamic processes while equilibrium variables are solutions of a system of algebraic equations corresponding to equilibrium processes.
- Constant variables that are assigned a constant value e.g.: kinetic constants;
- Formula variables have a functional relationship to other variables;
- Real list variables;
- Probe variables which make variables that are “locally” calculated within a compartment, “globally” visible.

Processes

Two types of processes exist:

- Dynamic processes are those defined by specifying the corresponding stoichiometry and kinetics i.e.: they can be described by means of a set of differential equations.’
- Equilibrium processes in which the kinetics are assumed to be fast enough, at least in comparison to the other processes of the system, to be neglected e.g.: physico-chemical processes.

Compartment

Numerous compartments can be described, however in this study only the mixed reactor compartment was considered. This compartment permitted the implementation of spatially homogeneous systems e.g. stirred reactors. However, to fully define the reactor, the active processes and variables, the initial conditions and reactor inputs had to be specified. A mixed reactor can operate either at a constant or variable volume. In the first case, if an input exists, then the outflow can be automatically set to equal the inflow; in the second case, the input and output flowrates i.e.: (Q_{in}) and (Q_{out}) respectively, can be independently set and the algorithm derives the resulting increase or decrease in volume (V_R), according to:

$$\frac{d}{dt} V_R = Q_{in} - Q_{out} \quad [4-2]$$

The Links

The various compartments of a complex system can be connected through two types of links:

- advective: these describe water and advective substance transport between compartments; they not only connect compartments, but also permit the development of bifurcations and junctions;
- diffusive: these describe diffusive boundary layers or membranes between compartments and can be diffusively permeated by substances.

4.7.2 Modelling Criteria

The pH-stat titration technique is applicable to any bioreaction involving pH variations. In the past, the main application of the pH-stat titration had been for nitrification monitoring, however with the extension of its use to anaerobic systems it was necessary to provide a theoretical model of pH-stat titration to predict the response to any reaction involving the production or consumption of protons, hydroxyl ions and inorganic carbon chemical species. A typical model could have the following assumptions:

1. The effect of the ionic strength on components concentration was neglected.
2. The dynamics of the $\text{CO}_2/\text{HCO}_3/\text{CO}_3$ equilibria were assumed to be much faster than the dynamics of the reaction:
3. The carbonic acid concentration was negligible.
4. The buffer capacity was assumed to be due only to the inorganic carbon species.

Assumption 1 was added because the ionic strength was low. Assumption 2 was appropriate because these chemical reactions are generally faster than biologically catalysed ones. Finally, Assumption 3 was justified by the fact that carbonic acid equivalently makes 0.2 % of the carbon dioxide concentration (Stumm and Morgan, 1996).

4.7.3 Model Application

The mathematical modelling of the anaerobic pH-stat system i.e.: MAIA was extensively investigated by (Remigi, 2001). However, the experimental work performed in this study suggested that the existing model could be improved. The Textile Study seemed to suggest that complex substrates may consist of two different substrate fractions i.e.: easily (S_F) and slowly (S_S) fermentable fractions or from earlier discussion RBCOD and SBCOD respectively (refer: **Figure [3-8]**). The existing mathematical model could not effectively simulate the sequential degradation of these substrate fractions. The model depends to a large extent upon a single step to assess fermentation. However, this approach proved to be inappropriate for the complex sequence of biochemical reactions that leads to acetate production. Further, MAIA provides data that are not completely compatible with the input requirements of the Remigi model because it is limited to evaluating pH changes associated with the production of either excess acidity or alkalinity.

Objectives

This study aims to provide a simplified mathematical model that consists of only two sequential degradative steps i.e.: acidogenesis and methanogenesis. In addition, it is proposed that these anaerobic processes are performed by just three microbial species i.e.: fermentative bacteria, acetoclastic and hydrogenotrophic methanogens (refer: equations [4-9] to [4-11]).



Symbol or Subscript	Definition
X	particulate biomass
S	soluble substrate
f	fermentative step
Ac	acetoclastic step
H ₂	hydrogenotrophic step

The study also aims to highlight the benefit of using mathematical modelling to improve the quality of experimental work. It is proposed that modelling can be used to effectively assess and consequently eliminate the influence of system interferences. In this regard, the model developed in this study will be used to simulate the effect of temperature on the titrimetric system.

Structure of the Model

The new model was developed in association with Remigi, (2003) and has the following characteristics:

- a) There are four uptake processes: fermentation of fast and slowly degradable soluble substrates to acetate and hydrogen i.e.: equation [4-9]; hydrogenotrophic methanogenesis i.e.: equation [4-10] and acetoclastic methanogenesis i.e.: equation [4-11]. The fermentative step was divided into two sub-processes that relate to the fermentation of the easily degradable and recalcitrant substrate fractions respectively. First, the readily accessible substrate (S_f) is immediately fermented and after a period of acclimation or preliminary conversion, the recalcitrant substrate (S_s) is utilised.
- b) The fermentative biomass performing both transformations was assumed to be the same with identical kinetic characteristics i.e.: k_{max} and Y (refer: **Table [4-13]**).
- c) The basic structure including the initial parameter and constant approximation was derived from the Anaerobic Digestion Model (ADM1) by Bastone et. al. (2002). It consists of two compartments i.e.: a liquid and a gas phase. An additional ‘virtual’ compartment, that mirrors the liquid phase, had to be defined in order to account for cumulative quantities i.e.: the volumes of acid or base titrated.

Table 4-11: Physico-Chemical Processes

Equation	Comment
$0 = K_{Ac} \cdot S_{Ac} - \left(k_{a,Ac} + [H^+] \right) \cdot [Ac^-]$	Dissociation Equilibrium: Acetate [4-12]
$0 = [OH^-] - \frac{K_{H_2O}}{[H^+]}$	Dissociation Equilibrium: Water [4-13]
$0 = [H^+] - [OH^-] - [Ac^-] + [Cat^+] - [An^-] - [HCO_3^-]$	Charge Balance [4-14]
$CO_2 + H_2O \left(\rightarrow H_2CO_3 \right) \leftrightarrow HCO_3^- + H^+$	Pseudo-Equilibrium: Inorganic Carbon [4-15]

- d) The biochemical processes i.e.: the uptake of substrates and the decay of microbial populations have been implemented as dynamic processes (refer: **Table [4-16]**). The equilibrium of inorganic carbon species e.g.: the association and dissociation of carbon dioxide/hydrogen carbonate system has also been included as a dynamic process.
- e) The impact of temperature on the physical-chemical equilibrium and consequently on the titration process has also been incorporated. This was achieved by making the inorganic carbon equilibrium as well as the carbon dioxide exchange between the liquid and gas phases, temperature dependent (refer: **Table [4-12]**).

Table 4-12: Constants used in the Mathematical Model

Constant	Unit	Comment
$K_{H,CO_2} = 1E+2.6747-0.0139 T$	mol.L ⁻¹ atm ⁻¹	Henry's Law Constant Carbon Dioxide
$pK_{[CO_2]} = 17052 T^{-1} + 215.21 \log T - 0.12675 T - 545.56$	-	Dissociation Constant CO ₂ /HCO ₃ ⁻ (-log ₁₀)
$pK_{[H_2O]} = -0.0361 T + 24.7599$	-	Dissociation Constant Water (-log ₁₀)

*T = Temperature in Kelvin

- f) All biological steps follow Michaelis-Menten kinetics (refer: **Table [4-13]**). However, no inhibition had been accounted for, although it was known that an increase in hydrogen partial pressure significantly affects acidogenesis i.e.: $K_I = 0.3$ to $1E-05$ gCOD/L (Batstone et. al., 2002). The decision not to include inhibition was based on the desire for a simple model; however the model can be easily modified to include inhibition kinetics. Further, it is known from literature that temperature affects the performance of microbial species: methanogens are more sensitive than acidogens in the anaerobic consortium and as a result unbalanced metabolism can occur at lower temperatures when the acidogens produce volatile acids faster than the methanogens can convert them to methane (Speece, 1996).

Specifically, temperature can affect biochemical reactions in five main ways:

- Increase in reaction rates with increasing temperature i.e.: Arrhenius equation;
- Decrease in reaction rate with increasing temperature above optimum e.g.: greater than 40°C for mesophilic range of microbial activity;
- Decrease in yields and an increase in (K_s) due to increased turnover and maintenance energy with increased temperatures;
- Shifts in yield and reaction pathways due to changes in thermodynamic yields and microbial populations;
- Increase in death rate due to increased lysis and maintenance.

Therefore, equation [4-16] was implemented in the model to describe how uptake rates can be varied with temperature.

$$k_{\max,i} = k_{\max,i,20} \cdot \exp[\theta \cdot (T - 293)] \quad [4-16]$$

Slow fermentative bacteria were introduced as:

$$k_{\max,S_f}^* = \left[1 - \exp\left[\frac{-\gamma \cdot t}{t_{\text{lag}} - t}\right] \right] \quad \text{if } t \leq t_{\text{lag}} \quad [4-17]$$

$$k_{\max,S_f}^* = k_{\max,S_f} \quad \text{if } t > t_{\text{lag}}$$

γ Empirical Rate Factor

t_{lag} Period required for Activation

- g) The model was designed to track the titration switches i.e.: acid and alkaline, when they occur. This means that the model has built into its design the ability to draw ‘stepped’ pH functions when the titration system switches from titrating alkali to acid. This addition was necessary because AQUASIM has an intrinsic inability to step over a discontinuity i.e.: it is impossible to define a step-like function. Nevertheless, it is possible to approximate the step function with a very steep line or by using a proper switch function e.g.: if, then and else logic statements. The latter option is difficult to implement because it does not permit the implementation of pH ranges. Further, the switch function does not allow the model to introduce titrant flowrate or concentration as parameters. Therefore, a steep line function was used to model titrant switches.

Table [4-13] and Table [4-14] summarise the parameter and physical constant values respectively used in the model.

Table 4-13: Parameters Values (Batstone, 2002)

Symbol	Value	Units	Comment
α	0.371	-	Fraction of Methanogens
β	0.786	-	Stoichiometric Fraction: Acetate from Solubles
γ	0.100	min	Activation Time Constant: Slow Fermentative Bacteria
$k_{d,F}$	5E-05	min ⁻¹	Decay rate: Fermentative species
$k_{d,Ac}$	1E-04	min ⁻¹	Decay rate: Acetoclastic Methanogens
$k_{d,H2}$	6E-06	min ⁻¹	Decay rate: Hydrogenotrophic Methanogens
$k_{m,f,20}$	0.020	gCOD.gVS ⁻¹ .min ⁻¹	Maximum Uptake Rate: Fast Fermentative Bacteria at 20°C
$k_{m,s,20}$	0.020	gCOD.gVS ⁻¹ .min ⁻¹	Maximum Uptake Rate: Slow Fermentative Bacteria at 20°C
$k_{m,Ac,20}$	0.022	gCOD.gVS ⁻¹ .min ⁻¹	Maximum Uptake Rate: Acetoclastic Methanogens at 20°C
$k_{m,H2,20}$	0.005	gCOD.gVS ⁻¹ .min ⁻¹	Maximum Uptake Rate: Hydrogenotrophic Methanogens at 20°C
$K_{S,f}$	0.500	gCOD/L	Half Saturation Constant: Fermentation
$K_{S,Ac}$	0.200	gCOD/L	Half Saturation Constant: Acetoclastic Methanogens
$K_{S,H2}$	5E-05	gCOD/L	Half Saturation Constant: Hydrogenotrophic Methanogens
θ	0*	-	Temperature Dependent Coefficient: Uptake Rates
t_{lag}	50*	min	Lag-time Activation: Fermentative Biomass
Y_f	0.272	gVS.gCOD ⁻¹	Yield Coefficient: Fermentative Biomass
Y_{Ac}	0.005	gVS.gCOD ⁻¹	Yield Coefficient: Acetoclastic Biomass
Y_{H2}	0.024	gVS.gCOD ⁻¹	Yield Coefficient: Hydrogenotrophic Biomass

Table 4-14: Physical Constant Values (Batstone, 2002)

Symbol	Value	Units	Comment
H_{CH_4}	0.0315	-	Henry's Law Constant: Methane
H_{N_2}	0.0162	-	Henry's Law Constant: Nitrogen
$K_{L,a} [CH_4]$	2E+05	min ⁻¹	Liquid/Gas Exchange Coefficient: Methane
$K_{L,a} [CO_2]$	10.000	min ⁻¹	Liquid/Gas Exchange Coefficient: Carbon Dioxide
$k_{kin} [CO_2]$	2E+03	min ⁻¹	Association/Dissociation Kinetic Constant: CO ₂ /HCO ₃
pK_{Ac}	4.7600	-	Dissociation Constant: Acetate (log ₁₀)
p_{atm}	1.0130	bar	Atmospheric Pressure
R	8.2E-05	bar.L.K ⁻¹ .mmol ⁻¹	Universal Gas Constant

Initial Conditions

It is crucial that the initial conditions are correctly set i.e.: the values of those variables that are solutions to the system of algebraic equations listed as [4-12] through [4-14] earlier, especially when dealing with such a sophisticated titrimetric technique. Otherwise, numerical inconsistencies could result and induce a titration in the model that is unrelated to biological activity. Therefore, the model requires that the user manually calculate and input these important values. The user would have to take into account the association/dissociation pseudo-equilibrium of inorganic carbon (refer: **Table [4-16]**, Process 1) as well as the gas-liquid exchange of carbon dioxide governed by Henry's Law:

$$[HCO_3^-] = [CO_2]_{liq} \cdot \left(1 \times 10^{pH - pK_{CO_2}} \right) \quad [4-18]$$

$$[CO_2]_{liq} = K_{H,CO_2} \cdot p_{CO_2}$$

As an example, **Table [4-15]** presents the initial conditions determined for a set-point pH = 6.80, T = 35°C and a carbon dioxide molar fraction of 50 %.

Table 4-15: Initial Condition

[H ⁺]	p _{CO2}	[HCO ₃] ⁻	[OH] ⁻	[Ac] ⁻	[Cat]	[An] ⁻
mmol.L ⁻¹	bar	mmol.L ⁻¹	mmol.L ⁻¹	gCOD.L ⁻¹	mmol.L ⁻¹	mmol.L ⁻¹
1.585 x 10 ⁻⁴	0.4735*	39.97	1.318 x 10 ⁻⁴	0	39.97	2.67 x 10 ⁻⁴

*The carbon dioxide partial pressure was calculated as 50 % of total pressure where $p_{tot} = p_{atm} - p_{H_2O}$

Table 4-16: Matrix of Dynamic Processes

Process	S_{CO_2}	S_{HCO_3}	$S_{Ac,F}$	$S_{Ac,S}$	S_{m,CH_4}	S_{m,H_2}	S_{CH_4}	X_{Ac}	X_{H_2}	X_{CH_4}	Bs	A	Rate
	mmol.L ⁻¹	mmol.L ⁻¹	g/L	g/L	g/L	g/L	g/L	g/L	g/L	g/L	mL	mL	mmol.min ⁻¹ or g.min ⁻¹
1 CO ₂ /HCO ₃	1	-1											$k_{eq,CO_2} \cdot (S_{HCO_3} \cdot [H^+] - k_{a1} \cdot S_{CO_2})$
2 Fast [F]	$6.166 \cdot (1 - Y_{Ac})$		-1		$\beta(1 - Y_{Ac})$	$(1 - \beta)(1 - Y_{Ac})$		Y_{Ac}					$k_{m,Ac,F} \cdot X_{Ac} \cdot \frac{S_{Ac,F}}{K_{S,Ac,F} + S_{Ac,F}}$
3 Slow [F]	$6.166 \cdot (1 - Y_{Ac})$			-1	$\beta(1 - Y_{Ac})$	$(1 - \beta)(1 - Y_{Ac})$		Y_{Ac}					$k_{m,Ac,S} \cdot X_{Ac} \cdot \frac{S_{Ac,S}}{K_{S,Ac,S} + S_{Ac,S}}$
4 H ₂ [m]	$-(1 - Y) / 64$					-1	$(1 - Y_{H_2})$		Y_{H_2}				$k_{m,m,H_2} \cdot X_{m,H_2} \cdot \frac{S_{m,H_2}}{K_{S,m,H_2} + S_{m,H_2}}$
5 Ac [m]	$15.625 \cdot (1 - Y_{m,CH_4})$					-1	$(1 - Y_{m,CH_4})$			Y_{H_2}			$k_{m,m,CH_4} \cdot X_{m,CH_4} \cdot \frac{S_{m,CH_4}}{K_{S,m,CH_4} + S_{m,CH_4}}$
6 Decay [Ff]								-1					$k_{d,Ac} \cdot X_{m,Ac}$
7 H ₂ Decay									-1				$k_{d,m,H_2} \cdot X_{m,H_2}$
8 Ac Decay										-1			$k_{d,m,CH_4} \cdot X_{m,CH_4}$
9a Base Flow											1		$Flow \cdot TitrBs \cdot [Bs]^{-1}$
9b Acid Flow												1	$Flow \cdot TitrA \cdot [A]^{-1}$

1 Pseudo-Equilibrium for Inorganic Carbon
 4 Hydrogenotrophic Methanogenesis
 7 Decay of Hydrogenotrophic Methanogens

2 Fermentation: Readily Biodegradable Solubles
 5 Acetoclastic Methanogenesis
 8 Decay of Acetoclastic Methanogens

3 Fermentation: Slowly Biodegradable Solubles
 6 Decay of Fermenters
 9 Cumulative Alkaline and Acid Titration

Results and Discussion

Figure [4-32], compares the output of the general anaerobic mathematical model with plots from an experimental sample test. The intention of this comparison was to highlight the potential of using modelling to plan experiments with MAIA. It is clear from the figure that the model can effectively simulate the experimental trends, at least qualitatively. This was supported by the fact that both alkali plots have approximately the same shape and the model was able to predict an extended lag period prior to acid titration. However, this comparison cannot be used to draw quantitative deductions because the simulation had not been calibrated with the unique conditions of the sample test. Though, the model is capable of such calculation, it was not within the scope of this study to show that the model can simulate actual experimental data, but only to reveal the potential usefulness of modelling applications.

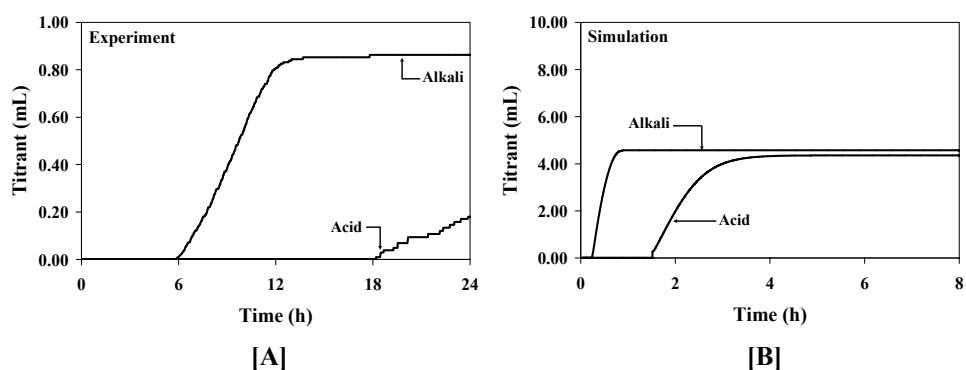


Figure [4-32]: Model Suitability. The simulation suggests that the model is capable of predicting experimental trends.

The Nutrient Study suggested that temperature disturbances could be a possible source of interference for the titrimetric system. This phenomenon was investigated further and it was concluded that temperature does affect the titrating ability of the instrument. However, this result was obtained only after performing additional experiments. The inability to identify and confirm potential sources of experimental error quickly can have negative implications e.g.: inefficient use of time and chemical resources, for the MAIA pre-screening system. However, modelling could reduce the risk of MAIA being side-lined as costly or unreliable, by ensuring that experiments are better planned and performed. Therefore, it was decided to use the mathematical modeling to assess the impact of temperature on the system. This was achieved by simulating two model scenarios. The temperature adjusted model uses temperature as a variable parameter i.e.: temperature varies from 28 to 35°C (refer: equation [4-16]) while the other model case keeps temperature constant. In this way it was possible to highlight the impact of temperature on the titrating ability of the instrument. The results are depicted in **Figure [4-33]**. The figure shows that a varying temperature condition causes the system to titrate a greater volume of titrant than in the case where temperature is held constant. This is evident by the great disparity between the titrant curves (refer: **Figure [4-33], (a)**) shows the quantitative difference in alkaline titration between both models. Further, temperature fluctuation seems to impact on the lag period for acid titration as well (refer: **Figure [4-33], (b)**). Consequently, this result confirmed the earlier experimental deductions and reinforced the idea that modelling is a valuable tool when working with MAIA.

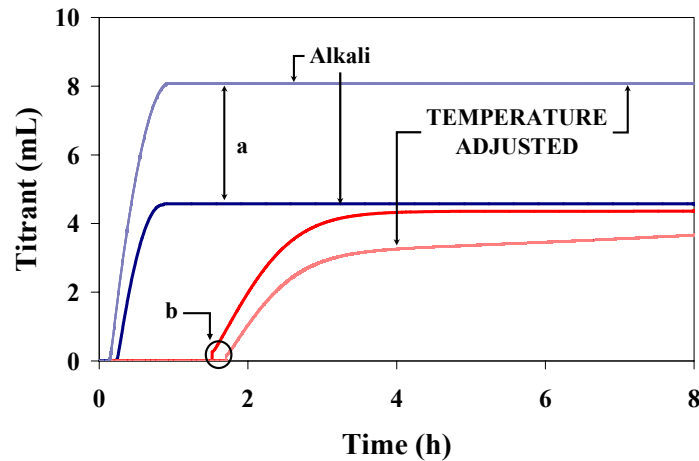


Figure [4-33]: Improvements to the Existing Model. The new model accounted for the impact of temperature to the anaerobic process. The figure compares both model outputs.

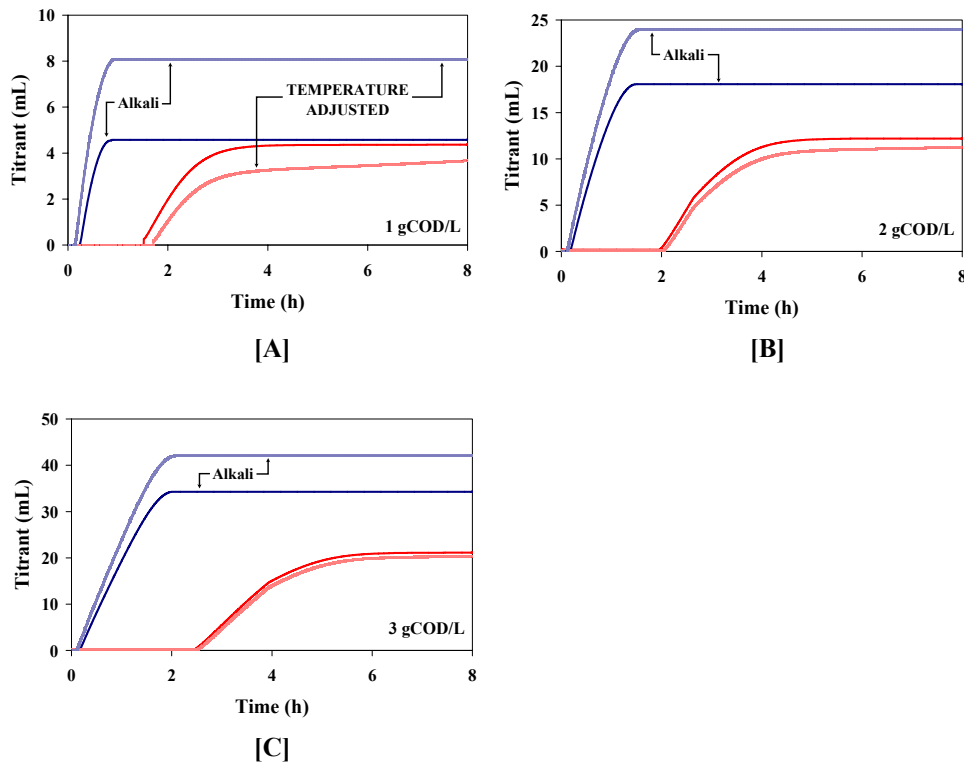


Figure [4-34]: Significance of the Model. The impact of changing substrate concentration on the model output suggested that modelling can enhance the quality of experimental work.

It is also possible that modelling can enhance the quality of experimental work by extending the scope of the research field. Earlier investigation into the impact of temperature on the system did not consider the combined effect of increasing both temperature and substrate concentration on the system. However, modelling this scenario produced this interesting result. Looking specifically at the alkali plots in **Figure [4-34]**, the disparity between the adjusted and unadjusted titration curves reduces from 70 % to 25 % in **Plot [A]** to **Plot [C]** respectively. This suggests that at higher substrate concentrations the effect of temperature is reduced (refer: **Figure [4-35]**).

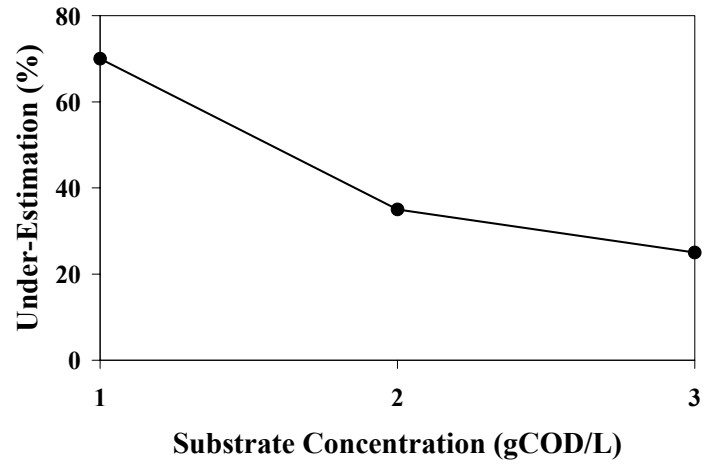


Figure [4-35]: There is a decrease in the error of titration as the substrate concentration and temperature increases.

Despite the fact that this result was not experimentally validated, it does suggest that modelling can be a valuable extension to the pre-screening potential of MAIA.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

It can be concluded from the experimental results of this study that:

1. MAIA has potential to be utilised as an anaerobic pre-screening test. However, the current system design is neither suitably refined enough to perform accurate biodegradability tests on complex substrate systems. Alkaline titration in the hydrolytic and fermentative period of complex substrate breakdown complicates the measurement of microbial activity i.e.: methanogenic, and makes the assessment of biodegradability very difficult. Unless the chemical stoichiometry behind alkaline titration is better understood, the titrimetric method has an intrinsic inability to effectively track the path of methanogenic activity in a short period i.e.: 24 h.
2. The existing system has shown a high susceptibility to temperature changes which can seriously affect the interpretation of experimental results. In addition, the lack of uniform mixing conditions in the headspace has prevented the possibility of accurately relating methane gas production with either alkaline or acid titration rates. Moreover, the limitation of the existing system design to allow for the easy feed of large substrate volumes, after the reactor has been sealed, makes it difficult to internally acclimate the biomass to the optimal temperature set-point prior to a sample test being initiated.
3. It was determined that the use of a nutrient medium did not improve the quality of biodegradation of the textile size effluent but that its buffering property is essential to reduce the impact of interferences, especially temperature and pH.
4. The titrimetric method is comparable to the serum bottle method if a qualitative assessment of toxicity and biodegradability is needed. The titrimetric method also produces results in a much shorter period of time i.e.: 1 d compared to the serum bottle method i.e.: 7 d. However, the current titrimetric method cannot challenge the reliability of the serum bottle method to provide quantitative results. The reliability of the serum bottle method has been established through years of screening tests and MAIA cannot consistently compete with the serum bottle method.
5. The Remigi mathematical model was determined to be too rigorous to effectively simulate the anaerobic processes occurring during the pre-screening study. A new, more simplified model was developed which can assess essential experimental parameters like acidification and methanogenesis while at the same time accounting for temperature and physico-chemical changes.
6. It was possible to compile a more detailed User Manual which could be used to form the basis for future pre-screening protocols (refer: **APPENDIX D**).

Based on the work conducted in this study, the following is recommended:

1. It is imperative that the initial experimental conditions e.g.: gas phase composition; temperature and biomass concentration, be accurately established. The sensitivity of the carbon dioxide/hydrogen carbonate equilibrium necessitates the use of a carbon dioxide and nitrogen gas mixture for the equilibration process. Unless suitable i.e.: reliable gas phase mixing equipment is available, separate cylinders of carbon dioxide and nitrogen gas should not be used to provide the equilibration gas mixture. Further, the duration of the equilibration process should not be reduced to less than 20 min.

The effect of temperature has a pronounced impact upon the titrimetric process and the temperature fluctuations should be reduced to less than 1 °C. It is suggested that the biomass be acclimated internally i.e.: within the reactor, to ensure that the system is at optimum temperature i.e.: 35°C when pre-screening tests are initiated. However, internal acclimation may warrant a reactor and system design modification. The current design does not allow for the easy introduction of large quantities of substrate into the reactor. It is proposed that a refined reactor design with the possible inclusion of a pump be considered to allow the efficient feed and withdrawal of substrate and liquid samples into and out of the reactor respectively. It is proposed that future screening tests should be standardised using set amounts of biomass e.g.: 7 g of biomass, rather than set volumes. This would help increase the level of reproducibility of screening tests conducted with MAIA.

2. More research is conducted to improve the understanding of the chemistry and impact of alkaline titration on the screening process.
3. The use of a nutrient medium, primarily for its buffering properties is recommended for all future study with MAIA. Buffering limited the impact of temperature fluctuation and improved the pH sensitivity of the instrument i.e.: MAIA. Homogenous mixing of the system, especially the gas phase, must be addressed. Non-uniform mixing conditions, in the reactor headspace, could be improved with the implementation of a mechanical stirrer passing through the headspace and into the liquor. The use of acidified water as the displacement fluid or barrier solution is not recommended unless the headspace mixing limitation is eliminated. Analysis of total gas measurements depend heavily on uniformly mixed gas samples. However, the use of a concentrated solution of sodium hydroxide i.e.: 0.5 M, is a better alternative.
4. It is suggested that MAIA be operated only as a multi-channel system when conducting screening tests. Such a system application could greatly improve the speed at which results could be obtained as well as reduce associated experimental costs.
5. The future provision of MAIA should include a software package that includes the mathematical model. The inclusion of the model would allow the user to critically assess experimental findings and improve the quality of experimental study.

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APPENDICES

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APPENDIX A

REAGENT PREPARATION

Acidified Water

$$\text{pH} \quad (\text{HCl})_{\text{aq}} = 2$$

$$\text{Log} \quad [\text{H}^+] = 2$$

$$[\text{H}^+] = 0.01 \text{ mol.L}^{-1}$$



$$[\text{HCl}] = [\text{H}^+]$$

$$[] = n / V$$

$$n = [] \cdot V$$

$$= (0.01 \times 2) \text{ mol.L}^{-1} \times \text{L}$$

$$= 0.02 \text{ mol}$$

$$n = m / M_m$$

$$M_m = 36.46 \text{ g.mol}^{-1}$$

$$m = (0.02 \times 36.46) \text{ mol} \times \text{g.mol}^{-1}$$

$$= 0.7292 \text{ g}$$

Using a 32 % (w/w) solution of HCl

$$0.32 : 0.7292 \text{ g}$$

$$1 : X$$

$$X = 2.279 \text{ g}$$

Hydrochloric Acid Titrant

$$\begin{aligned}
 [\text{HCl}]_{\text{aq}} &= 0.5 \text{ M} \\
 n &= [] \cdot V \\
 &= (0.5 \times 1) \text{ mol.L}^{-1} \times \text{L} \\
 &= 0.5 \text{ mol} \\
 n &= m / M_m \\
 M_m &= 36.46 \text{ g.mol}^{-1} \\
 m &= (0.5 \times 36.46) \text{ mol} \times \text{g.mol}^{-1} \\
 &= 18.23 \text{ g}
 \end{aligned}$$

Using a 32 % (w/w) solution of HCl

$$\begin{aligned}
 0.32 &: 18.23 \text{ g} \\
 1 &: X \\
 X &= 56.96 \text{ g}
 \end{aligned}$$

Sodium Bicarbonate Titrant

Using the McCarthy Plot for a pH change from 6.50 to 6.88 units requires:

$$\begin{aligned}
 [\text{HCO}_3]_{\text{aq}} &= 0.027 \text{ M} \\
 \text{Therefore:} \\
 n &= [] \cdot V \\
 &= (0.027 \times 1) \text{ mol.L}^{-1} \times \text{L} \\
 &= 0.027 \text{ mol}
 \end{aligned}$$

Using Anhydrous Sodium Bicarbonate of 99 % purity.

$$\begin{aligned}
 n &= m / M_m \\
 M_m &= 84 \text{ g.mol}^{-1} \\
 m &= (0.027 \times 84) \text{ mol} \times \text{g.mol}^{-1} \\
 &= 2.27 \text{ g}
 \end{aligned}$$

Acetic Acid Titrant

$$\begin{aligned}[\text{CH}_2\text{COOH}]_{\text{aq}} &= 0.5 \text{ M} \\ n &= [] \cdot V \\ &= (0.5 \times 1) \text{ mol.L}^{-1} \times \text{L} \\ &= 0.5 \text{ mol}\end{aligned}$$

Using Glacial Acetic Acid of 99.7 % purity.

$$\begin{aligned}n &= m / M_m \\ M_m &= 60.05 \text{ g.mol}^{-1} \\ m &= (0.5 \times 60.05) \text{ mol} \times \text{g.mol}^{-1} \\ &= 30.03 \text{ g}\end{aligned}$$

Sodium Acetate Spike

$$\begin{aligned}[\text{C}_2\text{H}_3\text{NaO}_2]_{\text{aq}} &= 2.5 \text{ M} \\ n &= [] \cdot V \\ &= (2.5 \times 1) \text{ mol.L}^{-1} \times \text{L} \\ &= 2.5 \text{ mol}\end{aligned}$$

Using Sodium Acetate Anhydrous of 98 % purity.

$$\begin{aligned}n &= m / M_m \\ M_m &= 82.03 \text{ g.mol}^{-1} \\ m &= (2.5 \times 82.03) \text{ mol} \times \text{g.mol}^{-1} \\ &= 205.08 \text{ g}\end{aligned}$$

Sodium Hydroxide Titrant

$$\begin{aligned}
 [\text{NaOH}]_{\text{aq}} &= 0.5 \text{ M} \\
 n &= [] \cdot V \\
 &= (0.5 \times 1) \text{ mol.L}^{-1} \times \text{L} \\
 &= 0.5 \text{ mol}
 \end{aligned}$$

Using Sodium Hydroxide pellets of 98 % purity.

$$\begin{aligned}
 n &= m / M_m \\
 M_m &= 40.00 \text{ g.mol}^{-1} \\
 m &= (0.5 \times 40.00) \text{ mol} \times \text{g.mol}^{-1} \\
 &= 20.00 \text{ g}
 \end{aligned}$$

Potassium Hydrogen Phosphate Standard

For a $20 \mu\text{g O}_2 \cdot \text{mL}^{-1}$ Standard Solution

$$\begin{aligned}
 20 \text{ mg O}_2 &= 2 \times 10^{-5} \text{ g.O}_2 \cdot \text{mL}^{-1} \\
 &= 20 \mu\text{g} \cdot \text{O}_2 \cdot \text{mL}^{-1}
 \end{aligned}$$

There is 1.176 mg O₂ per 1 mg of KHP

Therefore:

Dissolve 17.01 mg KHP in 1L

Buffer Solution

Table A-1: Buffer Solution Constituents

CHEMICAL	CONCENTRATION
	mg / L
KH ₂ PO ₄	300
K ₂ HPO ₄	350 – 1750
Na ₂ HPO ₄ .12H ₂ O	1000
NH ₄ Cl	360 – 530
NaHCO ₃	1200 - 6000

Nutrient Medium

Table A-2: Nutrient Medium Constituents

STOCK SOLUTION	COMPOSITION	CONCENTRATION
		g / L
S2	RESAZURIN	1.00
S4	CaCl ₂ .2H ₂ O	16.70
	NH ₄ Cl	26.60
	MgCl ₂ .6H ₂ O	120.00
	KCl	86.7
	MnCl ₂ .4H ₂ O	1.33
	CoCl ₂ .6H ₂ O	2.00
	H ₃ BO ₃	0.38
	CuCl ₂ .2H ₂ O	0.18
	Na ₂ MoO ₄ .2H ₂ O	0.17
	ZnCl ₂	0.14
S5	FeCl ₃ .6H ₂ O	503
S6	Na ₂ S.9H ₂ O	500
S7	BIOTIN	0.002
	FOLIC ACID	0.002
	PYRIDOXINE HYDROCHLORIDE	0.010
	RIBOFLAVIN	0.005
	THIAMIN	0.005
	NICOTINIC ACID	0.005
	PANTHOTHENIC ACID	0.005
	p-AMINOBENZOIC ACID	0.005

STEP	METHOD	VOLUME ml	MASS g
1	Add 1 L of de-ionised water to a 2 L Pyrex bottle		
2	Add: S2 S4	1.80 27.0	
3	Top with de-ionised water up to 1.8 L		
4	Boil for 15 min while flushing with OFN gas		
5	Cool to room temperature		
6	Add: S7 S5 S6	18.0 1.80 1.80	
7	Add NaHCO ₃		8.40
8	Flush with OFN gas until pH is 7.1		
9	Autoclave at 121°C for 30 min		
10	Store at 4°C until use		

Preparation of Iron (III) Chloride

In the absence of any Iron (II) Chloride, it was necessary to assess how much Iron (III) Chloride should be used instead.

Required:

$$\begin{aligned}
 m(\text{FeCl}_2 \cdot 4\text{H}_2\text{O}) &= 370 \text{ g} \\
 M_m(\text{Fe}) &= 55.845 \text{ g/mol} \\
 M_m(\text{Cl}) &= 35.453 \text{ g/mol} \\
 M_m(\text{H}) &= 1.0079 \text{ g/mol} \\
 M_m(\text{O}) &= 15.999 \text{ g/mol}
 \end{aligned}$$

$$\begin{aligned}
 x(\text{Fe}) &= 0.2809 \\
 m(\text{Fe}) &= 104 \text{ g}
 \end{aligned}$$

Therefore:

$$\begin{aligned}
 x(\text{Fe [III]}) &= 0.2066 \\
 m(\text{FeCl}_3 \cdot 6\text{H}_2\text{O}) &= 503 \text{ g}
 \end{aligned}$$

APPENDIX B

EQUIPMENT

GAS CHROMATOGRAPH

Type: GOW MAC 350

Column: Haysep D
L = 4 m
ID = 2.2 mm
OD = 3.2 mm

Carrier: Helium

Settings:

Attenuation = 1
Detector Current = 100 mA
Detector Temperature = 25 C

INTEGRATOR

Type: Varian 4270

Settings:

- Switch power on.
- Press the DIALOG key.
- Enter a FILE NAME if desired, and then press ENTER.
- Set TT = 0.01, press ENTER. Set TF = AZ, press ENTER. Set TV = 1, press ENTER.
- Set TT = 0.01, press ENTER. Set TF = CS, press ENTER. Set TV = 0.5, press ENTER.
- Set TT = 0.01, press ENTER. Set TF = PM, press ENTER. Set TV = 1, press ENTER.
- Set TT = 0.01, press ENTER. Set TF = AT, press ENTER. Set TV = 1, press ENTER.
- Set TT = 5, press ENTER. Set TF = ER, press ENTER. Set TV = 1, press ENTER.
- At the next prompt, simply press ENTER to exit.
- Press ENTER, to END DIALOG.
- Press PRINT FILE to display programme code.

PUMP

Type: Watson Marlow

EQUIPMENT CALIBRATION**GAS CHROMATOGRAPH**

Requirements:

- Sampling line with attached septum and pressure gauge.
- Retort stand.
- Gas-lock syringe.
- Pure grade (Nitrogen, Methane, Carbon Dioxide).
- Thermometer.

Procedure:

- Set up the sampling line.
- Make sure that the integrator and GC settings are correct.
- Check the septum seal on the sampling line.
- Look at the reading on the pressure gauge and ensure that there is no fluctuation.
- Record the ambient temperature and the gauge pressure.
- Use the syringe to draw out a sample of gas and record the volume taken.
- Lock the gas sample in the syringe until the point of injection.
- Inject gas sample into the GC and wait for integrator analysis.
- Record the retention time of the gas with the highest area percentage.
- Perform steps 1 – 9 for all the other sample gases.
- Perform steps 1 – 8 for gas with lowest retention time i.e.: nitrogen.
- Perform steps 1 – 8 for nitrogen for three different sample volumes e.g.: 0.2, 0.3, 0.4 μL .
- For each sample volume, injections should continue until 3 peak area values coincide within 2 % of their average value.
- Once step 13 has been successfully conducted for nitrogen, continue the calibration with the gas that has the next highest retention time i.e.: methane.

Sample Calculation:

Table B-1: Obtaining Peak Area

Type	Nitrogen			
Injection	Volume μL	Gauge Pressure kPa	% Area	Peak Area
1	0.3	750	99.91	319 834
2	0.3	700	99.14	309 420
3	0.3	700	99.86	314 019
4	0.4	700	99.74	403 419
5	0.4	700	99.77	403 598
6	0.4	700	99.91	396 574
7	0.2	600	98.08	189 024
8	0.2	550	100.00	167 790
9	0.2	550	99.64	160 233

Average Peak Area

$$\begin{aligned} \text{Sum} &= (319\,834 + 309\,420 + 314\,019) = 943\,273 \\ \text{Ave} &= (943\,273 / 3) = 314\,424 \end{aligned}$$

Deviation from Average

$$\begin{aligned} \text{Dev}_1 &= [(319\,834 - 314\,424) / (314\,424)] \times 100 = 1.7\% \\ \text{Dev}_2 &= [(309\,420 - 314\,424) / (314\,424)] \times 100 = 1.6\% \\ \text{Dev}_3 &= [(314\,019 - 314\,424) / (314\,424)] \times 100 = 0.1\% \end{aligned}$$

Moles of Gas Sampled

$$Z = (B.P / R.T) + 1$$

$$P.V = Z.n.R.T$$

$$N = (P.V) / (Z.R.T)$$

where:

$$\begin{aligned} B &= \text{Virial Coefficient} \\ P &= \text{Total Pressure} = \text{Gauge} + \text{Atmospheric} \\ R &= \text{Ideal Gas Constant} \\ T &= \text{Temperature} \\ V &= \text{Gas Volume} \\ n &= \text{Number of Moles} \end{aligned}$$

Virial Coefficient Models

- The estimation of (B) values were obtained from **The Virial Coefficients of Pure Gases and Mixtures**
- Only Class One Models were chosen. The error in the (B) values for this class, are less than 2 %.

Table B-2: Virial Coefficient Models

Gas	Model	Reference
Methane	1	Michels, A. (1935)
	2	Schamp, H.W. (1958)
	3	Schafer, K. (1969)
Nitrogen	1	Michels, A. (1936)
	2	Michels, A. (1951)
	3	Gunn, R.D. (1958)
Carbon Dioxide	1	Michels, A. (1935)
	2	Butcher, E.G. (1964)
	3	Dadson, R.S. (1967)

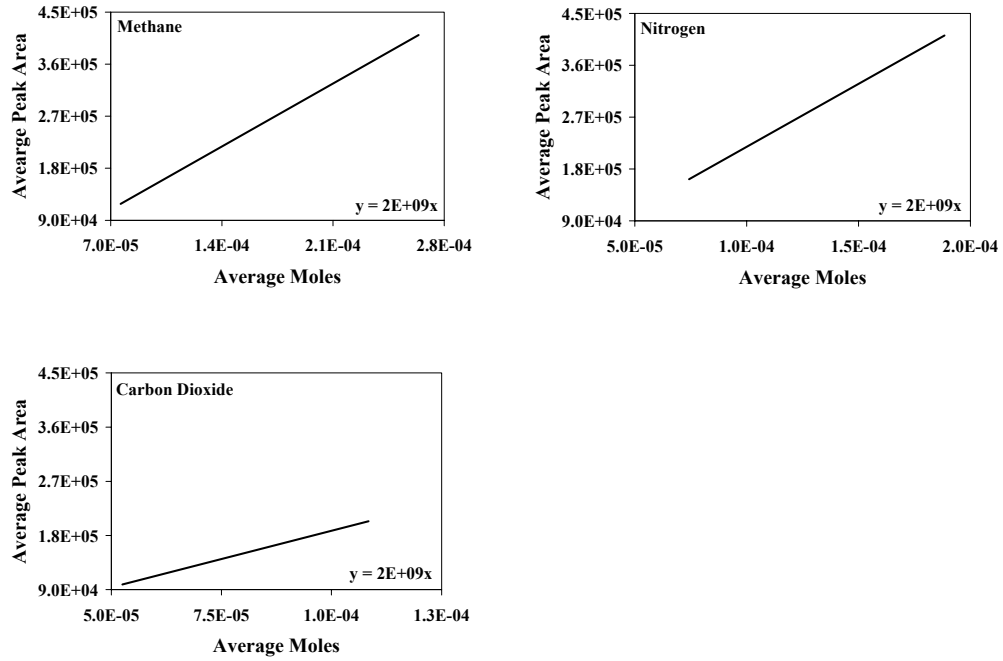


Figure [B-1]: Chromatograph Calibration Curves

PUMP

Table B-3: Pump Calibration Data

Setting	Time			Flowrate			
	1	2	3	1	2	3	Average
rpm	s	s	s	mL.s ⁻¹	mL.s ⁻¹	mL.s ⁻¹	mL.s ⁻¹
0	0	0	0	0.0000	0.0000	0.0000	0.0000
10	363	380	384	0.0138	0.0132	0.0130	0.0133
16	246	214	241	0.0203	0.0233	0.0207	0.0215
25	144	143	142	0.0347	0.0350	0.0352	0.0350
31	125	121	123	0.0401	0.0415	0.0407	0.0407
40	83	82	82	0.0600	0.0608	0.0608	0.0605
46	77	76	74	0.0646	0.0656	0.0678	0.0660
55	68	67	66	0.0731	0.0744	0.0758	0.0744
61	64	62	63	0.0779	0.0801	0.0794	0.0791

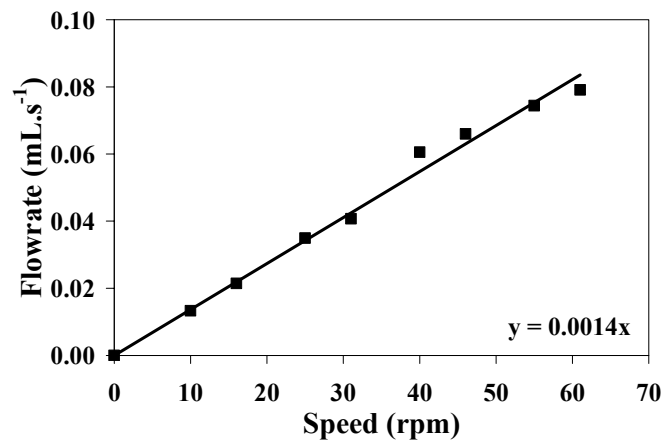
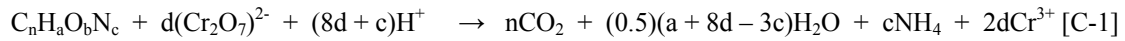
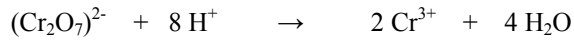


Figure [B-2]: Pump Calibration Curve

APPENDIX C

Estimating Organic Carbon Content

The theoretical COD of a compound can be determined by using a simple equation:



where:

$$d = (2/3)n + (1/6)a - (1/3)b - (1/2)c \quad [\text{C-2}]$$

$$\text{COD} = (3/2)d \quad [\text{C-3}]$$

This calculation was used to determine the theoretical COD of certain mass balance components. An example of this calculation is presented and the results summarised in **Table [C-2]**.

For this calculation the molecular mass of the reacting species must be known. Some of those used are shown in **Table [C-1]**.

Table C-1: Molecular Formula and Weights

Compound	Formula	Molecular Mass g/mol
Biomass	$\text{C}_5\text{H}_7\text{O}_2\text{N}$	113
Starch	$\text{C}_6\text{H}_{10}\text{O}_5$	102
Acetate	CH_3COONa	82

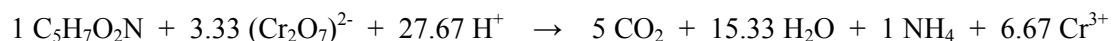
Biomass:

$$\begin{aligned} n &= 5 \\ a &= 7 \\ b &= 2 \\ c &= 1 \end{aligned}$$

Substituting into the equation [C-2]:

$$d = 3.33 \text{ moles of O}_2$$

Substituting into equation [C-1]:



Therefore, using equation [C-3]:

$$\begin{aligned} 1 \text{ mole C}_5\text{H}_7\text{O}_2\text{N} &= [(3/2) d] \\ &= 5 \text{ moles of O}_2 \end{aligned}$$

but, 1 mole C₅H₇O₂N = 113 g
 1 mole O₂ = 32 g

This means that for the complete oxidation of 113 g of C₅H₇O₂N, 160 g of oxygen is required.

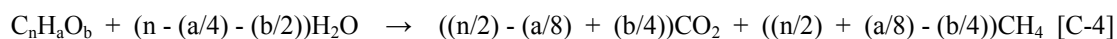
Thus: 1 g C₅H₇O₂N requires 1.416 gO₂ or is equivalent to 1.416 gCOD.

Table C-2: Theoretical Estimation of Organic Carbon Content

Compound	Formula	n	a	b	c	d	gCOD
Biomass	C ₅ H ₇ O ₂ N	5	7	2	1	3.33	1.416
Starch	C ₆ H ₁₀ O ₅	6	10	5	0	4.00	1.882
Acetate	CH ₃ COONa	2	3	2	1	0.67	0.392

Determining Biogas Composition

When organic material is degraded anaerobically, the end result is carbon in its most oxidized form i.e.: carbon dioxide and in its most reduced form i.e.: methane. The ratio between these gases depends on the oxidation state of the carbon present in the organic material. The earliest definition of the stoichiometry of anaerobic digestion was presented by (Buswell, 1939).



The equation reflects algebraically that the higher the oxidation state of the carbon in the organic substrate, the lower the proportion of methane in the biogas. **Figure [C-1]**, is a graphical representation of [C-4].

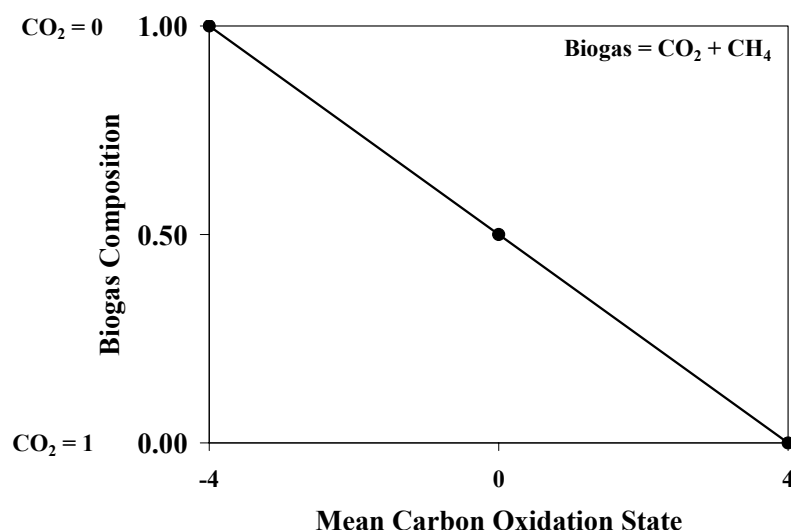


Figure [C-1]: The Buswell Equation.

Calculation

Starch:

$$\begin{array}{lcl} n & = & 6 \\ a & = & 10 \\ b & = & 5 \end{array}$$

Therefore, equation [C-4] is:



This implies that the biogas produced from the anaerobic degradation of starch will be 50 % methane gas.

APPENDIX D

STAGE ONE

The biomass sampled from an anaerobic digester may have to be stored before it can be concentrated. Therefore, the raw sludge should be kept in the dark, at 4°C with no substrate.

Biomass Concentration

- Remove raw sludge sample from 4°C fridge.
- Filter the sludge using a sieve to remove any large particulates.
- Centrifuge for 30 min at 3 000 rpm.
- Concentrate sludge by removing as much of supernatant as possible.

Biomass Acclimation

- Remove concentrated biomass from 4°C fridge.
- Add biomass to a 2 L beaker.
- Gasify contents of beaker for 10 min with 50 % nitrogen/carbon dioxide mixture.
- Seal beaker with Parafilm.
- Place beaker on a stirrer unit in a temperature controlled room (37°C).
- Set stirrer motor on low mixing speed i.e.: fast enough to allow for uniform mixing.
- Acclimate for at least 24 h.

Effluent Preparation

Depending on the physical properties of the effluent being tested e.g.: presence of solids, the test substrate/effluent may have to be prepared before use. In this study only the size effluent needed to be specially prepared.

Textile Size

- Remove effluent from 4°C fridge.
- Centrifuge effluent at 3000 rpm for 30 min.
- Place 500 mL (each) of the pellet and supernatant respectively into a 1L vessel.

STAGE TWO

Start-up

- Locate MAIA main socket. Check that the amplifier i.e.: white box and computer plug are plugged in.
- Check that the amplifier is connected to MAIA via the black wire and to the computer via the white wire.
- Switch on the MAIA main switch: six LED's on the front should light up.
- Switch on the computer and in the (DOS) environment start the programme i.e.: (C:\anita\anita_31.exe)
- The screen should read 'serial port initialised' and 'port activate'.
- Check that the response of modules 4060 and 4017 display on the screen as (01) and (02) respectively.
- A blue presentation screen should appear, follow the instruction to the main menu.

Preparation

- Ensure that there is sufficient gas in the 50 % nitrogen/carbon dioxide cylinder.
- Check that you have the buffer solutions (pH 4 and 7).
- Remove the effluent from cold room and bring to room temperature.
- Attach the heating tube to the bioreactor and switch on water bath pump.
- Ensure that the water bath temperature has been maintained between 35 and 40 °C.
- Ensure there is sufficient acidified water (pH = 2) for the displacement bottle. It is also possible to use a concentrated solution of sodium hydroxide (0.5 M) as the displacement fluid. The latter solution will dissolve carbon dioxide gas and will consequently prevent total gas determinations.
- Remove biomass from temperature controlled room.
- Measure out biomass, effluent, sodium acetate spike and distilled water volumes to be placed into the reactor.
- Place a stirrer bar into the reactor along with the biomass, effluent and distilled water. Turn the stirrer unit on (low speed).

Valves Statement

The parameters of the control system which manage the valve statement were kept constant i.e.: the frequency of measurements at 60 s; valve opening time and minimum closing time at 0,80 s and 0,10 s respectively.

- To access the valve statement menu, press (5) on the main menu page.
- Locate the cursor at the bottom left of the screen. If the process parameters are correct press (Y) and return to the main menu, without modifications, otherwise press (N) and the cursor will move onto the first line.
- Type in the frequency for data recording on the (DAT file), the opening time for the valves and the closing time for the valves. After each value press ENTER.
- Confirm by pressing (Y) and return to the main menu page. The new process parameters will be recorded in the VALVES.DAT file.

Valves Calibration

The calibration involves setting the flowrate of the acid and base dosing valves. From the information about the volume of titrant dosed per pulse it is possible to determine the total volume dosed into the reactor. Prior to any test being conducted, air bubbles have to be removed from the line connecting the reservoir tanks with their respective valves. This is

achieved by selecting either the 'open acid' or 'open base' option from the valve calibration menu screen.

The volume delivered by the valve for a set number of pulses must be entered following the software instructions. This entails placing a measuring cylinder underneath the delivery line of each valve and waiting for a set volume of titrant to be dosed.

- Press (4) from the main menu to activate the valve calibration menu.
- Check that both titrant bottles (HCl and NaOH) are full and connected correctly. Ensure that there are no air bubbles present in these connection lines and that the level of atmospheric pressure in the Mariotte bottle i.e.: the level of constant head, is correctly set i.e.: an air bubble should be visible at the outlet of the capillary tube.
- Ensure that the E2 and E1 signal wire is plugged to the left and right valve respectively.
- Place a beaker under the tube connected to the acid valve and press (1), the valve will open and acid should flow into the beaker. Wait till the capillary in the HCl bottle is empty (this is to have a fixed liquid level above the valve).
- Press SPACE BAR to close the valve.
- Perform the same procedure for the base valve by reading the options in the calibration menu.
- Press (4) to activate the acid valve calibration. The screen should read 'acid dosage calibration'.
- Press any key and select the number of repetitions required e.g.: 3. Place a graduated cylinder under the tube (the tube should be connected to a syringe needle).
- Press the SPACE BAR and the valve should start to dose acid in small doses (hits) producing a characteristic hit sound.
- When the volume in the cylinder reaches a fixed value e.g.: 3 mL, press SPACE BAR and the dosing will stop. The screen should display a (?) symbol.
- Enter in the volume dosed until that moment and press ENTER. Repeat for each repetition.
- Press (C) to confirm the calibration.
- Insert the needle tipped tubes into the septa of lid 1 and close the lid on the reactor.

Probe Calibration

The pH electrode is calibrated, using pH 4 and pH 7 buffer solutions. The software shows and records the probe signal in millivolts. Usually the pH probe is immersed into the lower pH buffer solution and then into the higher one. It is necessary to wait until a stable signal is obtained before proceeding to a new buffer solution. The temperature probe is calibrated by placing the instrument into beakers containing cold water (4°C) and hot water (60°C) respectively. The software displays and records the probe signal in millivolts.

The Temperature Probe

- Press (3) from the main menu to activate the temperature calibration menu.
- Select option (2) to calibrate the probe which goes into the beaker (PT100A).
- Wipe the probe and place it in a beaker containing cold water. Press SPACE BAR and the corresponding voltage value will be displayed.
- Wait till the value is stable in a restricted range.
- Immerse a mercury thermometer into the beaker, read of the temperature and input the value into the computer and press ENTER.
- Repeat the procedure for hot water.
- Press (C) to confirm and you will return to the temperature calibration menu.
- Place the probe into the reactor and fasten lid 2 shut.

The pH-Probe

- Press (2) from the main menu to view the pH calibration function.
- Check the safe lock device of the pH-probe is closed i.e.: the hole under the safe lock is not visible, and remove the pH-probe cap.
- Rinse the probe with distilled water and dry with towelling paper.
- Immerse the probe into buffer solution of pH = 7.
- Press SPACE BAR and the corresponding voltage will be displayed. Wait till the value is stable in a restricted range (don't expect it to be constant but merely notice that no increasing or decreasing trend is visible).
- Press SPACE BAR and enter the value of the buffer, 7 then press ENTER.
- Rinse and dry the probe and repeat the procedure for buffer solution of pH = 4.
- Press (C) to confirm the inputs and return to the main menu.
- Insert the pH-probe into the reactor and seal lid 3.

Set Point Adjustment

- Press (1) from the main menu.
- Press SPACE BAR to change the values listed or CTRL-U to abort.
- Type in the pH-value which has to be kept constant during the test and press ENTER.
- Type in the range of pH variability (usually 0.020) and press ENTER. During the test MAIA will dose acid when the pH is above the fixed pH plus the variability range. To start a test at pH = 6.880 with a variability of 0.020, the fixed pH must be 6.860. Acid is dosed when the pH is above 6.880.
- The other values are not of interest for anaerobic applications, so they can be skipped by pressing ENTER.
- Press (Y) to confirm and return to the main menu.

Pre-test Preparation

- Press (6) from the main menu. The test option menu will be displayed.
- Connect the gas tube to the bioreactor and open the gas valve.
- Ensure that the stirrer unit is operating and that the gas is bubbling in slowly.
- Keep the outlet line from the reactor connected to the displacement bottle but do not close lid 4, thus allowing gas to pass into and out the displacement bottle.
- Press (1) from the test option menu to monitor the pH in the reactor.
- Observe the pH, it is expected to decrease because of CO₂ dissolution.
- Continue the gas bubbling for between 20 to 45 min until the pH reaches a stable value.
- Close the gas valve, lid 5 on the reactor and lid 4 on the displacement bottle.
- If the pH after gas sparging settles at a value below the set-point, use the McCarthy Plot to determine the mass of sodium bicarbonate to add to raise the pH. Dissolve the bicarbonate in a small volume of distilled water e.g.: 10 ml and inject it into the reactor using a syringe.
- Draw a sample of gas for GC analysis and record the result.
- Using a syringe, inject the test compound spike (s) e.g.: sodium acetate into the reactor through the septa on lid 1.
- Press CTRL-U to stop the pH monitoring and return to the test option menu.
- Select (2) from the menu to begin the test.

Test Monitoring

- At regular intervals e.g.: every 2 h or sooner depending on rate of activity, take gas samples from the reactor using a gas-lock syringe.
- Record the displacement of acidified water at the moment of gas sampling.
- During the test, it is important that the operator constantly monitor the instrument for problems. The apparatus is still in a prototype stage of development and it is possible that valves may clog; air may enter tubes; mixing may stop and so forth.

Shut-down

- Press CTRL-U to end the test.
- Remove all probes and tubes from the reactor.
- Rinse the pH-probe with distilled water and cap it. Make sure to place some potassium chloride solution into the cap before passing it over the probe.
- Rinse the temperature probe and dry it.
- All (DAT) files for the pH monitoring are named (N0) and for the tests (N1). They are saved in a directory named with the test date.

APPENDIX E

Temperature Interference

The determination of activity can be distorted by errors resulting from the impact of certain system interferences (refer: **Textile Study**). The Effluent Study seemed to suggest that temperature fluctuations at the start of the tests had a significant impact on the titrimetric ability of the instrument (refer: **Nutrient Study**). It was decided that this problem warranted further investigation.

The effect of temperature was investigated experimentally and using a mathematical model. Two experimental cases were considered: a buffered and unbuffered scenario. These experiments aimed to:

- assess the influence of temperature on the pH condition;
- evaluate the benefit of using a buffer to limit the impact of temperature fluctuations on the pH condition.

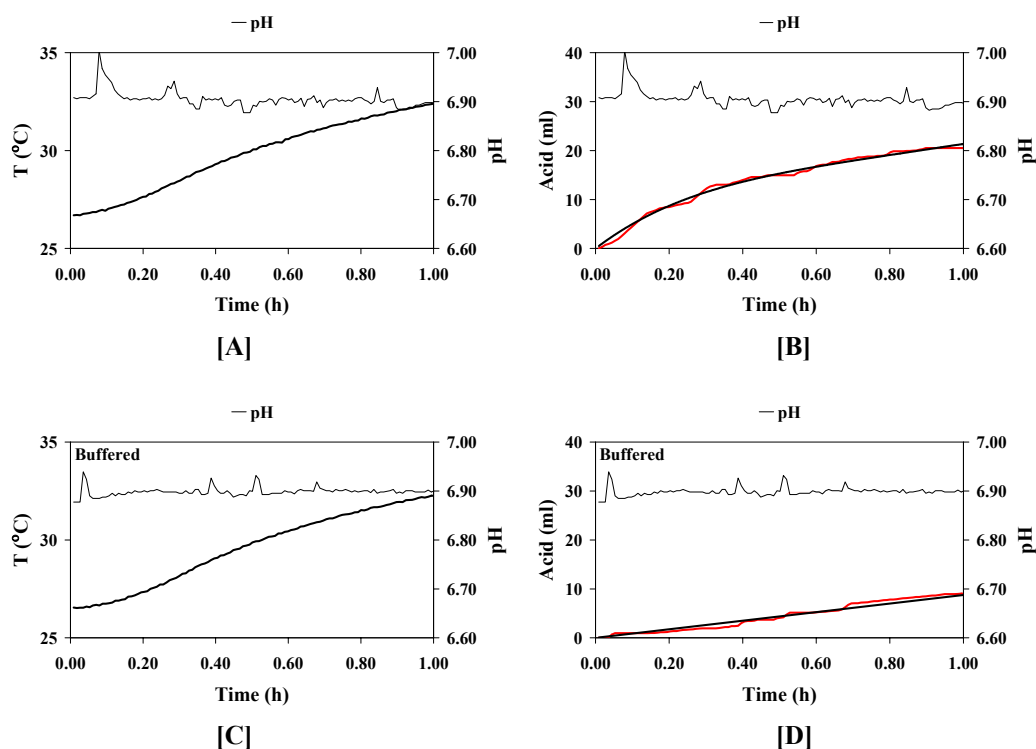


Figure [E-1]: Eliminating Temperature Interference. The figure shows how temperature interferences can be limited with the use of a nutrient medium.

It is clear from Plot B in **Figure [E-1]** that temperature significantly affects the ability of MAIA to track biological activity in the unbuffered case. The acid titration curve should ideally be a straight line (no slope) suggesting no external interference instead it seems to simulate the trend of the temperature curve in Plot A. The gradient of the acid titration curve begins sharply and then seems to settle towards a constant trend. The sharp increase in gradient is indicative of excessive titration of acid associated with the temperature change. Contrarily, buffering seems to limit the influence of temperature on the pH condition and consequently on the instrument's ability to evaluate activity (refer: **Plots C and D, Figure [E-1]**). Here, the gradient of acid titration curve increases gradually with an increase

in temperature. However, it is evident that buffering contributes to a more stable pH condition. The implication for an operator would be to neglect the temperature interference provided the titration by MAIA is not excessive with an increase in temperature.

Diffusion Limitation

The original MAIA system design assumed that efficient mixing of the liquor would be sufficient to promote a well mixed gas phase condition in the reactor headspace. Although, some convective transport patterns are initiated at the gas-liquid interface, this was insufficient to ensure uniform mixing conditions within the headspace. Therefore, some form of mechanical mixing is needed in the headspace to improve the sampling conditions. In an effort to support this view, a simple diffusive model based on Fick’s Law was considered. It was proposed that the model would:

- estimate the diffusion coefficient for the methane-carbon dioxide system;
- predict the methane flux at different positions along the length of the diffusion path;
- assess the impact of poor mixing upon the composition of gas samples taken at different positions along this path.

Diffusivity was predicted using the method outlined in **Mass Transfer: Fundamentals and Applications**.

$$D_{AB} = \frac{1.858 \times 10^{-27} \cdot T^{\frac{3}{2}}}{P \cdot \sigma_{AB}^2 \cdot \Omega_D} \left(\frac{1}{M_A} + \frac{1}{M_B} \right)^{\frac{1}{2}} \quad [E-1]$$

where:

- D_{AB} = Diffusivity [m²/s]
- T = Temperature [K]
- M = Molecular weight [g/mol]
- P = Pressure [atm]
- σ_{AB} = Collision Diameter [m]
- Ω_D = Collision integral

Calculation

Table E-1: Estimation of the Diffusion Coefficient

Parameter	Value	Unit
T	310	K
P	1	atm
M _A (CH ₄)	16	g/mol
M _B (CO ₂)	44	g/mol
σ_{AB}	3.8495 x 10 ⁻¹⁰	m
Ω_D	1.116	-
D _{AB}	1.770 x 10 ⁻⁵	m ² .s ⁻¹

However, it was virtually impossible to provide the boundary conditions necessary to perform the flux calculation. Further, without any experimental data, it was not possible to place the value of the diffusion coefficient in perspective i.e.: there is no way to assess if the value is too high or too low. Nevertheless, the numerical order of the diffusivity result suggested that the system needed to be modified, but it was not within the scope of this study to institute

such a change. However, it is reasonable to assume that mechanical mixing will reduce if not eliminate the diffusion limitation of the system.

APPENDIX F

The Data and Sample Calculations presented here are those of Test 1 of the Textile Size Effluent Study.

Titration Data

Time h	pH units	Acid mL	Alkali mL
0.00	6.8570	0.0000	0.0000
0.02	6.8526	0.0000	0.0000
0.03	6.8526	0.0000	0.0000
0.05	6.8526	0.0000	0.0000
0.07	6.8526	0.0000	0.0000
0.09	6.8548	0.0000	0.0000
0.10	6.8548	0.0000	0.0000
0.12	6.8526	0.0000	0.0000
0.14	6.8526	0.0000	0.0000
0.16	6.8526	0.0000	0.0000
0.17	6.8526	0.0000	0.0000
0.19	6.8503	0.0000	0.0000
0.21	6.8548	0.0000	0.0000
0.22	6.8548	0.0000	0.0000
0.24	6.8526	0.0000	0.0000
0.26	6.8548	0.0000	0.0000
0.28	6.8548	0.0000	0.0000
0.29	6.8537	0.0000	0.0000
0.31	6.8548	0.0000	0.0000
0.33	6.8548	0.0000	0.0000
0.35	6.8548	0.0000	0.0000
0.36	6.8570	0.0000	0.0000
0.38	6.8593	0.0000	0.0000
0.40	6.8604	0.0000	0.0000
0.41	6.8615	0.0000	0.0000
0.43	6.8615	0.0000	0.0000
0.45	6.8615	0.0000	0.0000
0.47	6.8615	0.0000	0.0000
0.48	6.8615	0.0000	0.0000
0.50	6.8637	0.0000	0.0000
0.52	6.8637	0.0000	0.0000
0.54	6.8637	0.0000	0.0000
0.55	6.8637	0.0000	0.0000
0.57	6.8637	0.0000	0.0000
0.59	6.8615	0.0000	0.0000
0.60	6.8637	0.0000	0.0000
0.62	6.8660	0.0000	0.0000
0.64	6.8660	0.0000	0.0000
0.66	6.8682	0.0000	0.0000
0.67	6.8660	0.0000	0.0000
0.69	6.8649	0.0000	0.0000
0.71	6.8637	0.0000	0.0000
0.73	6.8637	0.0000	0.0000
0.74	6.8626	0.0000	0.0000
0.76	6.8637	0.0000	0.0000
0.78	6.8637	0.0000	0.0000
0.79	6.8626	0.0000	0.0000
0.81	6.8637	0.0000	0.0000
0.83	6.8637	0.0000	0.0000
0.85	6.8615	0.0000	0.0000
0.86	6.8615	0.0000	0.0000
0.88	6.8637	0.0000	0.0000
0.90	6.8626	0.0000	0.0000

Time h	pH units	Acid mL	Alkali mL
0.91	6.8615	0.0000	0.0000
0.93	6.8615	0.0000	0.0000
0.95	6.8637	0.0000	0.0000
0.97	6.8637	0.0000	0.0000
0.98	6.8637	0.0000	0.0000
1.00	6.8637	0.0000	0.0000
1.02	6.8637	0.0000	0.0000
1.04	6.8615	0.0000	0.0000
1.05	6.8615	0.0000	0.0000
1.07	6.8615	0.0000	0.0000
1.09	6.8637	0.0000	0.0000
1.10	6.8637	0.0000	0.0000
1.12	6.8615	0.0000	0.0000
1.14	6.8660	0.0000	0.0000
1.16	6.8660	0.0000	0.0000
1.17	6.8660	0.0000	0.0000
1.19	6.8615	0.0000	0.0000
1.21	6.8615	0.0000	0.0000
1.23	6.8615	0.0000	0.0000
1.24	6.8593	0.0000	0.0000
1.26	6.8593	0.0000	0.0000
1.28	6.8593	0.0000	0.0000
1.29	6.8615	0.0000	0.0000
1.31	6.8615	0.0000	0.0000
1.33	6.8593	0.0000	0.0000
1.35	6.8615	0.0000	0.0000
1.36	6.8593	0.0000	0.0000
1.38	6.8615	0.0000	0.0000
1.40	6.8615	0.0000	0.0000
1.42	6.8615	0.0000	0.0000
1.43	6.8593	0.0000	0.0000
1.45	6.8615	0.0000	0.0000
1.47	6.8615	0.0000	0.0000
1.48	6.8615	0.0000	0.0000
1.50	6.8615	0.0000	0.0000
1.52	6.8615	0.0000	0.0000
1.54	6.8615	0.0000	0.0000
1.55	6.8604	0.0000	0.0000
1.57	6.8615	0.0000	0.0000
1.59	6.8615	0.0000	0.0000
1.61	6.8615	0.0000	0.0000
1.62	6.8615	0.0000	0.0000
1.64	6.8593	0.0000	0.0000
1.66	6.8593	0.0000	0.0000
1.67	6.8593	0.0000	0.0000
1.69	6.8593	0.0000	0.0000
1.71	6.8593	0.0000	0.0000
1.73	6.8593	0.0000	0.0000
1.74	6.8593	0.0000	0.0000
1.76	6.8593	0.0000	0.0000
1.78	6.8593	0.0000	0.0000
1.80	6.8593	0.0000	0.0000
1.81	6.8570	0.0000	0.0000

DATA AND SAMPLE CALCULATIONS

Time	pH	Acid	Alkali
h	units	mL	mL
1.83	6.8593	0.0000	0.0000
1.85	6.8582	0.0000	0.0000
1.86	6.8593	0.0000	0.0000
1.88	6.8570	0.0000	0.0000
1.90	6.8582	0.0000	0.0000
1.92	6.8570	0.0000	0.0000
1.93	6.8570	0.0000	0.0000
1.95	6.8582	0.0000	0.0000
1.97	6.8570	0.0000	0.0000
1.99	6.8593	0.0000	0.0000
2.00	6.8615	0.0000	0.0000
2.02	6.8637	0.0000	0.0000
2.04	6.8637	0.0000	0.0000
2.05	6.8660	0.0000	0.0000
2.07	6.8660	0.0000	0.0000
2.09	6.8660	0.0000	0.0000
2.11	6.8682	0.0000	0.0000
2.12	6.8682	0.0000	0.0000
2.14	6.8682	0.0000	0.0000
2.16	6.8682	0.0000	0.0000
2.18	6.8682	0.0000	0.0000
2.19	6.8693	0.0000	0.0000
2.21	6.8704	0.0000	0.0000
2.23	6.8704	0.0000	0.0000
2.24	6.8704	0.0000	0.0000
2.26	6.8704	0.0000	0.0000
2.28	6.8704	0.0000	0.0000
2.30	6.8704	0.0000	0.0000
2.31	6.8704	0.0000	0.0000
2.33	6.8727	0.0000	0.0000
2.35	6.8716	0.0000	0.0000
2.36	6.8727	0.0000	0.0000
2.38	6.8727	0.0000	0.0000
2.40	6.8727	0.0000	0.0000
2.42	6.8727	0.0000	0.0000
2.43	6.8727	0.0000	0.0000
2.45	6.8727	0.0000	0.0000
2.47	6.8704	0.0000	0.0000
2.49	6.8704	0.0000	0.0000
2.50	6.8704	0.0000	0.0000
2.52	6.8727	0.0000	0.0000
2.54	6.8727	0.0000	0.0000
2.55	6.8749	0.0000	0.0000
2.57	6.8749	0.0000	0.0000
2.59	6.8727	0.0000	0.0000
2.61	6.8749	0.0000	0.0000
2.62	6.8749	0.0000	0.0000
2.64	6.8749	0.0000	0.0000
2.66	6.8749	0.0000	0.0000
2.68	6.8749	0.0000	0.0000
2.69	6.8749	0.0000	0.0000
2.71	6.8727	0.0000	0.0000
2.73	6.8749	0.0000	0.0000

Time	pH	Acid	Alkali
h	units	mL	mL
2.74	6.8749	0.0000	0.0000
2.76	6.8727	0.0000	0.0000
2.78	6.8727	0.0000	0.0000
2.80	6.8727	0.0000	0.0000
2.81	6.8727	0.0000	0.0000
2.83	6.8727	0.0000	0.0000
2.85	6.8727	0.0000	0.0000
2.87	6.8727	0.0000	0.0000
2.88	6.8727	0.0000	0.0000
2.90	6.8727	0.0000	0.0000
2.92	6.8704	0.0000	0.0000
2.93	6.8704	0.0000	0.0000
2.95	6.8704	0.0000	0.0000
2.97	6.8727	0.0000	0.0000
2.99	6.8727	0.0000	0.0000
3.00	6.8727	0.0000	0.0000
3.02	6.8727	0.0000	0.0000
3.04	6.8704	0.0000	0.0000
3.06	6.8704	0.0000	0.0000
3.07	6.8704	0.0000	0.0000
3.09	6.8704	0.0000	0.0000
3.11	6.8727	0.0000	0.0000
3.12	6.8727	0.0000	0.0000
3.14	6.8727	0.0000	0.0000
3.16	6.8704	0.0000	0.0000
3.18	6.8704	0.0000	0.0000
3.19	6.8704	0.0000	0.0000
3.21	6.8727	0.0000	0.0000
3.23	6.8693	0.0000	0.0000
3.25	6.8704	0.0000	0.0000
3.26	6.8727	0.0000	0.0000
3.28	6.8704	0.0000	0.0000
3.30	6.8704	0.0000	0.0000
3.31	6.8704	0.0000	0.0000
3.33	6.8704	0.0000	0.0000
3.35	6.8682	0.0000	0.0000
3.37	6.8682	0.0000	0.0000
3.38	6.8682	0.0000	0.0000
3.40	6.8704	0.0000	0.0000
3.42	6.8682	0.0000	0.0000
3.44	6.8682	0.0000	0.0000
3.45	6.8682	0.0000	0.0000
3.47	6.8682	0.0000	0.0000
3.49	6.8682	0.0000	0.0000
3.50	6.8682	0.0000	0.0000
3.52	6.8693	0.0000	0.0000
3.54	6.8682	0.0000	0.0000
3.56	6.8682	0.0000	0.0000
3.57	6.8693	0.0000	0.0000
3.59	6.8682	0.0000	0.0000
3.61	6.8682	0.0000	0.0000
3.62	6.8682	0.0000	0.0000
3.64	6.8682	0.0000	0.0000

DATA AND SAMPLE CALCULATIONS

Time	pH	Acid	Alkali
h	units	mL	mL
3.66	6.8704	0.0000	0.0000
3.68	6.8704	0.0000	0.0000
3.69	6.8704	0.0000	0.0000
3.71	6.8682	0.0000	0.0000
3.73	6.8682	0.0000	0.0000
3.75	6.8682	0.0000	0.0000
3.76	6.8660	0.0000	0.0000
3.78	6.8682	0.0000	0.0000
3.80	6.8660	0.0000	0.0000
3.81	6.8660	0.0000	0.0000
3.83	6.8660	0.0000	0.0000
3.85	6.8660	0.0000	0.0000
3.87	6.8649	0.0000	0.0000
3.88	6.8660	0.0000	0.0000
3.90	6.8660	0.0000	0.0000
3.92	6.8660	0.0000	0.0000
3.94	6.8660	0.0000	0.0000
3.95	6.8660	0.0000	0.0000
3.97	6.8637	0.0000	0.0000
3.99	6.8660	0.0000	0.0000
4.00	6.8637	0.0000	0.0000
4.02	6.8637	0.0000	0.0000
4.04	6.8637	0.0000	0.0000
4.06	6.8615	0.0000	0.0000
4.07	6.8637	0.0000	0.0000
4.09	6.8637	0.0000	0.0000
4.11	6.8615	0.0000	0.0000
4.13	6.8615	0.0000	0.0000
4.14	6.8615	0.0000	0.0000
4.16	6.8626	0.0000	0.0000
4.18	6.8615	0.0000	0.0000
4.19	6.8615	0.0000	0.0000
4.21	6.8637	0.0000	0.0000
4.23	6.8615	0.0000	0.0000
4.25	6.8615	0.0000	0.0000
4.26	6.8637	0.0000	0.0000
4.28	6.8593	0.0000	0.0000
4.30	6.8615	0.0000	0.0000
4.32	6.8615	0.0000	0.0000
4.33	6.8593	0.0000	0.0000
4.35	6.8615	0.0000	0.0000
4.37	6.8604	0.0000	0.0000
4.38	6.8593	0.0000	0.0000
4.40	6.8615	0.0000	0.0000
4.42	6.8615	0.0000	0.0000
4.44	6.8615	0.0000	0.0000
4.45	6.8615	0.0000	0.0000
4.47	6.8615	0.0000	0.0000
4.49	6.8615	0.0000	0.0000
4.51	6.8615	0.0000	0.0000
4.52	6.8604	0.0000	0.0000
4.54	6.8615	0.0000	0.0000
4.56	6.8615	0.0000	0.0000

Time	pH	Acid	Alkali
h	units	mL	mL
4.57	6.8615	0.0000	0.0000
4.59	6.8615	0.0000	0.0000
4.61	6.8593	0.0000	0.0000
4.63	6.8593	0.0000	0.0000
4.64	6.8593	0.0000	0.0000
4.66	6.8593	0.0000	0.0000
4.68	6.8593	0.0000	0.0000
4.70	6.8593	0.0000	0.0000
4.71	6.8593	0.0000	0.0000
4.73	6.8570	0.0000	0.0000
4.75	6.8593	0.0000	0.0000
4.76	6.8593	0.0000	0.0000
4.78	6.8593	0.0000	0.0000
4.80	6.8593	0.0000	0.0000
4.82	6.8593	0.0000	0.0000
4.83	6.8570	0.0000	0.0000
4.85	6.8593	0.0000	0.0000
4.87	6.8570	0.0000	0.0000
4.89	6.8593	0.0000	0.0000
4.90	6.8570	0.0000	0.0000
4.92	6.8570	0.0000	0.0000
4.94	6.8570	0.0000	0.0000
4.95	6.8570	0.0000	0.0000
4.97	6.8570	0.0000	0.0000
4.99	6.8570	0.0000	0.0000
5.01	6.8570	0.0000	0.0000
5.02	6.8548	0.0000	0.0000
5.04	6.8548	0.0000	0.0000
5.06	6.8548	0.0000	0.0000
5.07	6.8526	0.0000	0.0000
5.09	6.8548	0.0000	0.0000
5.11	6.8526	0.0000	0.0000
5.13	6.8526	0.0000	0.0000
5.14	6.8548	0.0000	0.0000
5.16	6.8503	0.0000	0.0000
5.18	6.8526	0.0000	0.0000
5.20	6.8526	0.0000	0.0000
5.21	6.8526	0.0000	0.0000
5.23	6.8526	0.0000	0.0000
5.25	6.8526	0.0000	0.0000
5.26	6.8526	0.0000	0.0000
5.28	6.8526	0.0000	0.0000
5.30	6.8548	0.0000	0.0000
5.32	6.8526	0.0000	0.0000
5.33	6.8526	0.0000	0.0000
5.35	6.8503	0.0000	0.0000
5.37	6.8503	0.0000	0.0000
5.39	6.8503	0.0000	0.0000
5.40	6.8526	0.0000	0.0000
5.42	6.8503	0.0000	0.0000
5.44	6.8503	0.0000	0.0000
5.45	6.8503	0.0000	0.0000
5.47	6.8503	0.0000	0.0000

DATA AND SAMPLE CALCULATIONS

Time	pH	Acid	Alkali
h	units	mL	mL
5.49	6.8503	0.0000	0.0000
5.51	6.8503	0.0000	0.0000
5.52	6.8481	0.0000	0.0000
5.54	6.8481	0.0000	0.0000
5.56	6.8503	0.0000	0.0000
5.58	6.8459	0.0000	0.0000
5.59	6.8459	0.0000	0.0000
5.61	6.8436	0.0000	0.0000
5.63	6.8436	0.0000	0.0000
5.64	6.8436	0.0000	0.0000
5.66	6.8436	0.0000	0.0000
5.68	6.8436	0.0000	0.0000
5.70	6.8392	0.0000	0.0010
5.71	6.8392	0.0000	0.0020
5.73	6.8459	0.0000	0.0020
5.75	6.8436	0.0000	0.0020
5.76	6.8425	0.0000	0.0020
5.78	6.8414	0.0000	0.0020
5.80	6.8414	0.0000	0.0020
5.82	6.8414	0.0000	0.0020
5.83	6.8414	0.0000	0.0020
5.85	6.8425	0.0000	0.0020
5.87	6.8414	0.0000	0.0020
5.89	6.8414	0.0000	0.0036
5.90	6.8436	0.0000	0.0036
5.92	6.8414	0.0000	0.0036
5.94	6.8436	0.0000	0.0071
5.95	6.8414	0.0000	0.0089
5.97	6.8414	0.0000	0.0089
5.99	6.8392	0.0000	0.0109
6.01	6.8414	0.0000	0.0109
6.02	6.8414	0.0000	0.0109
6.04	6.8414	0.0000	0.0130
6.06	6.8459	0.0000	0.0152
6.07	6.8414	0.0000	0.0152
6.09	6.8414	0.0000	0.0174
6.11	6.8459	0.0000	0.0174
6.12	6.8436	0.0000	0.0198
6.14	6.8414	0.0000	0.0198
6.16	6.8414	0.0000	0.0224
6.18	6.8414	0.0000	0.0224
6.19	6.8414	0.0000	0.0224
6.21	6.8436	0.0000	0.0251
6.23	6.8414	0.0000	0.0279
6.24	6.8436	0.0000	0.0308
6.26	6.8414	0.0000	0.0308
6.28	6.8425	0.0000	0.0338
6.29	6.8414	0.0000	0.0338
6.31	6.8414	0.0000	0.0338
6.33	6.8414	0.0000	0.0370
6.34	6.8436	0.0000	0.0402
6.36	6.8436	0.0000	0.0402
6.38	6.8414	0.0000	0.0402

Time	pH	Acid	Alkali
h	units	mL	mL
6.40	6.8436	0.0000	0.0470
6.41	6.8436	0.0000	0.0505
6.43	6.8425	0.0000	0.0540
6.45	6.8414	0.0000	0.0540
6.46	6.8414	0.0000	0.0540
6.48	6.8414	0.0000	0.0576
6.50	6.8414	0.0000	0.0576
6.51	6.8414	0.0000	0.0612
6.53	6.8436	0.0000	0.0649
6.55	6.8436	0.0000	0.0649
6.57	6.8414	0.0000	0.0686
6.58	6.8414	0.0000	0.0686
6.60	6.8414	0.0000	0.0723
6.62	6.8414	0.0000	0.0723
6.63	6.8436	0.0000	0.0723
6.65	6.8414	0.0000	0.0723
6.67	6.8414	0.0000	0.0723
6.69	6.8436	0.0000	0.0723
6.70	6.8414	0.0000	0.0723
6.72	6.8414	0.0000	0.0800
6.74	6.8414	0.0000	0.0839
6.75	6.8403	0.0000	0.0839
6.77	6.8414	0.0000	0.0879
6.79	6.8436	0.0000	0.0920
6.80	6.8414	0.0000	0.0920
6.82	6.8414	0.0000	0.0959
6.84	6.8436	0.0000	0.1000
6.86	6.8414	0.0000	0.1000
6.87	6.8436	0.0000	0.1000
6.89	6.8414	0.0000	0.1000
6.91	6.8414	0.0000	0.1041
6.92	6.8436	0.0000	0.1081
6.94	6.8414	0.0000	0.1122
6.96	6.8436	0.0000	0.1122
6.97	6.8414	0.0000	0.1122
6.99	6.8414	0.0000	0.1165
7.01	6.8436	0.0000	0.1208
7.03	6.8436	0.0000	0.1208
7.04	6.8436	0.0000	0.1208
7.06	6.8436	0.0000	0.1208
7.08	6.8414	0.0000	0.1208
7.09	6.8414	0.0000	0.1208
7.11	6.8436	0.0000	0.1252
7.13	6.8414	0.0000	0.1252
7.14	6.8425	0.0000	0.1298
7.16	6.8414	0.0000	0.1298
7.18	6.8414	0.0000	0.1343
7.20	6.8459	0.0000	0.1388
7.21	6.8414	0.0000	0.1388
7.23	6.8414	0.0000	0.1388
7.25	6.8414	0.0000	0.1388
7.26	6.8414	0.0000	0.1434
7.28	6.8414	0.0000	0.1434

DATA AND SAMPLE CALCULATIONS

Time	pH	Acid	Alkali
h	units	mL	mL
7.30	6.8436	0.0000	0.1479
7.32	6.8414	0.0000	0.1479
7.33	6.8414	0.0000	0.1479
7.35	6.8414	0.0000	0.1524
7.37	6.8436	0.0000	0.1570
7.38	6.8414	0.0000	0.1570
7.40	6.8436	0.0000	0.1616
7.42	6.8414	0.0000	0.1616
7.43	6.8414	0.0000	0.1664
7.45	6.8414	0.0000	0.1664
7.47	6.8414	0.0000	0.1713
7.48	6.8436	0.0000	0.1762
7.50	6.8414	0.0000	0.1762
7.52	6.8436	0.0000	0.1810
7.54	6.8436	0.0000	0.1810
7.55	6.8436	0.0000	0.1810
7.57	6.8425	0.0000	0.1810
7.59	6.8414	0.0000	0.1810
7.60	6.8414	0.0000	0.1810
7.62	6.8392	0.0000	0.1858
7.64	6.8436	0.0000	0.1858
7.66	6.8436	0.0000	0.1858
7.67	6.8436	0.0000	0.1906
7.69	6.8425	0.0000	0.1906
7.71	6.8414	0.0000	0.1906
7.72	6.8414	0.0000	0.1954
7.74	6.8436	0.0000	0.2003
7.76	6.8414	0.0000	0.2003
7.77	6.8414	0.0000	0.2052
7.79	6.8414	0.0000	0.2101
7.81	6.8414	0.0000	0.2150
7.83	6.8414	0.0000	0.2150
7.84	6.8392	0.0000	0.2199
7.86	6.8436	0.0000	0.2199
7.88	6.8448	0.0000	0.2199
7.89	6.8436	0.0000	0.2199
7.91	6.8414	0.0000	0.2199
7.93	6.8414	0.0000	0.2199
7.95	6.8414	0.0000	0.2250
7.96	6.8414	0.0000	0.2300
7.98	6.8414	0.0000	0.2300
8.00	6.8414	0.0000	0.2349
8.01	6.8414	0.0000	0.2349
8.03	6.8414	0.0000	0.2400
8.05	6.8414	0.0000	0.2400
8.06	6.8436	0.0000	0.2450
8.08	6.8459	0.0000	0.2501
8.10	6.8414	0.0000	0.2501
8.11	6.8436	0.0000	0.2552
8.13	6.8414	0.0000	0.2552
8.15	6.8414	0.0000	0.2604
8.17	6.8414	0.0000	0.2657
8.18	6.8414	0.0000	0.2709

Time	pH	Acid	Alkali
h	units	mL	mL
8.20	6.8414	0.0000	0.2709
8.22	6.8436	0.0000	0.2762
8.23	6.8414	0.0000	0.2762
8.25	6.8414	0.0000	0.2762
8.27	6.8414	0.0000	0.2813
8.28	6.8436	0.0000	0.2865
8.30	6.8436	0.0000	0.2917
8.32	6.8414	0.0000	0.2917
8.33	6.8414	0.0000	0.2968
8.35	6.8414	0.0000	0.2968
8.37	6.8436	0.0000	0.2968
8.39	6.8425	0.0000	0.2968
8.40	6.8436	0.0000	0.2968
8.42	6.8436	0.0000	0.2968
8.44	6.8436	0.0000	0.2968
8.46	6.8436	0.0000	0.3019
8.47	6.8436	0.0000	0.3019
8.49	6.8414	0.0000	0.3019
8.51	6.8425	0.0000	0.3070
8.52	6.8414	0.0000	0.3122
8.54	6.8414	0.0000	0.3122
8.56	6.8414	0.0000	0.3173
8.57	6.8414	0.0000	0.3173
8.59	6.8414	0.0000	0.3224
8.61	6.8414	0.0000	0.3276
8.62	6.8436	0.0000	0.3329
8.64	6.8436	0.0000	0.3329
8.66	6.8414	0.0000	0.3329
8.68	6.8414	0.0000	0.3383
8.69	6.8414	0.0000	0.3383
8.71	6.8414	0.0000	0.3383
8.73	6.8414	0.0000	0.3439
8.74	6.8436	0.0000	0.3550
8.76	6.8414	0.0000	0.3550
8.78	6.8414	0.0000	0.3550
8.80	6.8436	0.0000	0.3605
8.81	6.8414	0.0000	0.3660
8.83	6.8425	0.0000	0.3660
8.85	6.8436	0.0000	0.3715
8.86	6.8436	0.0000	0.3715
8.88	6.8436	0.0000	0.3715
8.90	6.8436	0.0000	0.3715
8.92	6.8414	0.0000	0.3715
8.93	6.8414	0.0000	0.3715
8.95	6.8459	0.0000	0.3769
8.97	6.8414	0.0000	0.3769
8.98	6.8414	0.0000	0.3823
9.00	6.8414	0.0000	0.3878
9.02	6.8414	0.0000	0.3878
9.03	6.8414	0.0000	0.3932
9.05	6.8414	0.0000	0.3986
9.07	6.8414	0.0000	0.3986
9.08	6.8436	0.0000	0.4039

DATA AND SAMPLE CALCULATIONS

Time	pH	Acid	Alkali
h	units	mL	mL
9.10	6.8436	0.0000	0.4039
9.12	6.8436	0.0000	0.4092
9.14	6.8414	0.0000	0.4092
9.15	6.8414	0.0000	0.4092
9.17	6.8436	0.0000	0.4147
9.19	6.8436	0.0000	0.4147
9.20	6.8414	0.0000	0.4147
9.22	6.8436	0.0000	0.4201
9.24	6.8414	0.0000	0.4201
9.25	6.8414	0.0000	0.4255
9.27	6.8414	0.0000	0.4255
9.29	6.8414	0.0000	0.4311
9.31	6.8414	0.0000	0.4366
9.32	6.8436	0.0000	0.4366
9.34	6.8459	0.0000	0.4422
9.36	6.8414	0.0000	0.4422
9.37	6.8414	0.0000	0.4422
9.39	6.8436	0.0000	0.4476
9.41	6.8414	0.0000	0.4476
9.42	6.8436	0.0000	0.4531
9.44	6.8436	0.0000	0.4585
9.46	6.8414	0.0000	0.4585
9.48	6.8414	0.0000	0.4585
9.49	6.8414	0.0000	0.4639
9.51	6.8414	0.0000	0.4694
9.53	6.8414	0.0000	0.4749
9.54	6.8436	0.0000	0.4805
9.56	6.8414	0.0000	0.4805
9.58	6.8436	0.0000	0.4805
9.59	6.8436	0.0000	0.4805
9.61	6.8414	0.0000	0.4805
9.63	6.8436	0.0000	0.4805
9.65	6.8414	0.0000	0.4861
9.66	6.8414	0.0000	0.4861
9.68	6.8414	0.0000	0.4916
9.70	6.8414	0.0000	0.4972
9.71	6.8414	0.0000	0.4972
9.73	6.8414	0.0000	0.4972
9.75	6.8414	0.0000	0.5086
9.77	6.8414	0.0000	0.5086
9.78	6.8392	0.0000	0.5143
9.80	6.8436	0.0000	0.5143
9.82	6.8403	0.0000	0.5143
9.83	6.8436	0.0000	0.5200
9.85	6.8414	0.0000	0.5257
9.87	6.8436	0.0000	0.5257
9.89	6.8436	0.0000	0.5257
9.90	6.8436	0.0000	0.5257
9.92	6.8436	0.0000	0.5257
9.94	6.8414	0.0000	0.5257
9.95	6.8414	0.0000	0.5313
9.97	6.8414	0.0000	0.5369
9.99	6.8414	0.0000	0.5369

Time	pH	Acid	Alkali
h	units	mL	mL
10.00	6.8414	0.0000	0.5426
10.02	6.8414	0.0000	0.5426
10.04	6.8414	0.0000	0.5481
10.06	6.8436	0.0000	0.5481
10.07	6.8436	0.0000	0.5590
10.09	6.8436	0.0000	0.5590
10.11	6.8436	0.0000	0.5590
10.12	6.8414	0.0000	0.5644
10.14	6.8436	0.0000	0.5699
10.16	6.8414	0.0000	0.5699
10.18	6.8414	0.0000	0.5699
10.19	6.8436	0.0000	0.5752
10.21	6.8414	0.0000	0.5805
10.23	6.8414	0.0000	0.5859
10.24	6.8414	0.0000	0.5912
10.26	6.8425	0.0000	0.5912
10.28	6.8414	0.0000	0.5966
10.29	6.8436	0.0000	0.5966
10.31	6.8414	0.0000	0.5966
10.33	6.8414	0.0000	0.6021
10.34	6.8414	0.0000	0.6075
10.36	6.8425	0.0000	0.6129
10.38	6.8436	0.0000	0.6129
10.40	6.8414	0.0000	0.6129
10.41	6.8436	0.0000	0.6184
10.43	6.8436	0.0000	0.6184
10.45	6.8436	0.0000	0.6184
10.46	6.8425	0.0000	0.6184
10.48	6.8436	0.0000	0.6237
10.50	6.8436	0.0000	0.6290
10.51	6.8436	0.0000	0.6290
10.53	6.8425	0.0000	0.6290
10.55	6.8414	0.0000	0.6341
10.57	6.8414	0.0000	0.6341
10.58	6.8414	0.0000	0.6394
10.60	6.8436	0.0000	0.6447
10.62	6.8414	0.0000	0.6447
10.63	6.8414	0.0000	0.6447
10.65	6.8414	0.0000	0.6447
10.67	6.8392	0.0000	0.6553
10.68	6.8414	0.0000	0.6553
10.70	6.8436	0.0000	0.6605
10.72	6.8414	0.0000	0.6605
10.74	6.8414	0.0000	0.6605
10.75	6.8436	0.0000	0.6657
10.77	6.8436	0.0000	0.6657
10.79	6.8436	0.0000	0.6708
10.80	6.8414	0.0000	0.6760
10.82	6.8425	0.0000	0.6813
10.84	6.8414	0.0000	0.6813
10.85	6.8414	0.0000	0.6813
10.87	6.8436	0.0000	0.6867
10.89	6.8436	0.0000	0.6867

DATA AND SAMPLE CALCULATIONS

Time	pH	Acid	Alkali
h	units	mL	mL
10.91	6.8436	0.0000	0.6867
10.92	6.8425	0.0000	0.6867
10.94	6.8414	0.0000	0.6867
10.96	6.8414	0.0000	0.6867
10.98	6.8414	0.0000	0.6867
10.99	6.8414	0.0000	0.6922
11.01	6.8414	0.0000	0.6976
11.03	6.8436	0.0000	0.6976
11.04	6.8414	0.0000	0.7029
11.06	6.8414	0.0000	0.7029
11.08	6.8414	0.0000	0.7083
11.09	6.8414	0.0000	0.7083
11.11	6.8436	0.0000	0.7138
11.13	6.8414	0.0000	0.7192
11.14	6.8436	0.0000	0.7192
11.16	6.8436	0.0000	0.7192
11.18	6.8414	0.0000	0.7192
11.20	6.8414	0.0000	0.7192
11.21	6.8436	0.0000	0.7246
11.23	6.8414	0.0000	0.7246
11.25	6.8436	0.0000	0.7301
11.26	6.8436	0.0000	0.7301
11.28	6.8414	0.0000	0.7301
11.30	6.8414	0.0000	0.7301
11.32	6.8436	0.0000	0.7355
11.33	6.8436	0.0000	0.7410
11.35	6.8459	0.0000	0.7410
11.37	6.8459	0.0000	0.7410
11.38	6.8436	0.0000	0.7410
11.40	6.8436	0.0000	0.7410
11.42	6.8436	0.0000	0.7410
11.44	6.8414	0.0000	0.7410
11.45	6.8414	0.0000	0.7410
11.47	6.8414	0.0000	0.7466
11.49	6.8414	0.0000	0.7523
11.50	6.8436	0.0000	0.7523
11.52	6.8436	0.0000	0.7523
11.54	6.8414	0.0000	0.7578
11.55	6.8436	0.0000	0.7635
11.57	6.8414	0.0000	0.7692
11.59	6.8414	0.0000	0.7692
11.61	6.8414	0.0000	0.7748
11.62	6.8414	0.0000	0.7748
11.64	6.8414	0.0000	0.7748
11.66	6.8414	0.0000	0.7748
11.67	6.8414	0.0000	0.7748
11.69	6.8425	0.0000	0.7805
11.71	6.8414	0.0000	0.7861
11.72	6.8414	0.0000	0.7861
11.74	6.8414	0.0000	0.7861
11.76	6.8436	0.0000	0.7918
11.78	6.8436	0.0000	0.7918
11.79	6.8436	0.0000	0.7973

Time	pH	Acid	Alkali
h	units	mL	mL
11.81	6.8459	0.0000	0.7973
11.83	6.8459	0.0000	0.7973
11.84	6.8436	0.0000	0.7973
11.86	6.8414	0.0000	0.7973
11.88	6.8414	0.0000	0.7973
11.90	6.8436	0.0000	0.8029
11.91	6.8436	0.0000	0.8029
11.93	6.8459	0.0000	0.8029
11.95	6.8459	0.0000	0.8029
11.96	6.8414	0.0000	0.8029
11.98	6.8414	0.0000	0.8029
12.00	6.8436	0.0000	0.8086
12.02	6.8436	0.0000	0.8086
12.03	6.8425	0.0000	0.8086
12.05	6.8414	0.0000	0.8086
12.07	6.8414	0.0000	0.8086
12.09	6.8436	0.0000	0.8086
12.10	6.8414	0.0000	0.8086
12.12	6.8414	0.0000	0.8140
12.14	6.8436	0.0000	0.8140
12.15	6.8481	0.0000	0.8140
12.17	6.8414	0.0000	0.8140
12.19	6.8414	0.0000	0.8197
12.20	6.8414	0.0000	0.8197
12.22	6.8414	0.0000	0.8197
12.24	6.8414	0.0000	0.8197
12.26	6.8436	0.0000	0.8197
12.27	6.8459	0.0000	0.8197
12.29	6.8414	0.0000	0.8197
12.31	6.8414	0.0000	0.8257
12.33	6.8436	0.0000	0.8257
12.34	6.8436	0.0000	0.8257
12.36	6.8425	0.0000	0.8257
12.38	6.8436	0.0000	0.8257
12.39	6.8459	0.0000	0.8257
12.41	6.8436	0.0000	0.8257
12.43	6.8414	0.0000	0.8257
12.45	6.8414	0.0000	0.8257
12.46	6.8414	0.0000	0.8257
12.48	6.8436	0.0000	0.8320
12.50	6.8414	0.0000	0.8320
12.51	6.8459	0.0000	0.8320
12.53	6.8436	0.0000	0.8320
12.55	6.8436	0.0000	0.8320
12.57	6.8436	0.0000	0.8320
12.58	6.8414	0.0000	0.8320
12.60	6.8414	0.0000	0.8320
12.62	6.8459	0.0000	0.8320
12.64	6.8436	0.0000	0.8320
12.65	6.8425	0.0000	0.8320
12.67	6.8436	0.0000	0.8320
12.69	6.8414	0.0000	0.8320
12.70	6.8414	0.0000	0.8320

DATA AND SAMPLE CALCULATIONS

Time h	pH units	Acid mL	Alkali mL
12.72	6.8459	0.0000	0.8320
12.74	6.8436	0.0000	0.8320
12.76	6.8414	0.0000	0.8320
12.77	6.8414	0.0000	0.8320
12.79	6.8425	0.0000	0.8320
12.81	6.8425	0.0000	0.8384
12.82	6.8436	0.0000	0.8384
12.84	6.8459	0.0000	0.8384
12.86	6.8414	0.0000	0.8384
12.88	6.8436	0.0000	0.8384
12.89	6.8414	0.0000	0.8384
12.91	6.8414	0.0000	0.8384
12.93	6.8436	0.0000	0.8384
12.95	6.8470	0.0000	0.8384
12.96	6.8436	0.0000	0.8384
12.98	6.8436	0.0000	0.8451
13.00	6.8414	0.0000	0.8451
13.01	6.8414	0.0000	0.8451
13.03	6.8414	0.0000	0.8451
13.05	6.8470	0.0000	0.8451
13.07	6.8436	0.0000	0.8451
13.08	6.8436	0.0000	0.8451
13.10	6.8436	0.0000	0.8451
13.12	6.8436	0.0000	0.8451
13.13	6.8459	0.0000	0.8451
13.15	6.8481	0.0000	0.8451
13.17	6.8436	0.0000	0.8451
13.19	6.8436	0.0000	0.8451
13.20	6.8436	0.0000	0.8451
13.22	6.8436	0.0000	0.8451
13.24	6.8459	0.0000	0.8451
13.26	6.8436	0.0000	0.8451
13.27	6.8436	0.0000	0.8451
13.29	6.8414	0.0000	0.8451
13.31	6.8414	0.0000	0.8451
13.32	6.8436	0.0000	0.8451
13.34	6.8459	0.0000	0.8451
13.36	6.8436	0.0000	0.8451
13.38	6.8436	0.0000	0.8451
13.39	6.8436	0.0000	0.8451
13.41	6.8436	0.0000	0.8451
13.43	6.8481	0.0000	0.8451
13.45	6.8436	0.0000	0.8451
13.46	6.8414	0.0000	0.8451
13.48	6.8414	0.0000	0.8451
13.50	6.8414	0.0000	0.8451
13.51	6.8459	0.0000	0.8451
13.53	6.8436	0.0000	0.8451
13.55	6.8436	0.0000	0.8451
13.57	6.8436	0.0000	0.8451
13.58	6.8436	0.0000	0.8451
13.60	6.8481	0.0000	0.8451
13.62	6.8414	0.0000	0.8451

Time h	pH units	Acid mL	Alkali mL
13.64	6.8436	0.0000	0.8451
13.65	6.8425	0.0000	0.8451
13.67	6.8414	0.0000	0.8451
13.69	6.8481	0.0000	0.8526
13.70	6.8436	0.0000	0.8526
13.72	6.8436	0.0000	0.8526
13.74	6.8436	0.0000	0.8526
13.76	6.8436	0.0000	0.8526
13.77	6.8481	0.0000	0.8526
13.79	6.8436	0.0000	0.8526
13.81	6.8436	0.0000	0.8526
13.82	6.8436	0.0000	0.8526
13.84	6.8436	0.0000	0.8526
13.86	6.8481	0.0000	0.8526
13.88	6.8436	0.0000	0.8526
13.89	6.8414	0.0000	0.8526
13.91	6.8436	0.0000	0.8526
13.93	6.8459	0.0000	0.8526
13.95	6.8436	0.0000	0.8526
13.96	6.8436	0.0000	0.8526
13.98	6.8436	0.0000	0.8526
14.00	6.8425	0.0000	0.8526
14.01	6.8481	0.0000	0.8526
14.03	6.8436	0.0000	0.8526
14.05	6.8414	0.0000	0.8526
14.07	6.8436	0.0000	0.8526
14.08	6.8436	0.0000	0.8526
14.10	6.8459	0.0000	0.8526
14.12	6.8436	0.0000	0.8526
14.14	6.8414	0.0000	0.8526
14.15	6.8436	0.0000	0.8526
14.17	6.8481	0.0000	0.8526
14.19	6.8436	0.0000	0.8526
14.20	6.8436	0.0000	0.8526
14.22	6.8436	0.0000	0.8526
14.24	6.8481	0.0000	0.8526
14.26	6.8436	0.0000	0.8526
14.27	6.8436	0.0000	0.8526
14.29	6.8436	0.0000	0.8526
14.31	6.8481	0.0000	0.8526
14.32	6.8436	0.0000	0.8526
14.34	6.8436	0.0000	0.8526
14.36	6.8436	0.0000	0.8526
14.38	6.8436	0.0000	0.8526
14.39	6.8436	0.0000	0.8526
14.41	6.8436	0.0000	0.8526
14.43	6.8436	0.0000	0.8526
14.45	6.8459	0.0000	0.8526
14.46	6.8481	0.0000	0.8526
14.48	6.8436	0.0000	0.8526
14.50	6.8436	0.0000	0.8526
14.51	6.8436	0.0000	0.8526
14.53	6.8436	0.0000	0.8526

DATA AND SAMPLE CALCULATIONS

Time	pH	Acid	Alkali
h	units	mL	mL
14.55	6.8414	0.0000	0.8526
14.57	6.8436	0.0000	0.8526
14.58	6.8448	0.0000	0.8526
14.60	6.8459	0.0000	0.8526
14.62	6.8436	0.0000	0.8526
14.64	6.8436	0.0000	0.8526
14.65	6.8481	0.0000	0.8526
14.67	6.8459	0.0000	0.8526
14.69	6.8436	0.0000	0.8526
14.70	6.8436	0.0000	0.8526
14.72	6.8470	0.0000	0.8526
14.74	6.8459	0.0000	0.8526
14.76	6.8436	0.0000	0.8526
14.77	6.8436	0.0000	0.8526
14.79	6.8459	0.0000	0.8526
14.81	6.8436	0.0000	0.8526
14.83	6.8436	0.0000	0.8526
14.84	6.8436	0.0000	0.8526
14.86	6.8470	0.0000	0.8526
14.88	6.8436	0.0000	0.8526
14.89	6.8436	0.0000	0.8526
14.91	6.8436	0.0000	0.8526
14.93	6.8481	0.0000	0.8526
14.95	6.8436	0.0000	0.8526
14.96	6.8436	0.0000	0.8526
14.98	6.8436	0.0000	0.8526
15.00	6.8481	0.0000	0.8526
15.02	6.8436	0.0000	0.8526
15.03	6.8436	0.0000	0.8526
15.05	6.8436	0.0000	0.8526
15.07	6.8481	0.0000	0.8526
15.08	6.8436	0.0000	0.8526
15.10	6.8436	0.0000	0.8526
15.12	6.8436	0.0000	0.8526
15.14	6.8503	0.0000	0.8526
15.15	6.8459	0.0000	0.8526
15.17	6.8436	0.0000	0.8526
15.19	6.8459	0.0000	0.8526
15.20	6.8459	0.0000	0.8526
15.22	6.8448	0.0000	0.8526
15.24	6.8436	0.0000	0.8526
15.26	6.8481	0.0000	0.8526
15.27	6.8436	0.0000	0.8526
15.29	6.8436	0.0000	0.8526
15.31	6.8448	0.0000	0.8526
15.33	6.8481	0.0000	0.8526
15.34	6.8436	0.0000	0.8526
15.36	6.8436	0.0000	0.8526
15.38	6.8459	0.0000	0.8526
15.39	6.8459	0.0000	0.8526
15.41	6.8459	0.0000	0.8526
15.43	6.8459	0.0000	0.8526
15.45	6.8481	0.0000	0.8526

Time	pH	Acid	Alkali
h	units	mL	mL
15.46	6.8459	0.0000	0.8526
15.48	6.8459	0.0000	0.8526
15.50	6.8459	0.0000	0.8526
15.52	6.8503	0.0000	0.8526
15.53	6.8459	0.0000	0.8526
15.55	6.8459	0.0000	0.8526
15.57	6.8481	0.0000	0.8526
15.58	6.8459	0.0000	0.8526
15.60	6.8459	0.0000	0.8526
15.62	6.8481	0.0000	0.8526
15.64	6.8503	0.0000	0.8526
15.65	6.8481	0.0000	0.8526
15.67	6.8481	0.0000	0.8526
15.69	6.8503	0.0000	0.8526
15.71	6.8503	0.0000	0.8526
15.72	6.8481	0.0000	0.8526
15.74	6.8481	0.0000	0.8526
15.76	6.8481	0.0000	0.8526
15.77	6.8459	0.0000	0.8526
15.79	6.8436	0.0000	0.8526
15.81	6.8481	0.0000	0.8526
15.83	6.8459	0.0000	0.8526
15.84	6.8459	0.0000	0.8526
15.86	6.8459	0.0000	0.8526
15.88	6.8459	0.0000	0.8526
15.90	6.8436	0.0000	0.8526
15.91	6.8436	0.0000	0.8526
15.93	6.8503	0.0000	0.8526
15.95	6.8459	0.0000	0.8526
15.96	6.8436	0.0000	0.8526
15.98	6.8503	0.0000	0.8526
16.00	6.8459	0.0000	0.8526
16.02	6.8459	0.0000	0.8526
16.03	6.8481	0.0000	0.8526
16.05	6.8481	0.0000	0.8526
16.07	6.8459	0.0000	0.8526
16.09	6.8459	0.0000	0.8526
16.10	6.8503	0.0000	0.8526
16.12	6.8459	0.0000	0.8526
16.14	6.8459	0.0000	0.8526
16.15	6.8503	0.0000	0.8526
16.17	6.8481	0.0000	0.8526
16.19	6.8459	0.0000	0.8526
16.21	6.8503	0.0000	0.8526
16.22	6.8481	0.0000	0.8526
16.24	6.8481	0.0000	0.8526
16.26	6.8503	0.0000	0.8526
16.28	6.8481	0.0000	0.8526
16.29	6.8481	0.0000	0.8526
16.31	6.8503	0.0000	0.8526
16.33	6.8481	0.0000	0.8526
16.34	6.8503	0.0000	0.8526
16.36	6.8503	0.0000	0.8526

DATA AND SAMPLE CALCULATIONS

Time	pH	Acid	Alkali
h	units	mL	mL
16.38	6.8481	0.0000	0.8526
16.40	6.8459	0.0000	0.8526
16.41	6.8503	0.0000	0.8526
16.43	6.8481	0.0000	0.8526
16.45	6.8481	0.0000	0.8526
16.46	6.8503	0.0000	0.8526
16.48	6.8481	0.0000	0.8526
16.50	6.8481	0.0000	0.8526
16.52	6.8503	0.0000	0.8526
16.53	6.8492	0.0000	0.8526
16.55	6.8481	0.0000	0.8526
16.57	6.8526	0.0000	0.8526
16.59	6.8470	0.0000	0.8526
16.60	6.8481	0.0000	0.8526
16.62	6.8503	0.0000	0.8526
16.64	6.8470	0.0000	0.8526
16.65	6.8481	0.0000	0.8526
16.67	6.8503	0.0000	0.8526
16.69	6.8481	0.0000	0.8526
16.71	6.8481	0.0000	0.8526
16.72	6.8503	0.0000	0.8526
16.74	6.8459	0.0000	0.8526
16.76	6.8459	0.0000	0.8526
16.78	6.8526	0.0000	0.8526
16.79	6.8481	0.0000	0.8526
16.81	6.8481	0.0000	0.8526
16.83	6.8503	0.0000	0.8526
16.84	6.8459	0.0000	0.8526
16.86	6.8503	0.0000	0.8526
16.88	6.8481	0.0000	0.8526
16.90	6.8470	0.0000	0.8526
16.91	6.8526	0.0000	0.8526
16.93	6.8481	0.0000	0.8526
16.95	6.8481	0.0000	0.8526
16.97	6.8526	0.0000	0.8526
16.98	6.8481	0.0000	0.8526
17.00	6.8481	0.0000	0.8526
17.02	6.8503	0.0000	0.8526
17.03	6.8459	0.0000	0.8526
17.05	6.8503	0.0000	0.8526
17.07	6.8481	0.0000	0.8526
17.09	6.8492	0.0000	0.8526
17.10	6.8526	0.0000	0.8526
17.12	6.8481	0.0000	0.8526
17.14	6.8481	0.0000	0.8526
17.16	6.8503	0.0000	0.8526
17.17	6.8503	0.0000	0.8526
17.19	6.8503	0.0000	0.8526
17.21	6.8503	0.0000	0.8526
17.22	6.8503	0.0000	0.8526
17.24	6.8503	0.0000	0.8526
17.26	6.8503	0.0000	0.8526
17.28	6.8503	0.0000	0.8526

Time	pH	Acid	Alkali
h	units	mL	mL
17.29	6.8503	0.0000	0.8526
17.31	6.8503	0.0000	0.8526
17.33	6.8526	0.0000	0.8526
17.35	6.8503	0.0000	0.8526
17.36	6.8526	0.0000	0.8526
17.38	6.8503	0.0000	0.8526
17.40	6.8503	0.0000	0.8526
17.41	6.8526	0.0000	0.8526
17.43	6.8503	0.0000	0.8526
17.45	6.8526	0.0000	0.8526
17.47	6.8503	0.0000	0.8526
17.48	6.8503	0.0000	0.8526
17.50	6.8526	0.0000	0.8526
17.52	6.8503	0.0000	0.8526
17.54	6.8526	0.0000	0.8526
17.55	6.8503	0.0000	0.8526
17.57	6.8503	0.0000	0.8526
17.59	6.8526	0.0000	0.8526
17.60	6.8503	0.0000	0.8526
17.62	6.8548	0.0000	0.8526
17.64	6.8503	0.0000	0.8526
17.66	6.8548	0.0000	0.8526
17.67	6.8503	0.0000	0.8526
17.69	6.8526	0.0000	0.8526
17.71	6.8503	0.0000	0.8526
17.73	6.8503	0.0000	0.8526
17.74	6.8526	0.0000	0.8526
17.76	6.8503	0.0000	0.8526
17.78	6.8537	0.0000	0.8526
17.79	6.8526	0.0000	0.8526
17.81	6.8593	0.0000	0.8629
17.83	6.8682	0.0000	0.8629
17.85	6.8704	0.0000	0.8629
17.86	6.8704	0.0000	0.8629
17.88	6.8704	0.0000	0.8629
17.90	6.8727	0.0000	0.8629
17.91	6.8727	0.0000	0.8629
17.93	6.8704	0.0000	0.8629
17.95	6.8704	0.0000	0.8629
17.97	6.8727	0.0000	0.8629
17.98	6.8704	0.0000	0.8629
18.00	6.8727	0.0000	0.8629
18.02	6.8727	0.0000	0.8629
18.04	6.8749	0.0000	0.8629
18.05	6.8727	0.0000	0.8629
18.07	6.8727	0.0000	0.8629
18.09	6.8704	0.0000	0.8629
18.10	6.8727	0.0000	0.8629
18.12	6.8727	0.0000	0.8629
18.14	6.8771	0.0000	0.8629
18.16	6.8749	0.0000	0.8629
18.17	6.8749	0.0000	0.8629
18.19	6.8794	0.0000	0.8629

DATA AND SAMPLE CALCULATIONS

Time	pH	Acid	Alkali
h	units	mL	mL
18.21	6.8794	0.0096	0.8629
18.22	6.8749	0.0096	0.8629
18.24	6.8727	0.0096	0.8629
18.26	6.8749	0.0096	0.8629
18.28	6.8704	0.0096	0.8629
18.29	6.8749	0.0096	0.8629
18.31	6.8749	0.0096	0.8629
18.33	6.8727	0.0096	0.8629
18.35	6.8749	0.0096	0.8629
18.36	6.8727	0.0096	0.8629
18.38	6.8727	0.0096	0.8629
18.40	6.8749	0.0096	0.8629
18.41	6.8771	0.0096	0.8629
18.43	6.8760	0.0096	0.8629
18.45	6.8794	0.0096	0.8629
18.47	6.8771	0.0193	0.8629
18.48	6.8704	0.0291	0.8629
18.50	6.8693	0.0291	0.8629
18.52	6.8704	0.0291	0.8629
18.53	6.8704	0.0291	0.8629
18.55	6.8704	0.0291	0.8629
18.57	6.8727	0.0291	0.8629
18.59	6.8727	0.0291	0.8629
18.60	6.8749	0.0291	0.8629
18.62	6.8749	0.0291	0.8629
18.64	6.8749	0.0291	0.8629
18.65	6.8749	0.0291	0.8629
18.67	6.8704	0.0386	0.8629
18.69	6.8704	0.0386	0.8629
18.71	6.8693	0.0386	0.8629
18.72	6.8727	0.0386	0.8629
18.74	6.8749	0.0386	0.8629
18.76	6.8727	0.0386	0.8629
18.78	6.8749	0.0386	0.8629
18.79	6.8727	0.0386	0.8629
18.81	6.8704	0.0386	0.8629
18.83	6.8704	0.0386	0.8629
18.84	6.8727	0.0386	0.8629
18.86	6.8727	0.0386	0.8629
18.88	6.8749	0.0386	0.8629
18.90	6.8727	0.0386	0.8629
18.91	6.8749	0.0386	0.8629
18.93	6.8749	0.0386	0.8629
18.95	6.8704	0.0386	0.8629
18.97	6.8704	0.0386	0.8629
18.98	6.8749	0.0386	0.8629
19.00	6.8771	0.0386	0.8629
19.02	6.8749	0.0386	0.8629
19.03	6.8749	0.0386	0.8629
19.05	6.8727	0.0386	0.8629
19.07	6.8704	0.0386	0.8629
19.09	6.8727	0.0386	0.8629
19.10	6.8771	0.0386	0.8629

Time	pH	Acid	Alkali
h	units	mL	mL
19.12	6.8727	0.0386	0.8629
19.14	6.8727	0.0386	0.8629
19.15	6.8749	0.0386	0.8629
19.17	6.8794	0.0386	0.8629
19.19	6.8749	0.0386	0.8629
19.21	6.8749	0.0386	0.8629
19.22	6.8749	0.0386	0.8629
19.24	6.8794	0.0386	0.8629
19.26	6.8749	0.0386	0.8629
19.28	6.8749	0.0484	0.8629
19.29	6.8704	0.0484	0.8629
19.31	6.8749	0.0484	0.8629
19.33	6.8749	0.0484	0.8629
19.34	6.8749	0.0484	0.8629
19.36	6.8716	0.0484	0.8629
19.38	6.8749	0.0484	0.8629
19.40	6.8749	0.0484	0.8629
19.41	6.8749	0.0484	0.8629
19.43	6.8749	0.0484	0.8629
19.45	6.8749	0.0484	0.8629
19.47	6.8749	0.0484	0.8629
19.48	6.8749	0.0484	0.8629
19.50	6.8794	0.0484	0.8629
19.52	6.8794	0.0484	0.8629
19.53	6.8749	0.0689	0.8629
19.55	6.8727	0.0689	0.8629
19.57	6.8727	0.0689	0.8629
19.59	6.8727	0.0689	0.8629
19.60	6.8704	0.0689	0.8629
19.62	6.8682	0.0689	0.8629
19.64	6.8704	0.0689	0.8629
19.65	6.8749	0.0689	0.8629
19.67	6.8704	0.0689	0.8629
19.69	6.8704	0.0689	0.8629
19.71	6.8727	0.0689	0.8629
19.72	6.8727	0.0689	0.8629
19.74	6.8704	0.0689	0.8629
19.76	6.8727	0.0689	0.8629
19.78	6.8704	0.0689	0.8629
19.79	6.8704	0.0689	0.8629
19.81	6.8704	0.0689	0.8629
19.83	6.8749	0.0689	0.8629
19.84	6.8727	0.0689	0.8629
19.86	6.8727	0.0689	0.8629
19.88	6.8704	0.0689	0.8629
19.90	6.8727	0.0689	0.8629
19.91	6.8727	0.0689	0.8629
19.93	6.8727	0.0689	0.8629
19.95	6.8727	0.0689	0.8629
19.97	6.8727	0.0689	0.8629
19.98	6.8716	0.0689	0.8629
20.00	6.8749	0.0689	0.8629
20.02	6.8749	0.0689	0.8629

DATA AND SAMPLE CALCULATIONS

Time	pH	Acid	Alkali
h	units	mL	mL
20.03	6.8738	0.0689	0.8629
20.05	6.8749	0.0689	0.8629
20.07	6.8771	0.0689	0.8629
20.09	6.8760	0.0689	0.8629
20.10	6.8771	0.0689	0.8629
20.12	6.8771	0.0689	0.8629
20.14	6.8794	0.0689	0.8629
20.15	6.8771	0.0813	0.8629
20.17	6.8727	0.0938	0.8629
20.19	6.8704	0.0938	0.8629
20.21	6.8727	0.0938	0.8629
20.22	6.8727	0.0938	0.8629
20.24	6.8749	0.0938	0.8629
20.26	6.8693	0.0938	0.8629
20.28	6.8704	0.0938	0.8629
20.29	6.8704	0.0938	0.8629
20.31	6.8704	0.0938	0.8629
20.33	6.8704	0.0938	0.8629
20.34	6.8704	0.0938	0.8629
20.36	6.8704	0.0938	0.8629
20.38	6.8727	0.0938	0.8629
20.40	6.8727	0.0938	0.8629
20.41	6.8727	0.0938	0.8629
20.43	6.8749	0.0938	0.8629
20.45	6.8771	0.0938	0.8629
20.46	6.8727	0.0938	0.8629
20.48	6.8727	0.0938	0.8629
20.50	6.8727	0.0938	0.8629
20.52	6.8727	0.0938	0.8629
20.53	6.8727	0.0938	0.8629
20.55	6.8704	0.0938	0.8629
20.57	6.8704	0.0938	0.8629
20.59	6.8727	0.0938	0.8629
20.60	6.8727	0.0938	0.8629
20.62	6.8727	0.0938	0.8629
20.64	6.8727	0.0938	0.8629
20.65	6.8749	0.0938	0.8629
20.67	6.8749	0.0938	0.8629
20.69	6.8749	0.0938	0.8629
20.71	6.8771	0.0938	0.8629
20.72	6.8727	0.0938	0.8629
20.74	6.8727	0.0938	0.8629
20.76	6.8727	0.0938	0.8629
20.78	6.8704	0.0938	0.8629
20.79	6.8704	0.0938	0.8629
20.81	6.8704	0.0938	0.8629
20.83	6.8716	0.0938	0.8629
20.84	6.8727	0.0938	0.8629
20.86	6.8727	0.0938	0.8629
20.88	6.8727	0.0938	0.8629
20.90	6.8727	0.0938	0.8629
20.91	6.8749	0.0938	0.8629
20.93	6.8749	0.0938	0.8629

Time	pH	Acid	Alkali
h	units	mL	mL
20.95	6.8771	0.0938	0.8629
20.97	6.8749	0.0938	0.8629
20.98	6.8727	0.0938	0.8629
21.00	6.8727	0.0938	0.8629
21.02	6.8727	0.0938	0.8629
21.03	6.8749	0.0938	0.8629
21.05	6.8727	0.0938	0.8629
21.07	6.8727	0.0938	0.8629
21.09	6.8749	0.0938	0.8629
21.10	6.8749	0.0938	0.8629
21.12	6.8738	0.0938	0.8629
21.14	6.8727	0.0938	0.8629
21.16	6.8749	0.0938	0.8629
21.17	6.8760	0.0938	0.8629
21.19	6.8749	0.0938	0.8629
21.21	6.8727	0.0938	0.8629
21.22	6.8716	0.0938	0.8629
21.24	6.8727	0.0938	0.8629
21.26	6.8727	0.0938	0.8629
21.28	6.8727	0.0938	0.8629
21.29	6.8727	0.0938	0.8629
21.31	6.8749	0.0938	0.8629
21.33	6.8749	0.0938	0.8629
21.35	6.8749	0.0938	0.8629
21.36	6.8749	0.0938	0.8629
21.38	6.8771	0.0938	0.8629
21.40	6.8771	0.0938	0.8629
21.41	6.8749	0.1075	0.8629
21.43	6.8727	0.1075	0.8629
21.45	6.8727	0.1075	0.8629
21.47	6.8704	0.1075	0.8629
21.48	6.8704	0.1075	0.8629
21.50	6.8727	0.1075	0.8629
21.52	6.8704	0.1075	0.8629
21.53	6.8727	0.1075	0.8629
21.55	6.8727	0.1075	0.8629
21.57	6.8727	0.1075	0.8629
21.59	6.8749	0.1075	0.8629
21.60	6.8749	0.1075	0.8629
21.62	6.8749	0.1075	0.8629
21.64	6.8749	0.1075	0.8629
21.66	6.8749	0.1075	0.8629
21.67	6.8738	0.1075	0.8629
21.69	6.8749	0.1075	0.8629
21.71	6.8749	0.1075	0.8629
21.72	6.8749	0.1075	0.8629
21.74	6.8749	0.1075	0.8629
21.76	6.8749	0.1075	0.8629
21.78	6.8749	0.1075	0.8629
21.79	6.8749	0.1075	0.8629
21.81	6.8749	0.1075	0.8629
21.83	6.8749	0.1075	0.8629
21.85	6.8771	0.1075	0.8629

DATA AND SAMPLE CALCULATIONS

Time	pH	Acid	Alkali
h	units	mL	mL
21.86	6.8771	0.1075	0.8629
21.88	6.8749	0.1075	0.8629
21.90	6.8771	0.1075	0.8629
21.91	6.8738	0.1075	0.8629
21.93	6.8749	0.1075	0.8629
21.95	6.8749	0.1075	0.8629
21.97	6.8760	0.1075	0.8629
21.98	6.8771	0.1075	0.8629
22.00	6.8749	0.1075	0.8629
22.02	6.8749	0.1075	0.8629
22.04	6.8749	0.1075	0.8629
22.05	6.8749	0.1075	0.8629
22.07	6.8771	0.1075	0.8629
22.09	6.8749	0.1075	0.8629
22.10	6.8771	0.1075	0.8629
22.12	6.8771	0.1075	0.8629
22.14	6.8749	0.1205	0.8629
22.16	6.8749	0.1205	0.8629
22.17	6.8727	0.1205	0.8629
22.19	6.8749	0.1205	0.8629
22.21	6.8727	0.1205	0.8629
22.22	6.8749	0.1205	0.8629
22.24	6.8727	0.1205	0.8629
22.26	6.8749	0.1205	0.8629
22.28	6.8749	0.1205	0.8629
22.29	6.8771	0.1205	0.8629
22.31	6.8760	0.1205	0.8629
22.33	6.8749	0.1205	0.8629
22.34	6.8749	0.1334	0.8629
22.36	6.8749	0.1334	0.8629
22.38	6.8749	0.1334	0.8629
22.40	6.8749	0.1334	0.8629
22.41	6.8749	0.1334	0.8629
22.43	6.8749	0.1334	0.8629
22.45	6.8727	0.1334	0.8629
22.47	6.8727	0.1334	0.8629
22.48	6.8749	0.1334	0.8629
22.50	6.8749	0.1334	0.8629
22.52	6.8749	0.1334	0.8629
22.53	6.8749	0.1334	0.8629
22.55	6.8771	0.1334	0.8629
22.57	6.8749	0.1334	0.8629
22.59	6.8749	0.1334	0.8629
22.60	6.8749	0.1334	0.8629
22.62	6.8749	0.1334	0.8629
22.64	6.8749	0.1334	0.8629
22.66	6.8749	0.1334	0.8629
22.67	6.8749	0.1334	0.8629
22.69	6.8749	0.1334	0.8629
22.71	6.8771	0.1334	0.8629
22.72	6.8771	0.1334	0.8629
22.74	6.8771	0.1334	0.8629
22.76	6.8771	0.1456	0.8629

Time	pH	Acid	Alkali
h	units	mL	mL
22.78	6.8749	0.1456	0.8629
22.79	6.8749	0.1456	0.8629
22.81	6.8749	0.1456	0.8629
22.83	6.8749	0.1456	0.8629
22.84	6.8749	0.1456	0.8629
22.86	6.8771	0.1456	0.8629
22.88	6.8771	0.1456	0.8629
22.90	6.8771	0.1456	0.8629
22.91	6.8771	0.1456	0.8629
22.93	6.8760	0.1456	0.8629
22.95	6.8794	0.1456	0.8629
22.97	6.8771	0.1456	0.8629
22.98	6.8771	0.1456	0.8629
23.00	6.8771	0.1456	0.8629
23.02	6.8794	0.1456	0.8629
23.03	6.8760	0.1581	0.8629
23.05	6.8749	0.1581	0.8629
23.07	6.8749	0.1581	0.8629
23.09	6.8727	0.1581	0.8629
23.10	6.8749	0.1581	0.8629
23.12	6.8749	0.1581	0.8629
23.14	6.8749	0.1581	0.8629
23.15	6.8749	0.1581	0.8629
23.17	6.8771	0.1581	0.8629
23.19	6.8749	0.1581	0.8629
23.21	6.8749	0.1581	0.8629
23.22	6.8771	0.1581	0.8629
23.24	6.8771	0.1581	0.8629
23.26	6.8771	0.1581	0.8629
23.28	6.8771	0.1581	0.8629
23.29	6.8771	0.1581	0.8629
23.31	6.8749	0.1581	0.8629
23.33	6.8749	0.1581	0.8629
23.34	6.8749	0.1581	0.8629
23.36	6.8771	0.1581	0.8629
23.38	6.8749	0.1581	0.8629
23.40	6.8771	0.1581	0.8629
23.41	6.8794	0.1581	0.8629
23.43	6.8749	0.1704	0.8629
23.45	6.8771	0.1704	0.8629
23.47	6.8771	0.1704	0.8629
23.48	6.8771	0.1704	0.8629
23.50	6.8771	0.1704	0.8629
23.52	6.8749	0.1704	0.8629
23.53	6.8760	0.1704	0.8629
23.55	6.8749	0.1704	0.8629
23.57	6.8771	0.1704	0.8629
23.59	6.8760	0.1704	0.8629
23.60	6.8771	0.1704	0.8629
23.62	6.8771	0.1704	0.8629
23.64	6.8760	0.1704	0.8629
23.65	6.8771	0.1704	0.8629
23.67	6.8771	0.1704	0.8629

DATA AND SAMPLE CALCULATIONS

Time h	pH units	Acid mL	Alkali mL
23.69	6.8749	0.1704	0.8629
23.71	6.8771	0.1704	0.8629
23.72	6.8749	0.1704	0.8629
23.74	6.8771	0.1704	0.8629
23.76	6.8749	0.1704	0.8629
23.78	6.8771	0.1704	0.8629
23.79	6.8749	0.1704	0.8629
23.81	6.8794	0.1704	0.8629
23.83	6.8771	0.1704	0.8629
23.84	6.8771	0.1704	0.8629
23.86	6.8771	0.1704	0.8629
23.88	6.8760	0.1794	0.8629
23.90	6.8749	0.1794	0.8629
23.91	6.8771	0.1794	0.8629
23.93	6.8771	0.1794	0.8629
23.95	6.8771	0.1794	0.8629
23.96	6.8771	0.1794	0.8629
23.98	6.8771	0.1794	0.8629
24.00	6.8771	0.1794	0.8629

Gas Production Data

Table F-1: Gas Compositional Analysis

Time	Area	Composition			Area Units		
h	units	%N ₂	%CH ₄	%CO ₂	N ₂	CH ₄	CO ₂
0.00	35671	55.08	0.00	44.92	19648	0	16023
1.92	37607	50.83	3.39	45.78	19116	1275	17216
2.92	33983	48.86	5.81	45.33	16604	1974	15404
4.17	34971	44.47	7.88	47.65	15552	2756	16664
5.17	34506	41.09	9.80	49.11	14179	3382	16946
9.17	33935	32.46	16.82	50.73	11015	5708	17215
10.17	33188	31.74	17.94	50.33	10534	5954	16704
12.17	33272	30.05	20.22	49.74	9998	6728	16549
23.42	31874	32.97	25.09	41.93	10509	7997	13365
24.00	32121	22.19	27.49	50.15	7128	8830	16109

Table F-2: Gas Production

Time	Total Gas	N ₂	CH ₄	CO ₂
h	mL	mL	mL	mL
0.00	0	0	0	0
1.92	0	0	0	0
2.92	100	49	6	45
4.17	130	58	10	62
5.17	170	70	17	83
9.17	320	104	54	162
10.17	356	113	64	179
12.17	426	128	86	212
23.42	676	223	170	283
24.00	692	154	190	347

Biomass Data

Table F-3: Biomass Content

Crucible	Empty	Dry	Ignited	TS	VS
	g	g	g	g	g
1	42.8719	43.2359	43.0310	0.3640	0.2049
2	41.0464	41.4119	41.2036	0.3655	0.2083
3	42.6737	43.0251	42.8249	0.3514	0.2002
Average	42.1973	42.5576	42.3532	0.3603	0.2045
*TS and VS values reported here were calculated for a 20 mL sample of sludge.					

Methane Production

Table F-4: Titrimetric Method

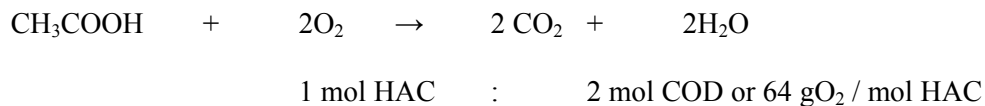
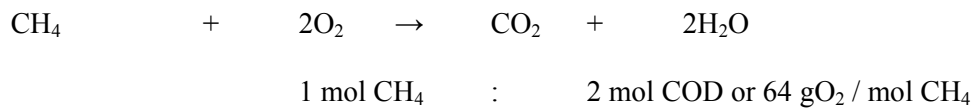
Time	Cumulative Gas	Titrant Dosed	Acetate	Methane
h	mL	mL	mol	mL
0.00	0	0.00	0.00E+00	0.00E+00
1.92	0	0.00	0.00E+00	0.00E+00
2.92	100	0.00	0.00E+00	0.00E+00
4.17	130	0.00	0.00E+00	0.00E+00
5.17	170	0.00	0.00E+00	0.00E+00
9.17	320	0.41	2.07E-04	4.64E+00
10.17	356	0.16	8.03E-05	1.80E+00
12.17	426	0.24	1.22E-04	2.74E+00
23.42	676	0.04	2.16E-05	4.84E-01
24.00	692	0.00	0.00E+00	0.00E+00

Table [F-4] presents the method for methane gas estimation using the titration data from the data tables. In **Table [F-4]**, the ‘**Titrant Dosed**’ column tabulates the volume of alkaline or acid titrant dosed in that period. Normally acid titration volumes would be used to determine methane gas production, however in this test alkaline titration dominated throughout the duration of the test (refer: Effluent Study). It was assumed that the alkaline titration was indirectly related to acetate production (refer: [section](#)).

The ‘**Acetate**’ column was obtained by determining the moles of alkali titrated into the reactor.

$$\begin{aligned}
 [\text{Alkali}] &= 0.5 \text{ M} \\
 [] &= n / V \\
 n &= (0.5)(0.41/1000) \quad [\text{mol/L}].L \\
 &= 2.07\text{E-}04 \quad [\text{mol}]
 \end{aligned}$$

The ‘**Methane**’ column was calculated by relating the amount of acetate converted to COD.



Thus, 1 mol HAC : 1 mol CH₄

Further (at STP), 1 mol COD : 350 mL CH₄ (Speece, 1996)

$$\begin{aligned}
 V &= (2.07\text{E-}04)(64)(350) \quad [\text{mol HAC}][\text{gO}_2 / \text{mol HAC}][\text{mL CH}_4 / \text{gO}_2] \\
 &= 4.64 \quad [\text{mL CH}_4]
 \end{aligned}$$

Table F-5: Gas Displacement Method

Time	Methane	
	GC Injection	Cumulative Total
h	mol / 30μL	mol
0.00	0.00E+00	0.00E+00
1.92	6.37E-07	0.00E+00
2.92	9.87E-07	0.00E+00
4.17	1.38E-06	5.97E-03
5.17	1.69E-06	9.58E-03
9.17	2.85E-06	3.04E-02
10.17	2.98E-06	3.53E-02
12.17	3.36E-06	4.78E-02
23.42	4.00E-06	9.01E-02
24.00	4.42E-06	1.02E-01

Using the compositional analysis presented in **Table [F-1]** in conjunction with the GC calibration curves (refer: **APPENDIX B**), it was possible to obtain the values tabulated in the ‘**GC Injection**’ column. The ‘**Cumulative Total**’ column was obtained by using the cumulative gas production values presented in **Table [F-4]**.

Estimating Substrate Usage

Sometimes, alkaline titration masks methanogenic activity i.e.: the production of methane gas occurs simultaneously with hydrolytic and fermentative processes. In this period the system cannot track methanogenic activity since no acid titration occurs. However, if it is assumed that alkaline titration is related to acetate production, then it is possible to determine the fraction of the substrate utilised prior to the system titrating acid. Specifically, the methane gas produced in this period is related to the fraction of the substrate already hydrolysed to acetate while the alkaline titration is associated with the fraction of the substrate that is still in the process of being degraded (refer: **Figure [4-8], Plot D**). The figure clearly shows that in the time interval $t = [4, 16]$, methanogenic activity was masked by alkaline titration.

Table F-6: Estimating Substrate Utilisation

Time	Methane	Titrant		Total	Substrate
h	gO ₂	mol	gO ₂	gO ₂	% Used
4.17	0.38	0.00E+00	0.00E+00	0.38	7.64
5.17	0.61	0.00E+00	0.00E+00	0.61	12.26
9.17	1.95	2.07E-04	1.33E-02	1.96	39.23
10.17	2.26	2.88E-04	1.84E-02	2.28	45.59
12.17	3.06	4.10E-04	2.62E-02	3.08	61.67
16.00	*	*	1.12E+00	4.21	84.10

*It was not possible to approximate these values

Using the values in **Table [F-5]** the ‘**Methane**’ column in **Table [F-6]** was drawn:

$$\begin{aligned}
 & 1 \text{ mol CH}_4 \quad : \quad 2 \text{ mol COD or gCH}_4 / 4\text{gO}_2 \\
 \text{Amount of COD} & = (5.97\text{E-}03)(16)(4) \quad [\text{mol}][\text{gCH}_4 / \text{mol}][\text{gO}_2 / \text{gCH}_4] \\
 & = 0.38 \quad [\text{gO}_2]
 \end{aligned}$$

The ‘**Titrant**’ column uses values from the ‘**Acetate**’ column (refer: **Table [F-4]**) and converts those values to COD equivalent values.

$$\begin{aligned}
 & 1 \text{ mol HAC} \quad : \quad 2 \text{ mol COD or } 64 \text{ gO}_2 / \text{ mol HAC} \\
 \text{Amount of COD} & = (2.07\text{E-}04)(2*32) \quad [\text{mol HAC}][\text{gO}_2 / \text{ mol HAC}] \\
 & = 1.33\text{E-}02 \quad [\text{gO}_2]
 \end{aligned}$$

Summing both fractions i.e.: fraction of substrate converted to methane and fraction of the substrate partially degraded to acetate, the values in the ‘**Total**’ column are obtained.

$$\begin{aligned}
 \text{Fraction Converted to CH}_4 & = 1.95 \quad [\text{gO}_2] \quad \text{refer: } \mathbf{Methane} \\
 \text{Fraction Partially Degraded} & = 1.33\text{E-}02 \quad [\text{gO}_2] \quad \text{refer: } \mathbf{Titrant} \\
 \text{Total Degraded} & = 1.96 \quad [\text{gO}_2]
 \end{aligned}$$

The COD equivalent of the substrate added to the system was determined (refer: Mass Balance).

$$\begin{aligned}
 \text{Amount of Substrate} & = 5.00 \quad [\text{gO}_2] \\
 \% \text{ Substrate Used} & = ((1.96) / (5.00))(100) \\
 & = 39.23 \quad [\%]
 \end{aligned}$$

The total amount of substrate utilised in the time interval $t = [9, 12]$ was assumed to occur in the interval $t = [12, 16]$ as well. By assessing the total amount of substrate used in period $t = [9, 10]$ and $t = [10, 12]$ respectively an estimate of the amount of substrate used in time interval $t = [12, 16]$ was approximated.

t = [9, 10]:

$$\begin{aligned}
 \text{Amount of Substrate Used} & = (2.28) - (1.96) \\
 & = 0.32 \quad [\text{gO}_2]
 \end{aligned}$$

t = [10, 12]:

$$\begin{aligned}
 \text{Amount of Substrate Used} & = 0.80 \quad [\text{gO}_2]
 \end{aligned}$$

t = [12, 16]:

$$\begin{aligned}
 \text{Amount of Substrate Used} & = 0.32 + 0.80 \\
 & = 1.12 \quad [\text{gO}_2] \\
 \% \text{ Substrate Used} & = 84 \quad [\%]
 \end{aligned}$$

Mass Balance

In order to perform an accurate mass balance, the inputs and outputs of the system have to be clearly defined. Special attention must be paid to the composition of individual components e.g.: if they are composed of solid and liquid fractions and the types of each. The size effluent had one solid fraction and one liquid fraction which were individually assessed and then combined to get an overall mixture property for the effluent. Further, the size effluent was specially prepared (refer: **APPENDIX D**) to ensure a 50 % (v/v) effluent solid concentration.

Table F-7: COD Mass Balance

Component				IN	OUT		
Biomass	Size (S)	Size (L)	Acetate	Total	Methane	Measured	Total
gO ₂ /L	gO ₂ /L	gO ₂ /L	gO ₂	gO ₂	gO ₂	gO ₂	gO ₂
35.77	210.10	83.64	1.28*	14.56	6.52	7.64	14.16

*Theoretical COD Estimate (refer: **APPENDIX C**)

System Properties:

Substrate = Textile Size

(S) = Solid Fraction

(L) = Liquid Fraction

Total Volume of Substrate = 32 [mL]

Total Volume of Biomass = 240 [mL]

Amount of Methane Produced = 1.02E-01 [mol] refer: Table [F-5]

= (1.02E-01)(16)(4) [gO₂]

= 6.52 [gO₂]

All COD concentrations i.e.: biomass and textile size, were obtained from open reflux tests. The '**Measured**' value was also determined from an open reflux test performed on the reactor contents after the sample test was completed. The size effluent was prepared so that 50 % of the total volume was solids only.

Total IN = (0.240*35.77) + (0.50*0.32*210.10) + (0.50*0.32*83.64) + (1.28)

= 14.56 [gO₂]

Total OUT = 6.52 + 7.64

= 14.16 [gO₂]

Standardising Simulated Activity

Simulated activity is based on the flowrate of an alkaline solution dosed into the bioreactor. From the pump curve (refer: **APPENDIX B**) for a pump setting of 50 rpm the flowrate of alkali is 0.070 mL/s. The concentration of alkali used for the calibration step was 0.50 M or meq/mL (refer: **Table F-8**)

Table F-8: Simulated Activity Data

Pump Setting	Flowrate	Alkali	Simulated Activity	
			rpm	mL/s
50	0.070	0.50	0.035	23.87

Therefore,

$$\begin{aligned} \text{Simulated Activity} &= (0.070 \times 0.50) && \text{[mL/s][meq/mL]} \\ &= 0.035 && \text{[meq/s] or [mmol/s]} \end{aligned}$$

The addition of alkali into the bioreactor during the calibration test is tracked by MAIA with the titration of acetic acid. Consequently, Measured Activity (A_m) is based on the titration of acetic acid. However, Simulated Activity (A_s) is only meaningful if it is standardised to biomass content and the titration of acetic acid. The average volatile solids content of the biomass over a six month period was determined to be 0.6756 g for a 20 mL sample. The volume of biomass added to the bioreactor for a typical MAIA test is 240 mL. Based on the biomass study result, a 240 mL sample of biomass should contain 8.1072 g of volatile solids. Therefore simulated activity based on biomass content for a typical MAIA test is:

$$\begin{aligned} \text{Simulated Activity} &= (0.035 \times 86\,400 \times 64) / (1000 \times 8.1072) \\ &= 23.87 && \text{[gCOD/gVS/d]} \end{aligned}$$

where:

$$\begin{aligned} 1 \text{ d} &= 86\,400 && \text{[s]} \\ 1 \text{ mole Acetic Acid} &= 64 && \text{[gCOD]} \end{aligned}$$

APPENDIX G