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Investigation into the effect of stripped gas liquor on the anaerobic digestion of Fischer-Tropsch reaction water

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As the candidate's Supervisor I agree/ do not agree to the submission of this dissertation.

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Abstract

The Fischer-Tropsch reaction technology is utilised in Sasol's Coal-to-liquid plant to produce liquid fuels from low grade coal. There are several processes on the Coal-to-liquid plant that generate aqueous streams which contain a high organic load and require treatment. The main contributors to the wastewater are the Phenosolvan plant, producing stripped gas liquor (SGL), and the Synthol plant, producing Fischer-Tropsch reaction water (FTRW). Stripped gas liquor contains water, organic acids, ammonia, and potentially toxic phenols. Fischer-Tropsch reaction water contains volatile fatty acids and alcohol. Stripped gas liquor is therefore nitrogen-rich while FTRW is nitrogen-deficient and requires nutrient supplementation for anaerobic treatment. Therefore co-treatment of the two streams could reduce nitrogen supplementation requirements.

This study is part of a larger project to determine the feasibility of anaerobically co-digesting FTRW and SGL.

This study has looked at the influence of SGL on the methanogenic activity of FTRW-acclimated sludge and involved the development of a method which allows accurate recording of the methanogenic activity in batch assays. Other studies involving the anaerobic digestion of high phenolic wastewaters showed that the phenol had an inhibitory effect on the specific methanogenic activity of the sludge, which was not acclimated to the phenol. The objective of this work was to test the hypotheses that (1) anaerobic sludge acclimated to FTRW will be inhibited by high molecular weight organics in SGL and (2) FTRW-acclimated sludge will not degrade phenolic compounds in SGL. This information will be used for designing process configurations for simultaneous treatment of the two streams with minimum contamination of the effluent stream.

The serum bottle was used as a small batch reactor and the biogas production was monitored as an indication of the state of the reaction. The biogas produced was collected and measured by the downward displacement of a sodium hydroxide solution, which absorbed the carbon dioxide and collected only the methane. A concentration of 1 g COD/ℓ FTRW was chosen as the reference test due to the reproducibility of the replicates within each experiment as well as its reproducibility across different batches of sludge. For the first inhibition test, the test units contained an additional 5% SGL (0.05 g COD/ℓ SGL) and an additional 15% SGL (0.15 g COD/ℓ SGL, i.e. 13% of the total COD load) respectively, added to 1 g COD/ℓ

FTRW. The 5% SGL test unit showed no inhibition compared to the reference unit. There was a reduction in the specific methanogenic activity of the 15% SGL test units compared to the reference unit. Since the total COD load was not the same in each unit, it cannot be conclusively stated that the SGL was responsible for the reduction in SMA, but this seems a reasonable possibility in the light of results from the reference test selection experiments which showed higher SMA at higher organic loading rates.

For the second inhibition test, the test units contained 85% FTRW (0.85 g COD/ℓ FTRW) and 15% SGL (0.15 g COD/ℓ SGL) to make up a total COD load of 1 g COD/ℓ. There was an increase in the specific methanogenic activity of the test unit compared to the reference unit. There was very little change in the phenol concentration.

Therefore, it was concluded the addition of SGL potentially reduced the SMA and that this could be an inhibitory effect, but that any inhibition would be a function of the concentration of potentially inhibitory substances in SGL and that these concentrations vary from batch to batch. However, the degree of SMA reduction is fairly low and would not prevent co-digestion of the two streams at the concentrations tested. It has been shown that FTRW anaerobic digestion can proceed adequately in the presence of SGL. There was some evidence that phenolics were degraded but at a much slower rate than COD. The percentage reduction in SMA due to additional SGL at concentrations and SGL:FTRW ratios tested was between 0 and 51%.

Ultimately, this work is a first step in the development of a co-digestion model relating organic loading rate, SGL:FTRW feed ratio to methane recovery and extent of biodegradation of phenol for use in the design and optimization of a co-digestion system.

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COD	–	Chemical oxygen demand
FAS	–	Ferrous ammonium sulphate
FTRW	–	Fischer-Tropsch reaction water
LCFA	–	Long chain fatty acids
SGL	–	Stripped gas liquor
SMA	–	Specific methanogenic activity
TSS	–	Total suspended solids
UASB	–	Upflow anaerobic sludge blanket
VFA	–	Volatile fatty acids
VSS	–	Volatile suspended solids

CHAPTER 1

1. Introduction

Sasol Technology Research and Development Environmental Science and Engineering are looking into the feasibility of anaerobically co-digesting two of the wastewater streams produced at the plant, that is, Fischer-Tropsch reaction water (FTRW) and stripped gas liquor (SGL). The logic behind this approach is that the Fischer-Tropsch reaction water is a nitrogen-deficient stream requiring nitrogen supplementation for anaerobic digestion, while the stripped gas liquor stream has significant concentrations of ammonia that could supply this nitrogen requirement.

In this Masters of Science in Chemical Engineering research project, the ratios of stripped gas liquor and Fischer-Tropsch reaction water at which biodegradation under anaerobic conditions is possible is investigated and it is determined whether there is any inhibition of the anaerobic digestion of Fischer-Tropsch reaction water caused by the stripped gas liquor and to what extent the organics in stripped gas liquor degrade using Fischer-Tropsch reaction water acclimatised sludge. In this Chapter, the project is put into perspective. The context and significance of the project within the Sasol plant is discussed.

1.1. Context of the Study

The Fischer-Tropsch reaction technology is utilised in Sasol's Coal-to-liquid (CTL) plant in order to produce liquid fuels from low grade coal. There are several processes on the Coal-to-liquid plant that generate aqueous streams which contain a high organic load and are required to be treated. The main contributors to the wastewater are the Phenosolvan plant, which produces stripped gas liquor (SGL), and the Synthol plant, where the Fischer-Tropsch reaction water (FTRW) is a by-product.

Stripped gas liquor from Phenosolvan's stripping unit contains water, organic acids, ammonia, as well as phenolic compounds. The Fischer-Tropsch reaction water contains volatile fatty acids and alcohol, of which acetic acid is a major portion.

Stripped gas liquor is therefore nitrogen-rich while Fischer-Tropsch reaction water is nitrogen-deficient and requires nutrient supplementation. The co-digestion of these two streams could reduce the nitrogen supplementation requirements. However, there is a risk that the higher molecular weight organics in stripped gas liquor, such as phenols, may inhibit the anaerobic activity of the Fischer-Tropsch reaction water.

In order to determine if co-digestion of stripped gas liquor and Fischer-Tropsch reaction water is possible, the project team has developed the concept of a co-digestion design model which relates dilution rate of a continuous reactor, ratio of stripped gas liquor to Fischer-Tropsch reaction water and the percentage of COD removed. The co-digestion design model shows how a co-digestion system might behave at different loading and substrate ratios. The dilution rate and the ratio of the stripped gas liquor to Fischer-Tropsch reaction water can be varied in order to obtain a feasible co-digestion region. The feasible range is where biomass populations that can degrade the organic constituents of the substrates have been established. The boundary of the feasible range is where any one of the micro-organism subpopulation gets severely inhibited or washed out. **Figure 1.1** shows an uncalibrated co-digestion model of a hypothetical labile substrate reduction when co-digested with an inhibitory substrate at different ratios of the two substrates (shown as a fraction of the labile substrate) and hydraulic loading rate (shown as dilution rate) (Foxon, unpublished). In order to obtain a calibrated model, hundreds of experiments to obtain digestion kinetics for all load and composition points would be required. Therefore, with this research, data can be provided to design a simple model which can then be extrapolated to predict the entire co-digestion design model from which the feasible co-digestion region can be located. These results can then be used to design experiments for a continuous reactor to investigate the co-digestion of stripped gas liquor and Fischer-Tropsch reaction water.

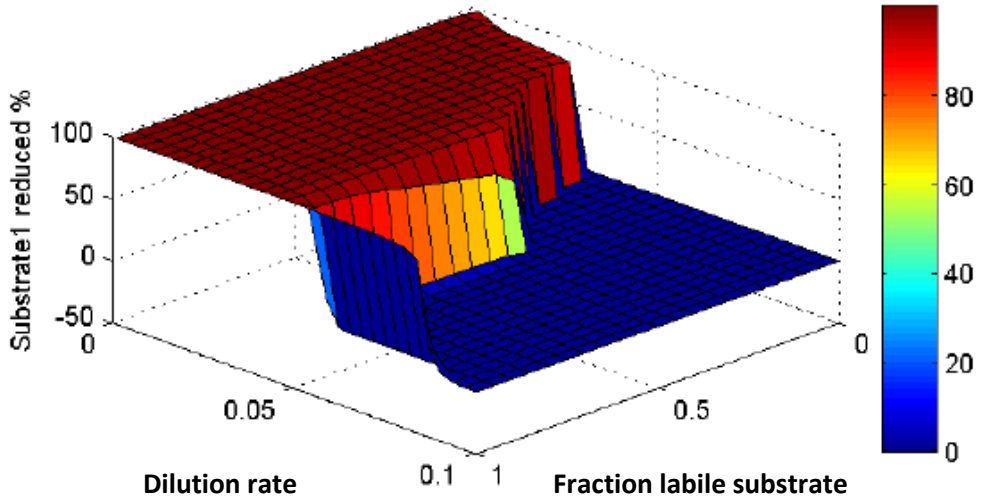

Figure 1.1: Graph showing an uncalibrated model of co-digestion a labile and inhibitory substrate (Foxon, unpublished)

The project team would ultimately like to propose a process design for the simultaneous treatment of the stripped gas liquor and Fischer-Tropsch reaction water streams with minimum contamination of the final effluent. Depending on the facility with which the streams can be co-digested, this may result in a single digester design, or two or more units in which the feasible co-digestion regions are exploited. The co-digestion design objectives of the broader project are to use the nitrogen from stripped gas liquor as a macronutrient for Fischer-Tropsch reaction water digestion, to design an anaerobic system with good organics removal efficiency, to identify the ratios of stripped gas liquor to Fischer-Tropsch reaction water which result in efficient design and to size the digesters.

This specific project will be investigating the conditions at the edge of the co-digestion model where the fraction of the labile substrate (Fischer-Tropsch reaction water) is near 1 and the fraction of the inhibitory substrate (stripped gas liquor) is relatively small. The effect of the stripped gas liquor on the anaerobic digestion of the Fischer-Tropsch reaction water will be investigated. This project is a preliminary study which is not focused on obtaining high precision kinetic and stoichiometric parameter values. Rather it concentrates on determining the extent to which the specific methanogenic activity changes when stripped gas liquor is anaerobically co-digested with the Fischer-Tropsch reaction water, compared to that when

only Fischer-Tropsch reaction water is digested. The fate of indicator compounds for potentially toxic higher molecular weight organics in the stripped gas liquor, such as phenols, will also be studied. This project is the first step towards answering the questions around the feasibility of co-digestion of Fischer-Tropsch reaction water and stripped gas liquor.

1.2. Significance of the Study

The significance of co-digesting Fischer-Tropsch reaction water and stripped gas liquor is to obtain a higher biodegradability of both wastewater streams. The emission of the nutrients, such as nitrogen, available in the stripped gas liquor, into the environment will be reduced and the nitrogen will instead be used to supplement the nitrogen-deficient Fischer-Tropsch reaction water reducing the need and cost of adding nutrients.

The wastewater streams from the Sasol processes are currently treated, for reuse as process cooling water, in an activated sludge process (Reddy, 2008). However, this process has many drawbacks. Since this process is aerobic, it requires the system to be continuously aerated to supply the micro-organisms with the oxygen they require for the breakdown of the organic matter. Aeration utilises large quantities of energy which increases the operating cost of the system (Wiseman, 1978). This process also produces large amounts of sludge which has to be handled and disposed of (Crueger et al., 1990).

Therefore the use of anaerobic digestion as a feasible option to treat the wastewater should be investigated. Anaerobic digestion will cut down significantly on the costs of treating the wastewater. This process does not require aeration therefore saving operating costs of an aeration system. The biomass production is much less than that for aerobic digestion therefore reducing handling costs (Crueger et al., 1990). Methane is also produced which can be used for electricity, heat or steam generation (Wiseman, 1978). However, anaerobic digestion is extremely sensitive to inhibition by various factors including the concentration of dissolved hydrogen, pH and nutrients therefore it is important to find a set of operating conditions that give stable operation (Batstone et al., 2002).

However the disadvantage of co-digesting Fischer-Tropsch reaction water and stripped gas liquor is that the higher molecular weight organics, such as phenols, in stripped gas liquor may reduce biological activity and therefore affect the capacity of the treatment plant. The

effluent that will be released to the environment may be contaminated with more toxic components, such as phenolics, than if Fischer-Tropsch reaction water was treated alone, therefore adversely affecting the system.

With the knowledge of whether Fischer-Tropsch reaction water and stripped gas liquor can be co-digested and in which ratios, a process design can be recommended to treat both the streams simultaneously with minimum contamination of the effluent stream, thus improving the efficiency and the profitability of the unit.

CHAPTER 2

2. Theory and Literature Review

In chapter 2, the relevant literature around the subject and state-of-the-art in terms of anaerobic digestion science and technology is presented and reviewed. In sections 2.1 and 2.2, the substrates used in the project are discussed, giving the origin of the substrate and its composition. In sections 2.3 and 2.4, the anaerobic digestion process is outlined. In section 2.5, co-digestion is defined and the reasons for co-digesting substrates are discussed. In section 2.6, the technique used to measure the biodegradability of the substrates is presented. It covers findings and protocols of other authors who have used the serum bottle test. In section 2.7, biogas collection and measurement techniques used in literature and the source of errors associated with the techniques is discussed. In sections 2.8 and 2.9, the anaerobic digestion of high alcohol wastewater and phenolic compounds is reviewed, respectively.

2.1. Fischer-Tropsch Reaction Water

The Synthol process is based on the Fischer-Tropsch process. In the Fischer-Tropsch process, carbon monoxide reacts with hydrogen over an iron or cobalt catalyst in order to produce hydrocarbons which can be used as fuels (Brady, 1981). The mixture of carbon monoxide and hydrogen is known as synthetic gas or syngas. Syngas is produced from the conversion of coal by coal gasification.

One of the by-products of the Fischer-Tropsch process is the reaction water. The reaction water contains volatile fatty acids and alcohol, of which the major portion is acetic acid (Reddy, 2008).

2.2. Stripped Gas Liquor

Gas liquor from the Tar Separation Units and other phenolic streams are sent to the Phenosolvan plant where they are used to recover valuable phenols and naphthas. The Phenosolvan plant consists of stripping units. The stripped gas liquor from the stripping units contains water, carbon dioxide, organic acids, nitrogen in the form of ammonia and some toxic phenols. The phenols include mono-hydric phenols as well as poly-hydric phenols. Examples of mono-hydric phenols are cresols, xylenols and ethyl phenols which contain one hydroxyl group each. The poly-hydric phenols have two or more hydroxyl groups, such as catechols and resorcinols. The composition of stripped gas liquor is not restricted to the components listed above as different companies have different coal supplies, gasification processes and conditions therefore causing the stripped gas liquor components to vary (Beychok, 1975).

2.3. Anaerobic Digestion

Anaerobic digestion is the consecutive biochemical breakdown of complex organic matter to methane, carbon dioxide, hydrogen and biomass, by a large range of organisms in an oxygen free environment.

There are several important advantages of using anaerobic digestion rather than aerobic digestion. Since the process takes place in the absence of oxygen, it does not require the expenditure of energy to aerate the system (Crueger et al., 1990). The anaerobic microorganisms are quite energy efficient and consequently exhibit a minimal energy exchange. Therefore the energy available for the growth of new cells is low and the production of biomass by anaerobic digestion is far less than that by the aerobic treatment. This significantly reduces the cost of solids handling and disposal (Crueger et al., 1990). Rather than being used to generate more organisms, the organic matter is converted to biogas, which is composed of methane and carbon dioxide. The methane generated by this process can be used as a source of energy.

The sequence of stages in the anaerobic process is: disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis and is presented in **Figure 2.1**.

- **Disintegration**

Disintegration is the process whereby the complex organic matter in the stream being treated is broken down, mechanically and by enzymes, into simpler substances including carbohydrates, proteins, lipids and inert compounds (Batstone et al., 2002).

- **Hydrolysis**

The carbohydrates, proteins and lipids are then broken down by extracellular hydrolases to monosaccharides, amino acids and long chain fatty acids respectively (Aldin, 2010). The enzymes which catalyse the process are produced by specific organisms which benefit from the hydrolysis products of certain compounds (Batstone et al., 2002).

- **Acidogenesis**

In this stage, acidogenic bacteria convert the products from the hydrolysis reaction, that is, monosaccharides and amino acids to short chain fatty acids (volatile fatty acids), releasing carbon dioxide and hydrogen (Crueger et al., 1990). Examples of the volatile fatty acids produced are acetic acid (CH_3COOH), propionic acid (CH_3CH_2COOH) and butyric acid ($CH_3CH_2CH_2COOH$). In wastewater treatment, organic acids are conventionally called volatile fatty acids and their common names (mentioned above) are preferred, rather than their IUPAC names (ethanoic acid, propanoic acid and butanoic acid, respectively).

- **Anaerobic oxidation**

Anaerobic oxidation is the conversion of long chain fatty acids, $CH_3(CH_2)_nCOOH$, to volatile fatty acids, namely, acetic acid and propionic acid. The process also releases hydrogen. If the "n" in the formula is an even number then the product is purely acetic acid. However if "n" is an odd number then propionic acid is also produced. In this case, one mole of substrate will generate one mole of propionic acid (Stieb et al., 1985).

- **Acetogenesis**

Acetogenesis is the process whereby the higher volatile fatty acids, that is, propionic acid and butyric acid, produced by the acidogenesis and anaerobic oxidation steps, are converted to acetic acid (Crueger et al., 1990). Although acidogenesis also produces small amounts of acetic acid and hydrogen, the bulk of these two products are obtained from the acetogenesis stage.

- **Homoacetogenesis**

In this process the hydrogen produced by the preceding stages, namely, acidogenesis, anaerobic oxidation and acetogenesis, reacts with carbon dioxide to produce acetic acid (Batstone et al., 2002).

- **Methanogenesis**

Methanogenesis is the final and most important stage of anaerobic digestion as it is the stage which removes COD from the stream being treated. Methane can be produced by methanogens by two different processes, namely, acetoclastic methanogenesis or hydrogenotrophic methanogenesis.

  - **Acetoclastic methanogenesis**

In this process, acetoclastic methanogens convert the acetic acid, produced by acidogenesis, anaerobic oxidation and acetogenesis, to methane and carbon dioxide (Batstone et al., 2002).

  - **Hydrogenotrophic methanogenesis**

This is an additional process that occurs when carbon dioxide and the dissolved hydrogen, produced by the system, react to produce methane and water (Goldman et al., 2009).

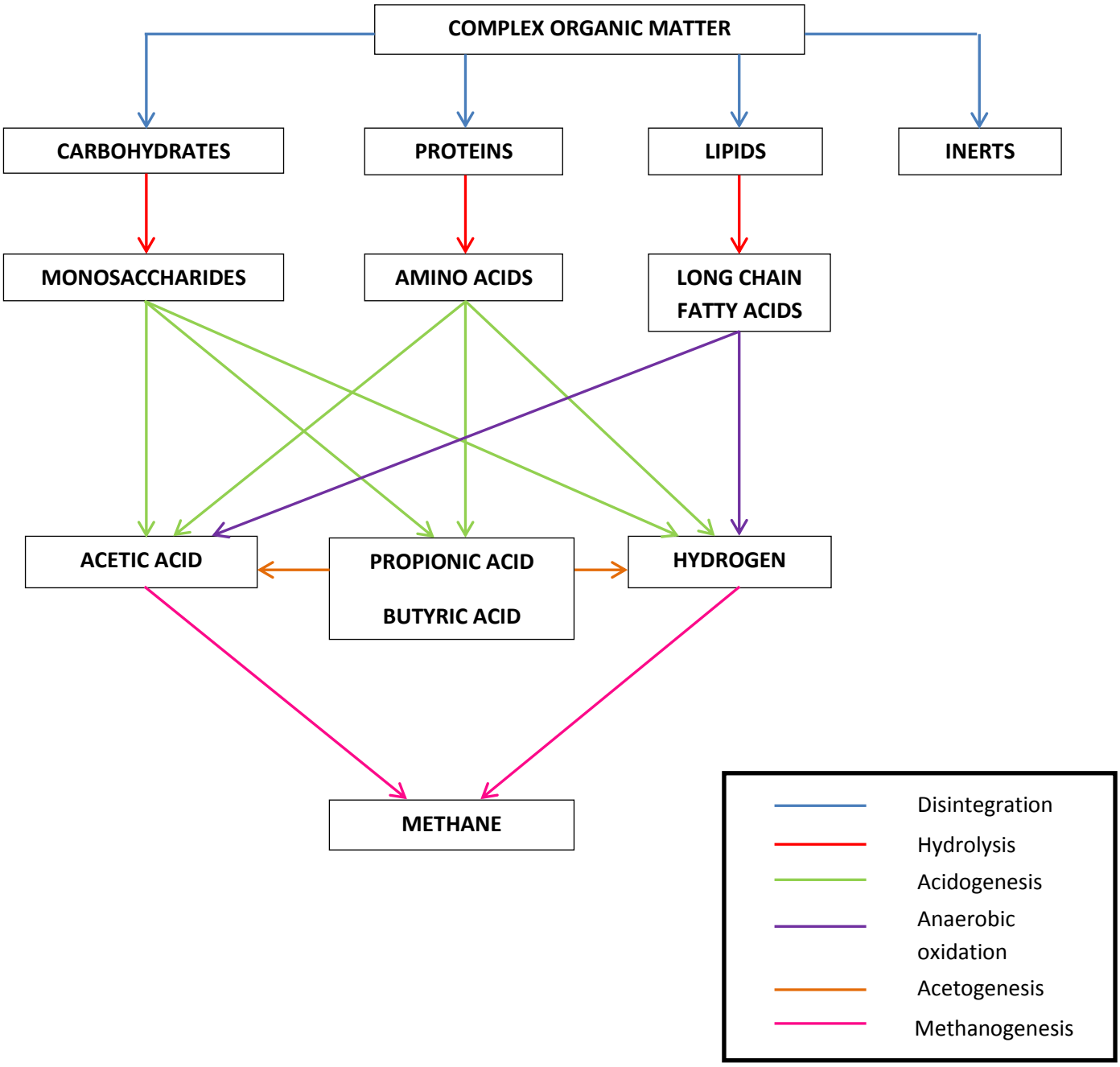

Figure 2.1: Flow diagram of the stages involved in anaerobic digestion (Adapted from Batstone et al., 2002)

2.4. Factors Affecting Anaerobic Digestion

The stability of the anaerobic digestion process is very sensitive to changes in operating parameters. Therefore factors such as the temperature, pH, nutrient composition and free acids and bases need to be monitored closely and controlled to prevent upsets in the system.

There are two types of inhibition, biocidal inhibition and biostatic inhibition (Batstone et al., 2002). Biocidal inhibition is caused by a reactive toxicity and this inhibition is usually irreversible. This means that the system will be unable to recover from the toxicity and cannot be re-established. Biocidal inhibitors include long chain fatty acids, aldehydes, cyanide etc. Biostatic inhibition is caused by a non-reactive toxicity and the growth ceases only temporarily. Once the conditions are favourable again, growth will resume. Examples of biostatic inhibition are pH inhibition, product inhibition and weak acid or base inhibition (Batstone et al., 2002).

2.4.1. Temperature

The temperature of the system can affect the reaction rate, decay rate and biomass yield. As the temperature increases, the reaction rate increases following the Arrhenius equation until the temperature optimum is reached. When the temperature increases above 40°C for mesophilic the reaction rate decreases and quickly drops to zero (Batstone et al., 2002).

Temperature changes also affect the pH value of the system. The methanogenic bacteria are very sensitive to changes in temperature (Dhadse et al., 2012). Therefore even low temperature changes impacts on the metabolic rate of the methanogens. This results in the accumulation of volatile fatty acids which lowers the pH.

2.4.2. pH

The optimal pH range for the anaerobic digestion process is generally taken to be between 6.5 and 7.5 although on a digester scale the range can be relatively wide and dependent on the substrate, the method and stage of digestion and the microorganisms present (Liu et al., 2007). Methanogenesis is extremely sensitive to changes in the pH value of the system. When the pH drops below 6.5 all processes, including methanogenesis, are inhibited. Therefore, because of the inhibition of methanogenesis, the volatile fatty acids, which are continuously

produced by acidogenesis, are not being converted to methane causing a buildup of acids and lowering the pH further (Conklin et al., 2008). This cycle continues until methanogenesis is inhibited entirely.

2.4.3. Weak acids and bases

Free acids and bases can pass through the cell membrane passively. Once the acid or base is in the cell, it dissociates thereby changing the pH and disrupting the cell homeostasis. The amount of free acids and bases are affected by the pH therefore the extent of the weak acid/base inhibition also depends on the pH. At lower pH values, inhibition is caused by free acids such as H_2S and free organic acids (e.g. acetic, propionic, butyric and valeric acids) with pK_a values from 4.7 – 4.9. While at higher pH values, free bases (e.g. free ammonia which is the main free base in anaerobic digesters and has a pK_a value of 9.25) cause inhibition (Batstone et al., 2002).

2.4.4. Nutrients

Micro-organisms require nutrients and trace metals in order to create new cells and to grow. The nutrients are only required in small quantities since the biomass production is low. However if the necessary nutrients are not available, the biodegradability of the substrate may be affected. The most important nutrients are nitrogen, phosphorus, sulphur and iron (Lettinga, 1995).

2.5. Co-digestion

Co-digestion is the simultaneous digestion of two different waste streams in the same digester making the disposal of challenging effluents possible without reducing the performance of the digester (Wu, 2007).

Co-digestion could be advantageous as it can enhance the digestion of the substrates and improve the biogas yield, by (Remigi et al., 2006; Wu, 2007):

- Providing an essential material or nutrient which may be lacking in one of the substrates, but present in the other

- Using a non-toxic stream to dilute a toxic stream to a concentration which does not inhibit the activity of the micro-organisms
- Improving the buffer capacity of the system

Unfortunately this process also has some disadvantages (Wu, 2007):

- One of the substrates may introduce toxic components to the non-toxic stream which cannot be degraded in the digester
- Biological activity may be reduced or completely inhibited
- Additional pre-treatment may be required

2.6. Inhibition

Inhibition is usually indicated by a decrease of the methane gas production rate and is caused by inhibitory substances such as ammonia, sulphide, heavy metals or organic chemicals which shift the microbial population or hinder bacterial growth. There are several mechanisms of inhibition (Chen et al., 2007):

- Free ammonia is hydrophobic and diffuses passively into the cell, causing proton imbalance, changing the intracellular pH and inhibiting specific enzyme reaction.
- In anaerobic digestion, sulphate is reduced to sulphide, by sulphate-reducing bacteria, which is toxic to various types of bacteria. There is competition for common organic and inorganic substrates from the sulphate-reducing bacteria, which reduces methane production. Also, H_2S, which is a toxic form of sulphide, can diffuse into the cell membrane and denature the proteins.
- Ions such as calcium, potassium, aluminium and sodium are important nutrients necessary for microbial growth. However, excessive amounts of these ions slow down and even inhibit microbial growth. Aluminium bonds to microbial cell membranes and affects its growth. With regards to the calcium ion, precipitation of carbonate and phosphate causes scaling of the biomass and reduces methanogenic activity. Potassium ions diffuse passively into the cell and neutralize the membrane potential.
- Heavy metals, such as iron, cobalt, copper and zinc, bind with thiol and other groups on protein molecules or replace naturally occurring metals and disrupt enzyme functions.

- Organic chemicals which are poorly soluble in water or are adsorbed onto the surfaces of sludge solids, such as alkyl benzenes, halogenated benzenes and phenol, accumulate in bacterial membranes and cause damage to the cell membrane.

2.7. Serum Bottle Test

The anaerobic activity of micro-organisms can be measured using a serum bottle test. The serum bottle test is simple to use and inexpensive (Owen et al., 1979). The serum bottle is used as a small batch reactor where the state of the reaction is monitored by biogas production.

Owen et al. (1979) presented techniques to measure the biodegradability (Biochemical Methane Potential) and the toxicity (Anaerobic Toxicity Assay) of a material undergoing anaerobic digestion. The biochemical methane potential is a simple test to determine the ability of the substrate to degrade without the presence of any other test compound. The control units for this experiment contain only sludge and nutrients to determine the reference biogas production due to residue on the sludge. The test units contain sludge, nutrients and the substrate. The anaerobic toxicity assay determines the adverse effect that the test material has on the micro-organisms. The control units for this test are the same as the test units for the biochemical methane potential and contain sludge, nutrients and the substrate. This is to determine the reference activity when the test material is not present. The test units for the anaerobic toxicity assay contain sludge, nutrients, the substrate and the test material and determine the inhibition caused by the test material.

Remigi et al. (2006) investigated the co-digestion of distillery effluent and textile size effluent. A three-step protocol was developed and verified. This involved (level 1) assessing the biodegradability of the reference substrate to obtain baseline information, (level 2) adding a test substrate to determine the effectiveness of co-digestion and finally (level 3) applying the acquired data to start-up a semi-continuous co-digestion experiment. It was found that the serum bottle test is a very accurate and reliable method for small-scale batch assays.

Shelton and Tiedjie (1984) investigated the ability of sludge from nine different municipal treatment plants to degrade nine different chemicals of varying susceptibility to degradation using the serum bottle test. The gas production was measured using a pressure transducer and multimeter. The excess gas was vented through a three-way valve. The sludges varied in

which substrates they degraded however no correlation with the waste properties could be determined. It was found that the results were reproducible and that triplicate bottles were sufficient to draw a conclusion about the biodegradability.

Conklin et al. (2008) used a serum bottle assay to determine the stability of the municipal wastewater sludge digester by measuring the maximum aceticlastic methane production rate and the acetate capacity number which measures the excess capacity of the digester and its capability to handle higher organic loading rates. It was concluded that the maximum acetoclastic methane production rate gives a good indication of the maximum acetate utilisation rate of the acetoclastic methanogens.

2.8. Biogas measurement techniques

Parajuli (2011) evaluated commonly used biogas measurement techniques and analysed the source of errors associated with the techniques. The liquid displacement method by downward displacement of gas was used for Parajuli's analysis. However a major drawback of the liquid displacement gas collecting and measuring system is the inaccuracy which arises from the dissolution or diffusion of the biogas carbon dioxide in the displaceable liquid. Therefore the solubility of carbon dioxide in different barrier solutions, such as simple tap water, carbonated water, acidified water and acidified brine solution, was investigated. Carbonated distilled water showed a high resistance to carbon dioxide diffusion at the beginning of the experiment; however, it also experienced a great loss of carbon dioxide as time passed. Acidified water with pH values of 2, 1 and 0.5 also did not perform well when it came to preventing carbon dioxide solubility. When an acidified brine solution was used as the barrier solution it was observed that as the concentration of salt increased, the solubility of the gas decreased and resulted in a lower gas loss. A saturated acidified brine solution showed lower carbon dioxide solubility among the tested solutions. Headspace gas chromatography analysis and its errors were also investigated. The analysis of the headspace biogas is affected by the balance between the liquid and gas phase in a closed anaerobic system. The solubility of analyte gases (methane and carbon dioxide) in the liquid phase, temperature and pressure can directly influence the balance of the gas concentration. It is a very laborious and time consuming task to maintain the syringe temperature at the experiment incubation temperature. More precise results can be achieved by the rapid injection of samples with no delaying and the use of insulated syringes for gas samples.

Carbon dioxide and methane concentrations in the biogas can also be determined without gas chromatograph analysis by using simple basic solutions such as potassium hydroxide or sodium hydroxide. The carbon dioxide will dissolve in the solution and the remaining gas can be assumed to be methane (Parajuli, 2011).

Bagudo et al. (2011) assessed the quality of biogas generated from various agricultural wastes, such as paper waste, saw dust, cow dung, rice husk and millet husk, by analysing the composition of the biogas in terms of methane, carbon dioxide and hydrogen sulphide content. Esposito et al. (2012) measured the biogas production from the digestion of two independent organic substrates (swine manure and greengrocery waste) and co-digestion of buffalo manure and maize silage. Both Bagudo et al. (2011) and Esposito et al. (2012) utilised the liquid displacement method with sodium hydroxide solution to collect and measure the biogas produced. The carbon dioxide was absorbed by the alkaline solution as shown by the following reaction (Bagudo et al., 2011):

$$CO_{2\,(g)} + 2\ NaOH_{\,(aq)} \rightarrow Na_2CO_3 + H_2O$$

The methane neither reacts nor dissolves in the alkaline solution and is collected by the downward displacement of the solution.

Walker et al. (2009) investigated the potential errors in the quantitative evaluation of biogas production. When a 3M sodium hydroxide solution was used to absorb the carbon dioxide and collect the methane, it was found that there was no observable change in the quantity of methane produced. However there was evidence of the inward diffusion of air as an air peak was observed on the gas chromatograph profile. The partial pressures of nitrogen and oxygen were lower in the collection column than in the air causing the air to enter the collection column.

2.9. Anaerobic Digestion of High Alcohol Wastewaters

Becarri et al. (2006) showed that an industrial wastewater, characterised by high organic load and high concentrations of potentially inhibiting long-chain alcohols, from a Fischer-Tropsch process, could be successfully treated under anaerobic conditions in a high rate packed bed biofilm reactor. A gradual start-up was employed in order to overcome the potential inhibition from the long-chain alcohols. The process was started with a low organic load of

3.4 g COD/ℓ/day and a modified synthetic wastewater with less or no long-chain alcohols. The organic load and the concentration of the long-chain alcohols were then increased and the COD removal, methane production and effluent concentration were monitored. It was found that the reactor could be operated up to an organic load of 20 g COD/ℓ/day with no loss of performance. A 96% COD removal and full conversion of the removed COD to methane was achieved. The effluent contained only acetic and propionic acid that were easily removed under aerobic conditions.

2.10. Anaerobic Digestion of High Phenolic Wastewaters and Phenolic Compounds

Anaerobic digester upsets and failures may be caused by a wide range of inhibitory substances which may be present in the wastewater in significant concentrations. Phenols are among those substances that are known to inhibit anaerobic processes (Chen et al., 2007).

Kayembe et al. (2013) evaluated the inhibitory effects of phenolic monomers on methanogenesis in anaerobic digestion. The effect of the number of hydroxyl groups in the aromatic structure on the inhibition of methane production by acetoclastic methanogens was also assessed. A serum bottle assay with a liquid displacement method to collect the biogas was used to observe the methane production and therefore the specific methanogenic activity. The biomass used was digested pig manure which had not been previously acclimated to any aromatic compounds. The substrate was composed of acetic acid. Naturally occurring phenolic compounds including phenol, resorcinol, hydroquinone and pyrogallol were used as the potentially inhibitory aromatic compounds and benzene was used as the reference aromatic compound. The inhibitory concentrations of benzene varied from 0 to 750 mg/ℓ. The inhibitory concentrations of phenol and resorcinol varied from 0 to 4500 mg/ℓ. The inhibitory concentrations of hydroquinone and pyrogallol varied from 0 to 10000 mg/ℓ. All of the concentrations of the aromatic compounds tested were observed to have an inhibitory effect on the specific acetoclastic methanogenic activity ranging from 0 – 100% inhibition. The concentrations of benzene, phenol, resourcinol, hydroquinone and pyrogallol which caused 50% inhibition of the methanogenic activity were 209 ± 6.32, 1249 ± 29.59, 1725 ± 5.27, 2745 ± 97.8 and 3173 ± 91.02 mg/ℓ, respectively. It was also found that as the number of hydroxyl groups on the aromatic compounds increased, the phenolic monomers

toxicity decreased. Therefore the toxicity of the phenolic monomers increases in the following order: pyrogallol < hydroquinone < resorcinol < phenol < benzene.

Fang et al. (1997) studied the effect of phenol on the methanogenic activity of three types of UASB granules, with different bacterial composition and microstructure, in batch tests. The three types of biogranules selected for these tests were from three different UASB reactors degrading acetate, propionate and benzoate individually. The batch tests were carried out in 157 mℓ serum vials placed in a 37 °C shaking water bath. The biogranules were added to the vials together with 100 mℓ of feed solution containing substrate, which was the same as the substrate used in the biogranule-cultivating reactors mentioned previously, nutrients, vitamins, trace metals and various concentrations of phenol. The control units contained a phenol-free solution. The concentrations of phenol that caused 50% inhibition of the methanogenic activity were 1750, 1000 and 1700 mg/ℓ for acetate-, propionate- and benzoate-degrading biogranules. The concentrations of phenol that caused 100% inhibition of the methanogenic activity were 3950, 2000 and 2200 mg/ℓ for acetate-, propionate- and benzoate-degrading biogranules. The specific methanogenic activities (SMA) were measured over 7 days. The toxicities of phenol were indicated by a decrease of the SMA relative to the control units. It was observed that the higher the phenol concentration, the greater the SMA reduction and therefore the higher the inhibition of the overall methanogenic activity.

Donaldson et al. (1987) evaluated a fluidised-bed bioreactor process for biooxidation of coal gasification wastewater. The wastewater used was obtained from METC (Blacksville Coal) and the majority of the sulphides and ammonia were stripped from the water using a steam stripper. The biomass used to start up the bioreactor was one that had been used in previous work. The bioreactor was started up on dilute phenol, mineral salts media and a small amount of METC water (10%). During the next 5 days, dilute phenol mixed with METC water was added to the bioreactor, first batch-wise in increasing amounts and then, after 3 days, with a dilute continuous feed. Once the bioreactor was operating in a stable manner, feed makeup was done once or twice a week which consisted of 2 ℓ METC water, 2 ℓ process water, 10 - 20 mℓ mineral salts media and 10 - 20 g of pure phenol. After approximately 3 months of operation the additional phenol was phased out. When the build-up of biomass caused the level of the fluidised-bed to become excessive about one-third of the bed volume was withdrawn. At higher concentrations of phenol (greater than 400 ppm of phenol) the growth of the biomass was slightly slower. During the stable operation of the bioreactor, its performance was monitored by analysing the phenol concentration of the feed and the

effluent wastewater streams. The phenol concentration was analysed using the 4-aminoantipyrine method and the gas chromatograph with Carbowax or cross-linked 5% phenyl column. Volumetric phenol degradation rates were calculated from the phenol concentrations at the inlet and outlet of the bioreactor, the liquid flow rate and the settled bed volume. Values in the range of 20 mg phenol/ℓ bed.min were achievable under proper operating conditions. The phenol concentration was typically reduced by 40 - 50% depending on the residence time.

Stepan (2006) investigated the technical feasibility of using an anaerobic process for the treatment of the Dakota Gasification Company (DGC) stripped gas liquor (SGL). The wastewater contained phenols, catechols, acids and alcohols. The phenolics concentration of the wastewater was 500 mg/ℓ. A sample of recycle sludge was obtained from an anaerobic digester (source of methanogenic bacteria) and was placed in a heated, mixed, glass reactor vessel for acclimation and biomass development. The SGL was added in increasing amounts to the reactor. Difficulty was experienced in establishing a high-rate catechol-degrading culture and the acclimation period was longer than expected. Three bioreactors were operated to acclimatise the biomass to the components of SGL. The reactor fed with synthetic SGL emphasising catechol showed good removals of the catechol. However, the reactors fed with actual SGL were inhibited and catechol degradation was negligible. It was suggested that the catechol may have undergone a polymerisation reaction instead of degrading and producing methane. The bioreactor system operation was continued and the feed dilution was decreased until the catechol degradation could be sustained without inhibition using full-strength SGL as the influent. The reactor feed was 5.3 ℓ/day and the gas production ranged from 3 - 4 ℓ/day. There was an improvement in the catechol degradation. The catechol degraded such that the gas produced was half of the calculated theoretical production if all of the catechol had been degraded. It was concluded that catechol can be degraded under anaerobic conditions under the right operating conditions.

2.11. Summary

Anaerobic digestion is an attractive method of degrading organic matter as it has several advantages; it does not require energy to aerate the system and it converts organic matter to methane gas which can be used as a source of energy. However factors such as temperature and pH have to be monitored closely to prevent system upsets. The serum bottle test is a

simple and reproducible technique to evaluate the anaerobic biodegradability of organic matter by monitoring the biogas production. There are different methods of collecting and measuring the biogas produced. The collection of the biogas by the downward displacement of a saturated acidified brine solution showed the lowest carbon dioxide solubility and the composition of the biogas could be analysed using a gas chromatograph. However the composition of the headspace biogas is easily altered by the solubility of analyte gases in the liquid phase resulting in inaccurate results. The use of the downward displacement of a sodium hydroxide solution eliminated the errors arising from gas chromatograph measurements by absorbing the carbon dioxide from the biogas produced, collecting only the methane.

There was no literature that provided direct information for designing a Fischer-Tropsch reaction water and stripped gas liquor co-digestion scheme. Literature did not provide an answer of whether the stripped gas liquor would inhibit Fischer-Tropsch reaction water digestion and to what extent and whether all of the components in stripped gas liquor can be degraded in an anaerobic digestion scheme with a sludge acclimated to Fischer-Tropsch reaction water only. There was no information regarding the anaerobic digestion kinetics of Sasol stripped gas liquor. However information about the anaerobic digestion of high alcohol wastewaters, similar to Fischer-Tropsch reaction water, and the anaerobic digestion of high phenolic wastewaters, similar to stripped gas liquor, independently, could be obtained.

Fischer-Tropsch reaction water, from the Synthol process, contains organic acids and alcohol. The anaerobic digestion of high alcohol wastewaters, similar to Fischer-Tropsch reaction water, was researched. Becarri et al. (2006) showed that wastewaters with a high concentration of long-chain alcohols can be successfully anaerobically digested by employing a gradual start-up to acclimate the sludge to the wastewater. Therefore it can be presumed that the Fischer-Tropsch reaction water used in this project will be readily degradable on its own, especially since the sludge was acclimated to the Fischer-Tropsch reaction water prior to the start of this project.

Stripped gas liquor, from the Phenosolvan plant, contains organic acids, ammonia and phenols. The results of anaerobic digestion of high phenolic wastewaters, such as stripped gas liquor, obtained from literature were reviewed. Kayembe et al. (2013) and Fang et al. (1997) observed that phenolic compounds tended to inhibit the specific methanogenic activity in the test units in comparison to the control units when the sludge was not acclimated to the

phenolic compounds. Since the sludge used in this project was not acclimated to the phenolic compounds in stripped gas liquor, it was postulated that these phenolic compounds may inhibit the anaerobic digestion of Fischer-Tropsch reaction water when stripped gas liquor is added to the batch reactor.

Donaldson et al. (1987) and Stepan (2006) showed that phenol and catechol in stripped gas liquor can be degraded under anaerobic conditions if the sludge is acclimated to the stripped gas liquor. However, acclimation of the sludge to the phenolic compounds in stripped gas liquor is very difficult. Stepan (2006) found that high-rate catechol-degrading cultures take a long period of time to establish and Donaldson et al. (1987) observed that biomass growth was slower at higher concentrations of phenol. Since the sludge used in this project was not acclimated to stripped gas liquor prior to the start of this project, it can be reasonably assumed that the anaerobic consortium that evolves during treatment of only Fischer-Tropsch reaction water is only able to execute a limited range of metabolic pathways, selecting for those micro-organisms that can most effectively metabolise short-chain volatile acids and alcohols as found in Fisher-Tropsch reaction water. Therefore the micro-organisms capable of metabolizing higher molecular weight organic contaminants, such as phenolic compounds, found in stripped gas liquor may be slower growing and it is possible that they will not establish to a significant degree in a mixed system. Thus by introducing stripped gas liquor to the Fischer-Tropsch reaction water digester, there is a risk that the phenolic compounds will not be treated. The final effluent will therefore be contaminated by these components, with implications for reuse and discharge.

This project will be investigating the influence of stripped gas liquor on Fischer-Tropsch reaction water digestion, the extent of inhibition of Fischer-Tropsch reaction water digestion by stripped gas liquor, if any, and whether the phenolic compounds in stripped gas liquor will be degraded by the Fischer-Tropsch reaction water acclimated sludge.

CHAPTER 3

3. Experiment Design

In chapter 3, the hypotheses of the project, which were developed based on the literature reviewed, are discussed, the research outcomes expected to be achieved by the project are stated and an experiment design in order to achieve the outcomes is presented. The materials used throughout the project and the methods used to sample these materials, determine the characteristics of the materials and to analyse the results obtained from the serum bottle tests were outlined. In section 3.4, the source of the materials that are used in the serum bottle tests and how these materials are sampled and stored are explained. In section 3.5, the methods used to analyse the physicochemical characteristics of the materials before the serum bottle tests are started and after the tests, to see the changes caused by the degradation of the materials, are described. The biogas produced by the serum bottle test consists of carbon dioxide and methane and it is important to know what composition of the biogas methane is in order to determine the extent of the degradation that occurred. The analysis of the composition of the biogas is covered in section 3.7. In section 3.8, an explanation of how the phenol concentration in the stripped gas liquor can be measured before and after the serum bottle tests, to obtain an indication of whether the FTRW acclimated sludge is capable of degrading the high molecular weight organics in stripped gas liquor, is given. In section 3.9 and 3.10, the interpretation of the data obtained is detailed.

3.1. Hypotheses

The first hypothesis is that the anaerobic sludge, which is acclimated to Fischer-Tropsch reaction water only, will be inhibited by the higher molecular weight organics such as phenols in stripped gas liquor.

The second hypothesis is that the Fischer-Tropsch reaction water acclimated sludge will not be able to degrade the phenolics in stripped gas liquor.

3.2. Research Outcomes

In order to test the hypotheses the following will be determined:

- The influence of stripped gas liquor on the specific methanogenic activity of Fischer-Tropsch reaction water acclimated sludge
- The fate of indicator compounds for potentially toxic higher molecular weight organics in stripped gas liquor, such as phenols, in batch anaerobic co-digestion experiments

3.3. Overview of Experiment Design

Since this project was only the first step of a broader project to investigate the feasibility of Fischer-Tropsch reaction water and stripped gas liquor co-digestion, there was no previous data to base this project's experiments on. Also, there was no information about the co-digestion of FTRW and SGL in literature. Therefore many experiments needed to be conducted to determine what loading rates and ratios of FTRW to SGL would be viable. Therefore a continuous process would not be economical to use since a continuous reactor takes time to start up and reach consistent steady state production. Thus, the serum bottle test, which is a batch process, was the technique used to measure the methanogenic activity of the Fischer-Tropsch reaction water and stripped gas liquor digested with a FTRW-acclimated sludge, under mesophilic conditions, since it is simple and inexpensive. Although the serum bottle technique does not give direct measurements of continuous process kinetics, it can generate the maximum methane production rate which gives a good indication of the maximum acetate utilisation rate of the aceticlastic methanogens (Conklin et al., 2008) and therefore an idea of how the process would behave in a continuous reactor.

A method was developed to successfully collect the biogas produced. The liquid displacement method was used and three different barrier solutions, to prevent the dissolution of the biogas in the displacement liquid were tested, that is, tap water, acidified brine solution and an alkaline solution.

Different COD loads of the FTRW were tested to determine what the base experiment should look like and a reference test was selected. The SGL was then added to the base experiment and the specific methanogenic activities of the reference units and test units were compared to investigate whether the SGL would cause any inhibitory effect on the anaerobic digestion of FTRW. The concentration of the indicator compounds in SGL (such as phenol) was tested at the beginning and end of the co-digestion experiments, using the colorimetric method and gas chromatograph, to determine the fate of the high molecular weight organics in SGL.

3.4. Materials

3.4.1. Sludge

The sludge used in the experiments were obtained from an upflow anaerobic sludge blanket (UASB) reactor which had been operated for 9 months prior to the start of this study, in the Pollution Research Group laboratory. The sludge had been acclimatised to Fischer-Tropsch reaction water. The reactor was constructed with a bottom inlet for the wastewater, which passed through the bed and the sludge blanket. When the sludge was sampled, the sludge was first pumped around for approximately 30 minutes to ensure even distribution of the granules. If the sludge was only to be used 1 – 2 days after sampling, it was stored in the cool room at 4 °C. The sludge was then incubated in a 2 ℓ Schott bottle in the water bath 24 hours before the serum bottle test to increase the temperature of the sludge to the required temperature of 35 °C. Otherwise the sludge was used directly after sampling.

3.4.2. Substrates

The substrates were the Fischer-Tropsch reaction water, to which the sludge was acclimatised, and the stripped gas liquor, which was the test substrate for the anaerobic co-digestion. Both the Fischer-Tropsch reaction water and the stripped gas liquor were received directly from the Sasol plant. The substrates were stored in the cool room at 4 °C. On the day that the substrate was to be used, it was removed from the cool room and allowed to reach room temperature.

3.4.3. Nutrients

The nutrients required to supplement the micro-organisms were prepared as described by Owen et al. (1979). The nutrients were a combination of trace nutrients, macro-nutrients and vitamins. The nutrients are said to be necessary for optimal growth of the micro-organisms which would affect the ability of the micro-organisms to degrade the substrate.

Table 3-1: Table showing the stock solutions for the preparation of the nutrients (Owen et al., 1979)

Solution	Compound	Concentration (g/ℓ)
S1	Resazurin	1
S2	$(NH_4)_2HPO_4$	26.7
S3	$CaCl_2.2H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2.6H_2O$	120
	KCl	86.7
	$MnCl_2.4H_2O$	1.33
	$CoCl_2.6H_2O$	2
	H_3BO_3	0.38
	$CuCl_2.2H_2O$	0.18
	$Na_2MoO_4.2H_2O$	0.17
	$ZnCl_2$	0.14
S4	$FeCl_2.4H_2O$	370
S5	$Na_2S.9H_2O$	500
S6	Biotin	0.002
	Folic acid	0.002
	Pyridoxine hydrochloride	0.01
	Riboflavin	0.005
	Thiamin	0.005
	Nicotinic acid	0.005
	Pantothenic acid	0.005
	B_{12}	0.0001
	ρ-aminobenzoic acid	0.005
	Thioctic	0.005

3.5. Physicochemical Characteristics of Substrates and Sludge

3.5.1. pH test

The pH of the Fischer-Tropsch reaction water, the stripped gas liquor and the mixed liquor from the serum bottles were measured using the Hanna pH meter (HI 255 combined meter). Since the serum bottle test was a batch test and there was no online system to measure the pH continuously, extra bottles were set up for the inhibition tests with the same composition of sludge and substrates as the test units. These extra bottles were then sacrificed before and after the serum bottle tests, as well as at intervals during the experiment to measure the pH during the experiment.

3.5.2. Alkalinity test

Alkalinity is the measure of the capacity of the water to neutralise a strong acid to keep the pH stable (Snoeyink et al., 1980). The alkalinity of a sample is a function of the concentrations of the hydroxyl, carbonate and bicarbonate ions present. The method used to determine the alkalinity involved adding a standardised acid to the sample until a colour change of the indicator was observed or a predetermined end point pH of 4.5 was reached. The indicator used was a mixture of bromocresol green and methyl red. However, since the SGL used had such a dark brown colour, it was difficult to observe a colour change therefore the end point was detected using a pH meter. Fischer-Tropsch reaction water had no alkalinity since the pH of FTRW was already below 4.5.

3.5.3. Solids test

The total solids can be measured by taking a known volume of the sample and evaporating the water contained in the sample by placing it in an oven at 103 – 105 °C and drying to a constant weight. The residue remaining is the total solids which is comprised of suspended solids and dissolved solids. Suspended solids are those solids that do not pass through a glass microfiber filter paper (1 μm, 47 mm). After filtration the filter paper with the wet solids are placed into the oven at a temperature of 103 – 105 °C for 30 minutes. The mass of solid residue on the filter paper after drying is the total suspended solids. The dissolved solids are the components, such as salts, in the sample filtrate that exist in the dissolved form but

precipitate when the sample is dried. The fixed solids can be determined by taking the suspended solids and igniting it to a constant weight by placing it in a furnace at 550 °C for 1 hour. The volatile solids are the weight lost during ignition, while the fixed solids are the mass remaining on the filter paper after ignition. These tests were performed on the substrates (FTRW and SGL) and the sludge before any experiments were started.

3.5.4. Chemical oxygen demand test

The chemical oxygen demand (COD) is the amount of oxygen necessary to oxidise any oxidisable material in a sample to water, carbon dioxide and ammonia. Chemical oxygen demand is a conserved property, as the COD of a sample before anaerobic digestion is equal to the sum of the COD of the substrate left and the COD removed and can be used to determine the extent of the biodegradation of the substrate. In anaerobic digestion, COD is only removed from the mixed liquor by conversion to gaseous methane, which escapes with the biogas. Therefore measurement of the methane produced can be used to estimate the COD removed from the mixed liquor by anaerobic digestion and hence the extent of biodegradation of the substrate.

The oxygen demand can also be estimated by conducting a test using a chemical oxidising agent. The COD is given as the amount of oxygen from the chemical oxidising agent used to break down the organic compounds in a particular volume of sample under hot, acidic conditions. The test is relatively quick and the results are reproducible. The most common oxidizing agents used are potassium dichromate or potassium permanganate (Wiseman, 1978). In this study, the COD of the substrates and sludge were measured before the experiments. Extra bottles were set up for the inhibition tests with the same composition of sludge and substrates as the test units and these were sacrificed before, during and after the experiment to measure the COD of the mixed liquor in the serum bottles. The samples were digested for 2 hours in an ETHOS One microwave using the closed reflux method. The program was set to ramp the temperature to 150 °C in 15 minutes, digest the sample at 150 °C for 30 minutes and then cool for 1 hour to 50 °C. The COD was then determined by back titration of the oxidising agent when all the COD of the sample has been used up.

3.6. Serum Bottle Test

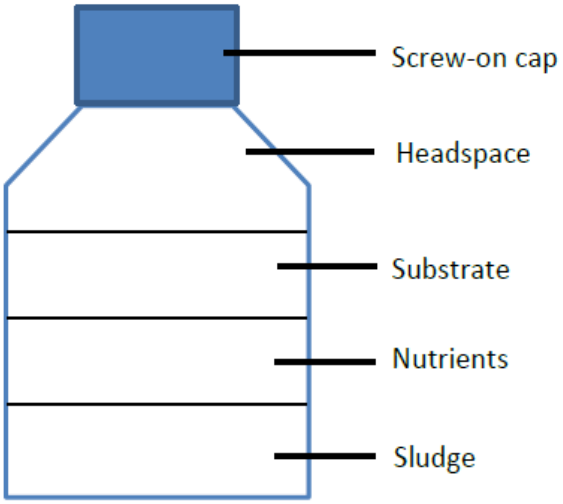

Figure 3.1: Diagram of a serum bottle, its components and contents

In a serum bottle test, the anaerobic sludge is placed in the vial together with the substrate to degrade and the nutrient and mineral solution to provide the necessary micronutrients, such as trace metals, amino acids and so forth, if necessary. The serum bottle is then kept at a constant temperature of about 35°C for between 7 to 30 days using a water bath. As the substrate is degraded, biogas is produced which consists of carbon dioxide and methane (Owen et al., 1978). The biogas is then collected using a liquid displacement method where the barrier solution could be either tap water, acidified brine solution or an alkaline solution. This liquid displacement method generates biogas production activity as a function of time. From this, the biogas production activity as a function of substrate concentration can be inferred. The collection of the biogas produced is described in detail in Chapter 4, Method Development.

3.7. Gas Composition Measurement

Since the biogas collected using the water displacement method and the acidified brine solution method contained both methane and carbon dioxide, the biogas had to be analysed to determine the percentage composition of each component. The Shimadzu gas chromatograph with the GC Real Time Analysis programme was used for this purpose. The column used was the Carboxen capillary column with an internal diameter of 2.1 mm and a length of 1.5 m,

which is suitable for the analyses of light hydrocarbons. A gas tight syringe was used to make the injections manually with an injection port temperature of 25 °C. The volume of the gas injected ranged from 0.1 to 0.4 mℓ. Helium was used as the carrier gas with a flow rate of 25 mℓ/min and the oven was held at a temperature of 100 °C for 8 minutes. The type of detector used was a thermal conductivity detector (TCD) at a temperature of 25 °C. The gas composition had to be measured as often as possible to obtain a reliable representation of how the gas compositions were changing as the anaerobic digestion took place. However since there were 12 serum bottles and triplicate readings were required for each bottle it was not possible to measure the gas composition more than once a day with the manual injection system available.

3.8. Phenol Measurement

One of the components in SGL that may inhibit biological activity is phenol. Although phenol is not the only inhibitor, it was used as an indicator compound to determine the fate of high molecular weight organics in SGL. Therefore it was important to measure the phenol concentration (mg/ℓ) before and after anaerobic digestion to determine if the phenol could be degraded by the FTRW acclimatised sludge and, if so, to what extent. There were two methods whereby the phenol concentration could be determined, namely, the colorimetric method and the gas chromatography. The colorimetric method was used to quantify all phenolic compounds in a sample, while the GC was used for qualitative assessment of phenolic compounds in the sample.

3.8.1. Colorimetric method

The first method used for the determination of the presence of phenols in the wastewater is the colorimetric method. It is based on the reaction, observed by Emerson (1943), whereby 4-aminoantipyrine reacts with phenol forming an intensely red or purple coloured compound in the presence of oxidising agents provided that the pH is high enough. The absorbance of light of the solution at a given wavelength is measured using a spectrophotometer.

The bonding of the 4-aminoantipyrine with the phenol takes place in the para-position which is opposite the OH group in the phenol. Therefore for the reaction to be possible the

para-position must either be free or occupied by a halogen, carboxyl, sulphonic acid, hydroxyl or methyl group which is expelled to accommodate the 4-aminoantipyrine (Emerson, 1943). **Figure 3.2** below shows how 4-aminoantipyrine and phenol bond:

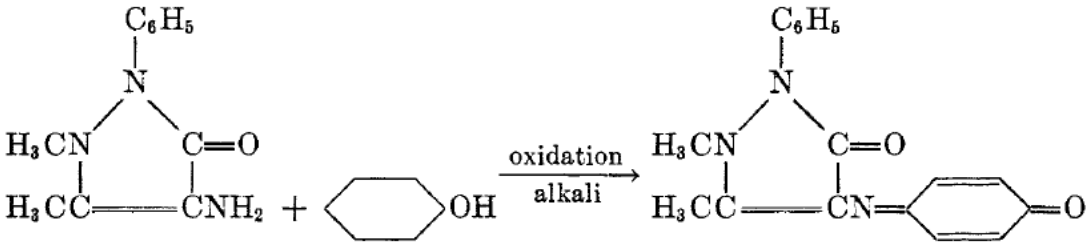

Figure 3.2: The reaction of 4-aminoantipyrine with phenol (Emerson, 1943)

If the pH is below 8, the 4-aminoantipyrine is instead oxidised to form the compound antipyrine red (Ochynski, 1960). Therefore Gottlieb and Marsh (1946) suggest that a pH range of 10.4 – 10.6 be used. Even a slight change in the pH may affect the intensity and the stability of the colours produced. However it is very difficult to obtain the exact pH required since the pH of the solution is usually analysed and accordingly adjusted before the addition of the reagents which cause a drop in the pH value. A way to decrease the pH drop is to add ammonium chloride which provides some buffering action and therefore stabilises the pH (Dannis, 1951). The buffer solution used in the colorimetric analyses for this project was made up of ammonium chloride and ammonium hydroxide.

The temperature is also a factor which affects the colour formation. To limit the effect that the temperature has on the results, the samples should be analysed quickly (Ettinger, 1951). If the samples are cloudy, intensely coloured or contain interfering compounds such as sulphites, metallic ions, aromatic amines or quinones, distillation may be advisable before any of the reagents are added. The distillation is done in a Pyrex all-glass distillation apparatus after addition of copper sulphate and adjustment with phosphoric acid to approximately pH 4 (Dannis, 1951). The phenolic compounds are separated out and the distillate is analysed using the colorimetric method. The samples used in this project were only slightly coloured therefore there was no need for distillation before the colorimetric analysis.

The oxidising agent used was potassium ferricyanide because it allows rapid colour formation, keeps the colour stable and has good sensitivity (Martin, 1949). 4-Aminoantipyrine is a good reagent due to the fact that it does not react with just the alkaline oxidising agents alone to form coloured products, unlike p-aminophenol and p-diamines which do (Emerson, 1943).

The absorbance was measured using a spectrophotometer at a wavelength of 510 nm. The method used to determine the concentration of the samples was the standard addition method. The standard addition method involved adding the buffer solution, aminoantipyrine solution, potassium ferricyanide as well as a standard volume of sample (10 mℓ) to varying concentrations of phenol. The absorbance was then plotted against the concentration and extrapolated to the x-axis to obtain the phenol concentration in the sample (refer to Appendix E4. Phenol measurement)

3.8.2. Gas chromatography

The Shimadzu gas chromatograph with the GC Solution programme was used to analyse the effluent from the serum bottles for the presence of phenols. The column used was the ZB-WAXplus column with an internal diameter of 0.25 mm and a length of 30 m, which is suitable for the analyses of phenols. An automatic sample injector was used to make the injections with an injection port temperature of 220 °C. The volume of the sample injected was 1 µℓ. Helium was used as the carrier gas with a constant flow rate of 0.23 mℓ/min. The oven temperature was programmed to ramp from 60 °C to 240 °C at a rate of 20 °C/min. The oven temperature was then held at 240 °C for 6 minutes. Hydrogen was used to ignite the flame ionisation detector (FID).

3.9. Data Interpretation

3.9.1. Cumulative volume

The cumulative volume of biogas can be plotted against the time of the test for the control units (blanks), which contains only sludge and nutrients, the reference units, which contain sludge, nutrients and Fischer-Tropsch reaction water and the test units, which contain sludge, nutrients, Fischer-Tropsch reaction water and stripped gas liquor.

The purpose of the blank is to provide a measure of the endogenous biogas production rate. It is assumed that the endogenous rate is due to cell death and decay, which occurs at the same rate in the test units as in the blanks. The net volume of biogas can be plotted against time, which can be calculated by subtracting the volume of methane measured from the blanks from the volume of biogas measured from the test units. This gives the biogas generated by the substrate only by eliminating the endogenous biogas production.

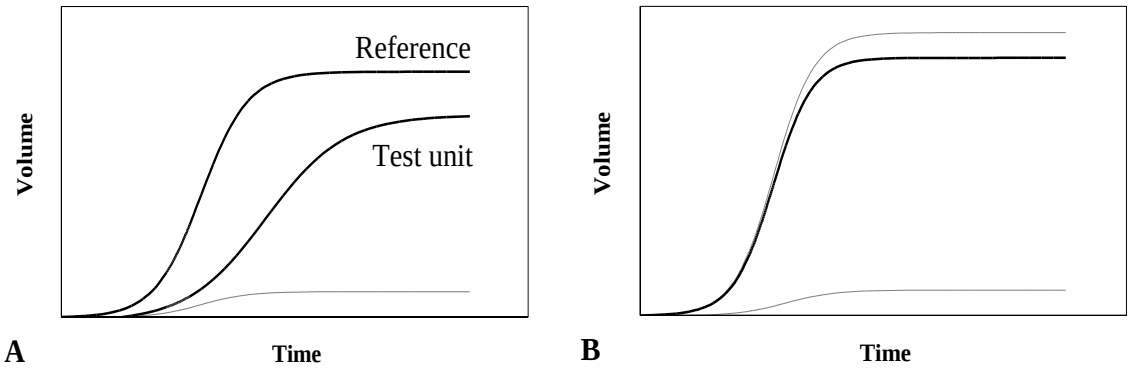

Figure 3.3: Representation of A) cumulative biogas volume vs time graphs of the control (dashed line), reference unit and test unit and B) a net volume of biogas vs time graph (solid line) (Source: Remigi et al., 2006)

There are various shapes (represented in **Figure 3.3**) that the cumulative biogas volume vs time graph can take on depending on the biodegradability of the test material:

1. The test material is readily biodegradable
2. The test material is biodegradable after a lag phase
3. There is partial inhibition
4. There is complete inhibition

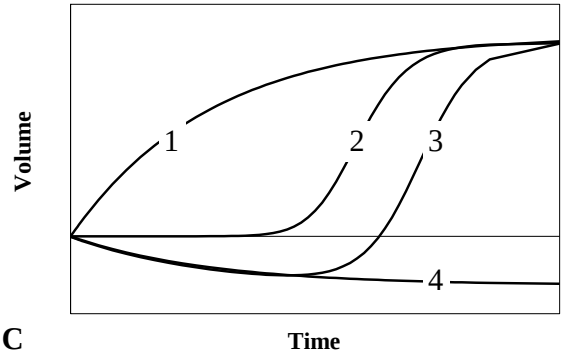

Figure 3.4: Possible shapes of the graph of cumulative biogas volume vs time (Source: Remigi et al., 2006)

3.9.2. Net methane volume

$$V_{CH_4,net} = V_{CH_4,test} - V_{CH_4,blank} \qquad \textbf{Equation 3-1}$$

Where:

$V_{CH_4,net}$ = Cumulative net volume of methane (mℓ) at time t

$V_{CH_4,test}$ = Cumulative methane volume of test unit (mℓ) at time t

$V_{CH_4,blank}$ = Cumulative methane volume of blank (mℓ) at time t

3.9.3. Conversion of moles of methane to COD

The chemical oxygen demand (COD) is the amount of oxygen necessary to oxidise any oxidisable material in a sample to water, carbon dioxide and ammonia. Therefore, to find the COD of 1 mole of methane, the number of moles of oxygen needed to convert the methane to carbon dioxide and water was determined:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \qquad \textbf{Equation 3-2}$$

Therefore 1 mole of methane = 2 moles COD

Since 1 mole of O_2 = 1 mole COD = 32 g

1 mole of methane = 64 g COD

3.9.4. Conversion of COD to volume of methane

From above **1 mole of methane = 64 g COD**

Using the ideal gas law, the volume of methane can be calculated

$$\boldsymbol{V = \frac{nRT}{P}} \qquad \textbf{Equation 3-3}$$

Where:

n = Number of moles of methane (mol)

R = Universal gas constant = 8.314 (Pa.m^3)/(mol.K)

T = Temperature (K)

P = Pressure (Pa)

$$V = \frac{1 \times 8.314 \times (35 + 273.15)}{101325} = 0.025\ m^3 = 25\ \ell$$

Therefore 25 ℓ CH_4 = 64 g COD

Giving **0.395 ℓ CH_4 = 1 g COD** (at atmospheric pressure and a reaction temperature of 35ºC)

3.9.5. Percentage COD recovery

In anaerobic digestion, COD is only removed from the mixed liquor by conversion to gaseous methane, which escapes with the biogas. The percentage COD recovery shows what percentage of the total COD added to the bottle was recovered as gaseous methane and thus the extent of the biodegradation of the substrate.

$$\%\ \boldsymbol{COD\ recovery = \frac{COD_{CH4}}{COD_0} \times 100} \qquad \textbf{Equation 3-4}$$

(Remigi et al., 2006)

Where:

COD_{CH4} = The COD value of methane at the end of the experiment

COD_0 = The initial COD value of substrate in the serum bottle

3.9.6. Specific methanogenic activity (SMA)

The specific methanogenic activity can be calculated as the amount of methane produced per day per mass of volatile suspended solids, which is a surrogate measurement for the amount of biomass. The specific methanogenic activity gives a good indication of the activity of the biomass, and with this, a direct comparison between different experiments and different substrates can be made.

$$\boldsymbol{SMA = \frac{R_{CH}}{X_0}} \qquad \textbf{Equation 3-5}$$

(Remigi et al., 2006)

Where:

R_{CH} = The net rate of methane production (mℓ/day)

X_0 = The biomass initially present in vial (gVSS)

The specific methanogenic activity of the sludge can then be plotted against time. These curves also have different patterns depending on the effect the test material has on the activity of the sludge (represented in **Figure 3.5**):

1. The test material is partially inhibitory but there is no lag phase or delay in the methanogenic activity
2. The test material is partially inhibitory and there is a delay in the methanogenic activity as the micro-organisms become acclimated to the test material
3. The test material is temporarily inhibitory and there is a lag phase before a specific methanogenic activity, which is similar to that of the reference material, is observed
4. The test material is completely inhibitory and the sludge does not adapt to the test material

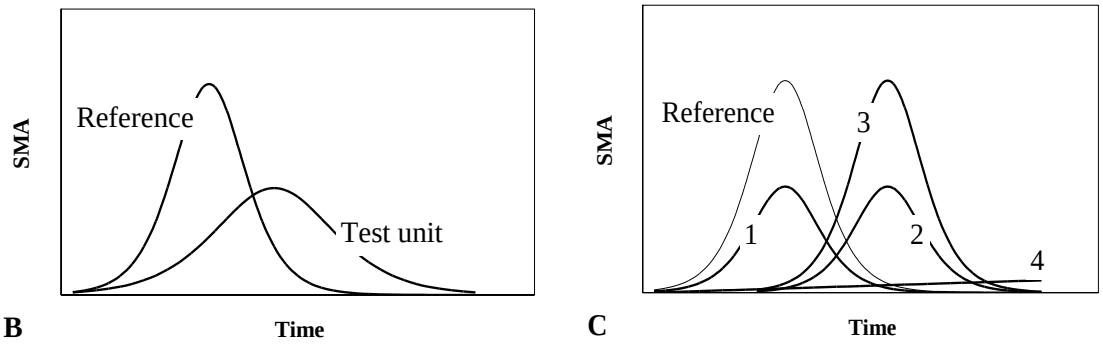

Figure 3.5: Representation of B) specific methanogenic activity vs time graphs and C) the possible shapes of the graph of specific methanogenic activity vs time (Source: Remigi et al., 2006)

3.9.7. Inhibition

The inhibition effect can be calculated from:

$$E = \frac{SMA_{test}}{SMA_{ref}} \qquad \textbf{Equation 3-6}$$

(Remigi et al., 2006)

Where:

SMA_{test} = Specific methanogenic activity of the test unit (mℓ/gVSS.day)

SMA_{ref} = Specific methanogenic activity of the reference unit (mℓ/gVSS.day)

Therefore the inhibition is:

$$I = 1 - E \qquad \textbf{Equation 3-7}$$

(Remigi et al., 2006)

3.10. Statistical Methods

3.10.1. Confidence limits for a mean

The confidence interval is an indication of the reliability of the data. For a 95% confidence interval, α = 0.025 since the confidence level is defined as (1 – 2α) for a two-tailed problem. The confidence limits can be calculated from (Davies and Goldsmith, 1977):

$$\bar{x} \pm t_{(n-1,1-\alpha/2)} \frac{s}{\sqrt{n}} \qquad \textbf{Equation 3-8}$$

Where:

$\bar{x}$ = Mean value of measurements variable x

t = t-statistic for $\emptyset_n$ degrees of freedom at a confidence level of (1 – 2α) for a two-tailed problem

n = Number of observations

α = Significance level

s = Estimated standard deviation of a population calculated from a sample

3.10.2. Difference between two means

In order to determine the confidence limits for the average difference between the means of two independent data sets the following calculation can be used (Davies and Goldsmith, 1977):

$$(\overline{x_1} - \overline{x_2}) \pm t_{(\emptyset,\ \alpha)} \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}} \qquad \textbf{Equation 3-9}$$

Where:

$\overline{x_1}$ and $\overline{x_2}$ = The mean values of measurements variables x_1 and x_2 respectively

t = t-statistic for $\emptyset_n$ degrees of freedom at a confidence level of (1 – 2a) for a two-tailed problem

$\emptyset$ = Number of degrees of freedom

n_1 and n_2 = Number of observations in data sets 1 and 2 respectively

α = Significance level

s_1 and s_2 = Estimated standard deviation of data sets 1 and 2 respectively

The number of degrees of freedom, ∅, can be calculated from:

$$\frac{1}{\emptyset} = \frac{1}{\emptyset_1}\left[\frac{\frac{s_1^2}{n_1}}{\frac{s_1^2}{n_1}+\frac{s_2^2}{n_2}}\right]^2 + \frac{1}{\emptyset_2}\left[\frac{\frac{s_2^2}{n_2}}{\frac{s_1^2}{n_1}+\frac{s_2^2}{n_2}}\right]^2 \qquad \textbf{Equation 3-10}$$

Where:

$\emptyset_1 = n_1 - 1$

$\emptyset_2 = n_2 - 1$

3.10.3. Paired two sample t-test

The paired two sample t-test is a widely used statistical method to compare the means of two data sets of paired samples. The paired t-test examines if the difference between the means is significantly different from the hypothesised value of zero, $\overline{x_1} - \overline{x_2} = 0$ (Park, 2009). Microsoft Excel was used to execute the t-test to compare the two sets of data. The P value displayed in the table of results describes the probability that the measured data would be obtained (assuming a normal distribution of measurement errors) if the null hypothesis is supported. Therefore a value of P = 0.05 means that there is a 5% probability that the calculated value between the measurement of the two means would have been obtained if there was no significant difference between the two measurement populations (Davies and Goldsmith, 1977).

CHAPTER 4

4. Method Development

The design of the method and the equipment used to attain the objectives of this research project are extremely important as they directly affect the quality and reliability of the results. In chapter 4, the details of the development of the method and equipment, from the water displacement method (Section 4.1) through to the acidified brine solution method (Section 4.2) and finally to the alkaline solution method (Section 4.3) is given, providing results which support the reasons for the changes made. The advantages and disadvantages of each method are compared. In section 4.4, the reference test, which contains only sludge and Fischer-Tropsch reaction water, is selected. This is to determine the reference activity when the test material is not present. Different COD loads of FTRW were tested to determine which gave the most reproducible results with different batches of sludge. Therefore there is a known methane production to which the results from the co-digestion experiments can be compared to determine whether the methanogenic activity is enhanced or inhibited by the addition of the stripped gas liquor. In sections 4.4.1 and 4.4.2, the results obtained when COD loads of 1, 1.5 and 2 g COD/ℓ of FTRW are used with different batches of sludge are presented. The results are compared in Section 4.4.3 to determine the reproducibility when different batches of sludge are used.

4.1. Water Displacement Method

The initial set-up consisted of twelve 250 mℓ serum bottles with screw-on caps, a water bath and temperature regulator, a metal rack to place the serum bottles in, 24 droppers, 12 pipes of the same length and a stand to attach the droppers to. The method used to collect the biogas was a water displacement method. The droppers were arranged in pairs so that one dropper acted as a gas collection dropper filled with water and the other dropper above it acted as an

overflow chamber to collect the displaced water. The biogas was collected by attaching one end of a pipe to the serum bottle in which the anaerobic digestion was taking place and the other end to the top of the gas collection dropper. As the biogas was produced it flowed directly into the gas collection dropper and displaced the water with a volume equal to the volume of gas produced. Therefore there was no need to vent the serum bottles so often. The gas volume could be read directly off the graduations on the dropper. Since the total biogas, which consists of methane and carbon dioxide, was collected in the gas collection dropper, the gas composition had to be analysed in a Shimadzu 2010 gas chromatograph.

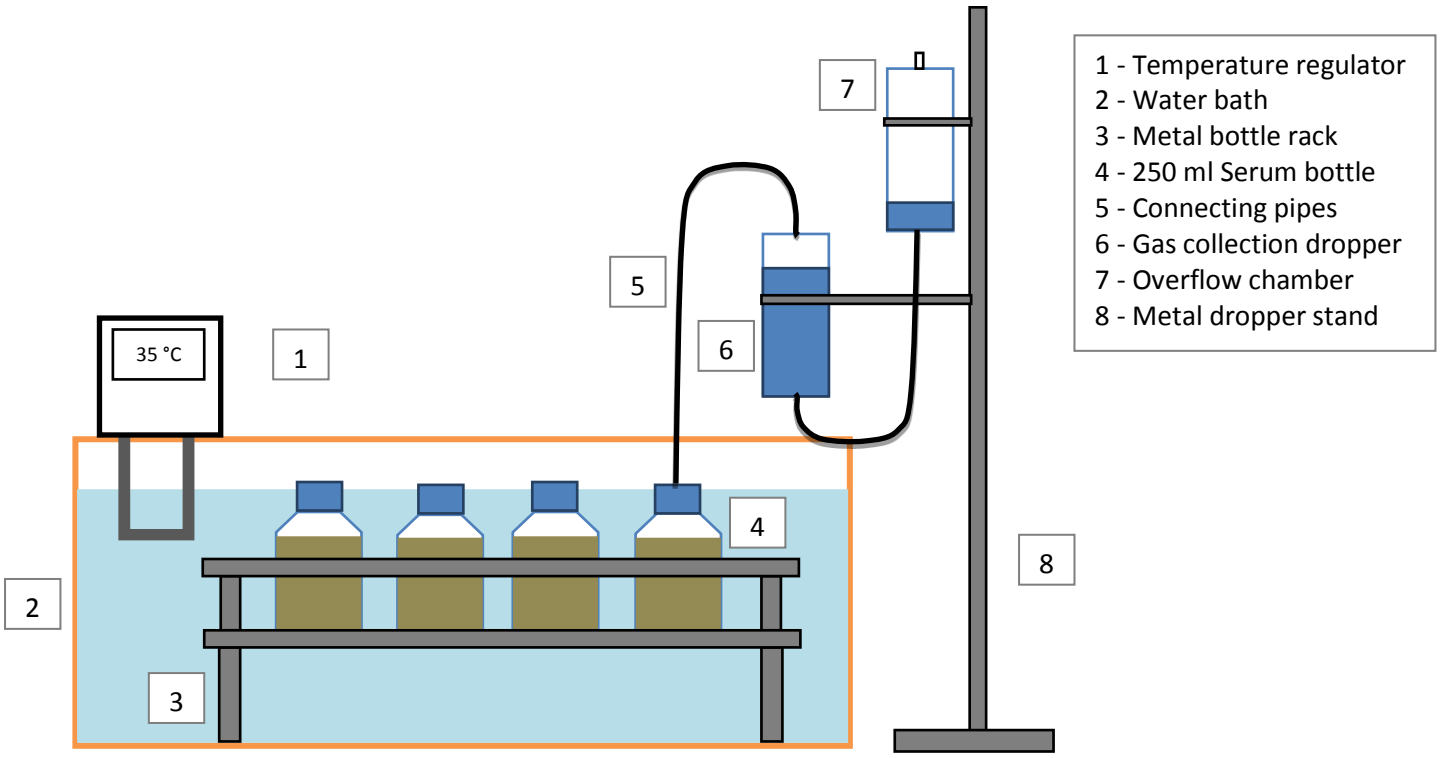

Figure 4.1: Set-up of the serum bottle test and water displacement method showing the dropper arrangement for one of the bottles

Figure 4.2: Photograph of the arrangement of the droppers with the bottom droppers being the gas collection droppers and the droppers above being the overflow chambers

Figure 4.3: Photograph showing the attachment of the serum bottles in the water bath to the gas collection droppers

Table 4-1: Table showing the concentration of the substrates and the type of gas measurement used in the method development experiments

Experiment	Type of gas measurement	Sodium acetate (g COD/ℓ)	FTRW (g COD/ℓ)	SGL (g COD/ℓ)	Sludge	Purpose of experiment
Trial run 1	Water displacement	2.5	0	0	Brewery	Proof of concept
Trial run 2	Water displacement	2.5	0	0	Brewery	Proof of concept
Set 1	Water displacement	0	1.2, 1.5, 1.8	0	UASB 1	Selection of F:M ratio
Set 2	Acidified brine solution	0	1.5	0	UASB 1	Test acidified brine solution displacement method
Set 3	Acidified brine solution	0	5	0	UASB 1	Test necessity of additional nutrient supplementation
Set 4	Acidified brine solution	0	1, 1.5, 2	0	UASB 2	Selection of F:M ratio
Set 5	Acidified brine solution & alkaline solution	0	1	0	UASB 2	Comparison of acidified brine solution method to alkaline solution method
Set 6	Alkaline solution	0	1, 1.5, 2	0	UASB 2	Selection of FTRW concentration
Set 7	Alkaline solution	0	1, 1.5, 2	0	UASB 2	Selection of FTRW concentration

4.1.1. Proof of concept

In order to prove that this set-up works, two trial runs were carried out using brewery sludge and acetate. A stock solution was made up using sodium acetate and distilled water such that when 5 mℓ of the solution was added to each bottle it provided a concentration of 2.5 g COD/ℓ. The calculations for the preparation of the stock solution and the theoretical amount of biogas that should be produced (approximately 436 mℓ) can be found in Appendix C. Although the experiments were not run to completion (i.e. until the methane production curve flattened out and there was no further production of methane) it can be seen

from **Figure 4.4** and **Figure 4.5** that the set-up works. The small error bars indicate that the serum bottle experiment gives good, reproducible results.

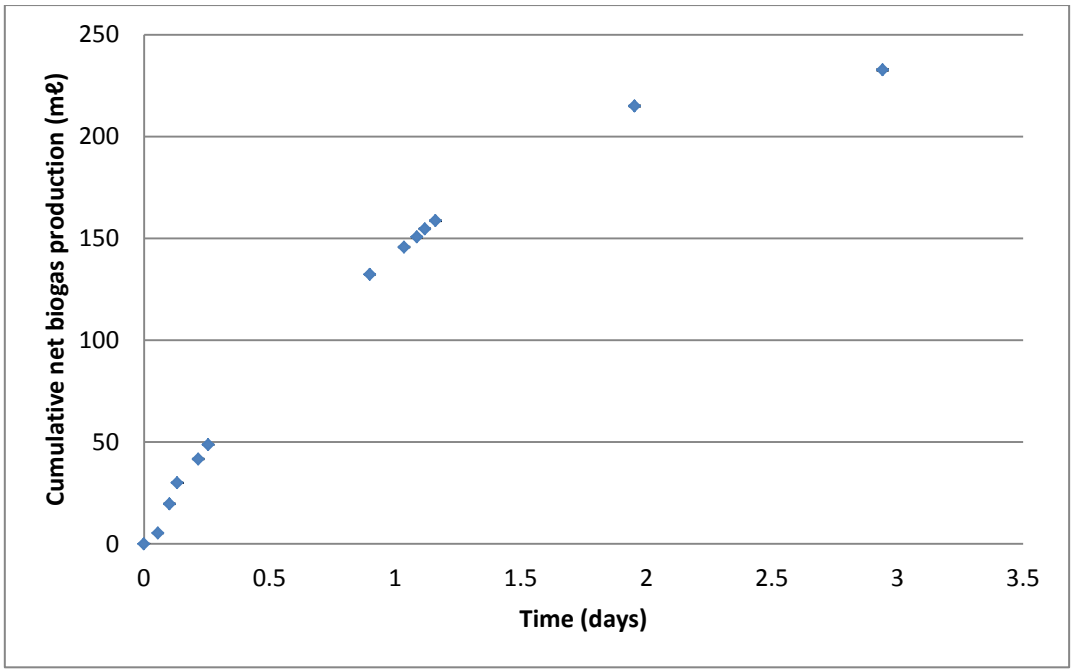

Figure 4.4: Graph showing the average cumulative net biogas production (mℓ) over time (days) in trial run 1

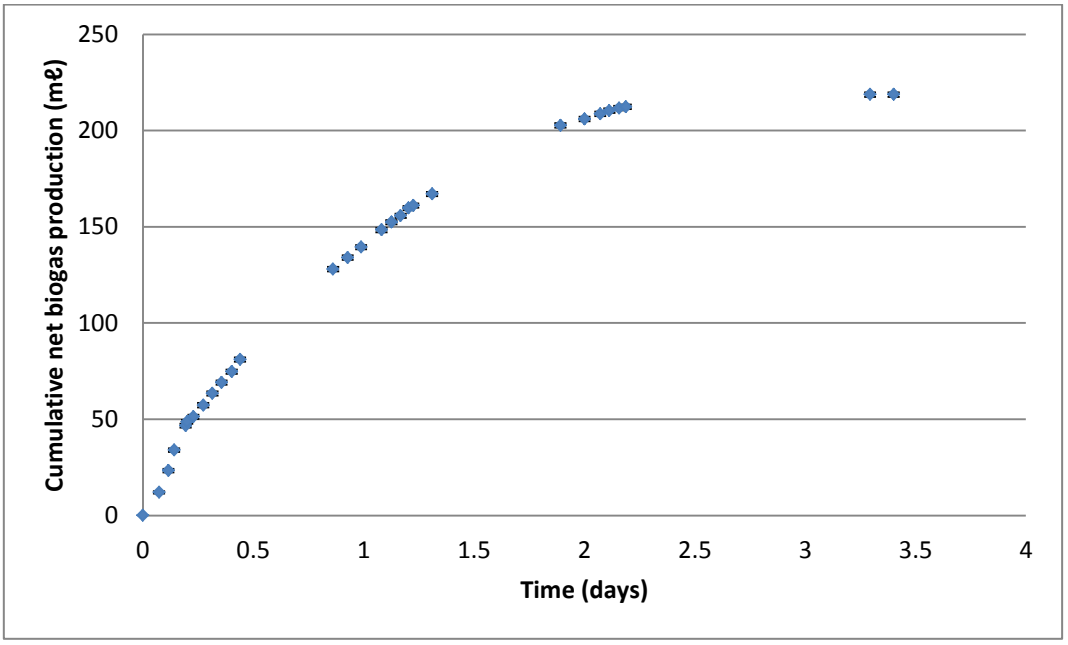

Figure 4.5: Graph showing the average cumulative net biogas production (mℓ) over time (days) in trial run 2

4.1.2. Set 1: Testing the water displacement method with FTRW and UASB 1 sludge

The aim of the experiments in set 1 was to test the water displacement method with different food: micro-organism ratios. Three ratios were tested (that is 0.2, 0.25 and 0.3 g COD/g VSS), with only FTRW to which the industrial sludge (UASB 1) was already acclimated. Each test was done in triplicate to check the reproducibility of the results and to ensure results will be available even if there is a problem with one of the bottles. There were 3 blanks which contained only sludge and distilled water. This was done to eliminate the biogas production from residual substrate from the previous application of the sludge and endogenous respiration, whereby the biomass continues to survive even without an external substrate by oxidising cellular materials (Hippeli, 1955). The net methane production, i.e. that attributable to anaerobic conversion of the supplied substrate, can be calculated by subtracting the blank unit gas production from the test unit gas production. These experiments were conducted using 250 mℓ bottles. The biogas was collected over water.

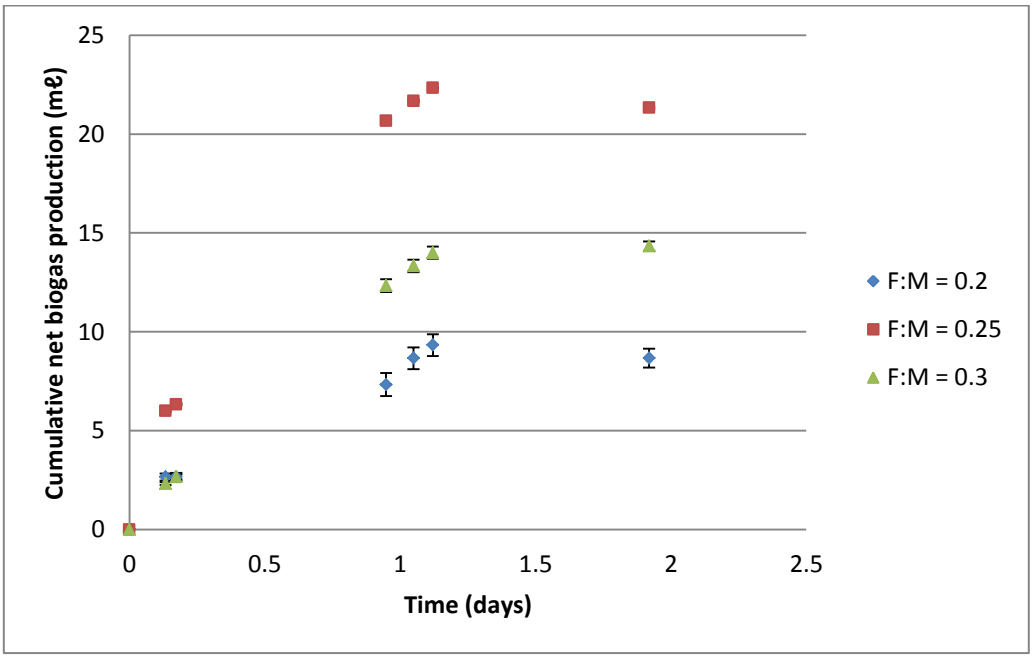

Figure 4.6: Graph showing the average cumulative net biogas produced (mℓ) over time (days) when food-to-micro-organism ratios of 0.2, 0.25 and 0.3 were used with only Fischer-Tropsch reaction water as the substrate

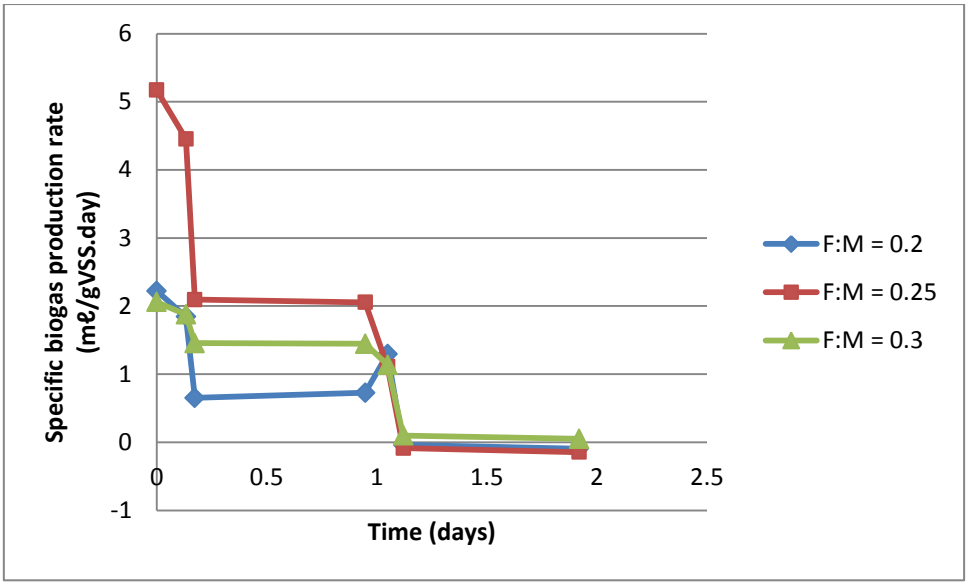

Figure 4.7: Graph showing the specific biogas production rate (mℓ/gVSS.day) over time (days) when food-to-micro-organism ratios of 0.2, 0.25 and 0.3 were used with only Fischer-Tropsch reaction water as the substrate

It can be observed in **Figure 4.6** that the average cumulative net biogas volume decreased after 1.2 days instead of increasing as expected. This gave a negative specific biogas production rate as shown in **Figure 4.7**. This decrease was reproducible in all 9 units that were tested in this experiment. This may be due to the gaseous carbon dioxide of the biogas which is produced being dissolved in or diffusing through the displacement liquid (Parajuli, 2011), in this case water.

4.2. Acidified Brine Solution Method

The acidified brine solution method is similar to the water displacement method. All of the equipment and the components are identical. However the water in the gas collection dropper is replaced with acidified brine solution to prevent the carbon dioxide collected in the dropper from dissolving in the displacement liquid (Iyagba, 2009).

4.2.1. Set 2: Testing the acidified brine solution displacement method with FTRW and UASB 1 sludge

From set 1 it was clear that the biogas could not be collected over water and that some sort of barrier was needed to prevent the carbon dioxide from dissolving. According to Iyagba, Mangibo and Mohammad (2009) an acidified brine solution could prevent the dissolution of the biogas. Therefore this method was tested in set 2. A solution was added to the droppers (which was prepared by adding sodium chloride to water until saturated and then adding a few drops of sulphuric acid to acidify it). A food: micro-organism ratio of 0.25 g COD/g VSS was used.

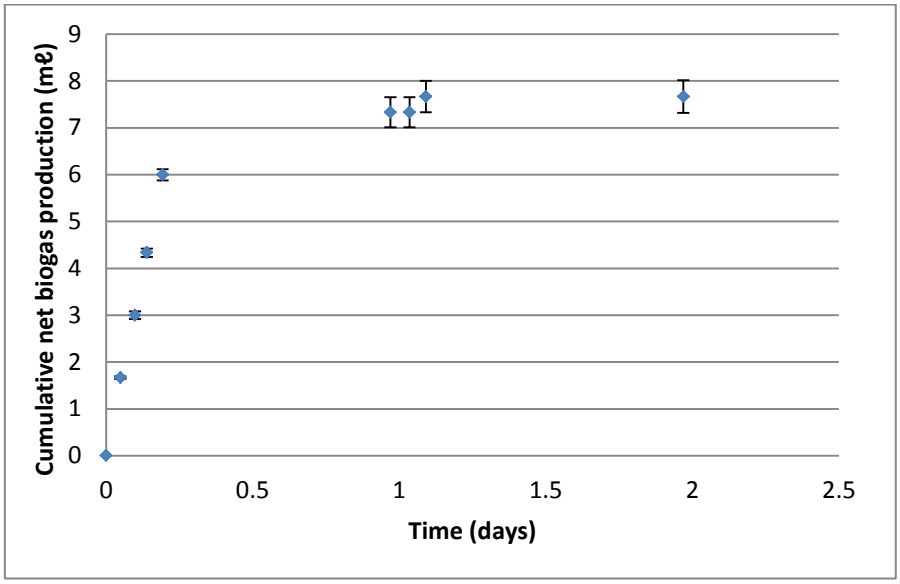

Figure 4.8: Graph showing the average cumulative net biogas production (mℓ) over time (days) with UASB 1 sludge, nutrients and Fischer-Tropsch reaction water as the substrate

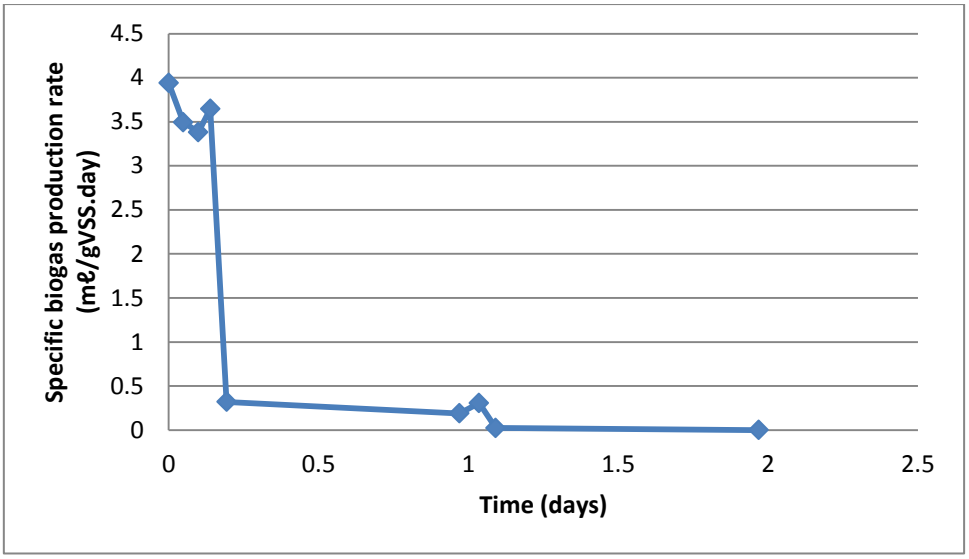

Figure 4.9: Graph showing the specific biogas production rate (mℓ/gVSS.day) over time (days) with UASB 1 sludge, nutrients and Fischer-Tropsch reaction water as the substrate

Figure 4.8 shows a smoother curve than that observed in **Figure 4.6**. The specific biogas production rate observed in **Figure 4.9** remained positive throughout the experiment indicating that the cumulative net biogas production in set 2 did not decrease during the experiment. This suggests that the acidified brine solution lowered the solubility of the carbon dioxide in the displacement liquid to a certain extent. Therefore this method of collecting the gas was continued in the subsequent tests.

4.2.2. Set 3: Testing the necessity of additional nutrient supplementation

Set 3 was testing whether the use of additional nutrients in the serum bottles to supplement the sludge was required. The preparation of the nutrients was presented in Section 3.4.3. Nutrients). A food: micro-organism ratio of 0.25 g COD/g VSS was used with FTRW as the only substrate and UASB 1 sludge. The biogas produced was collected using the acidified brine solution displacement method.

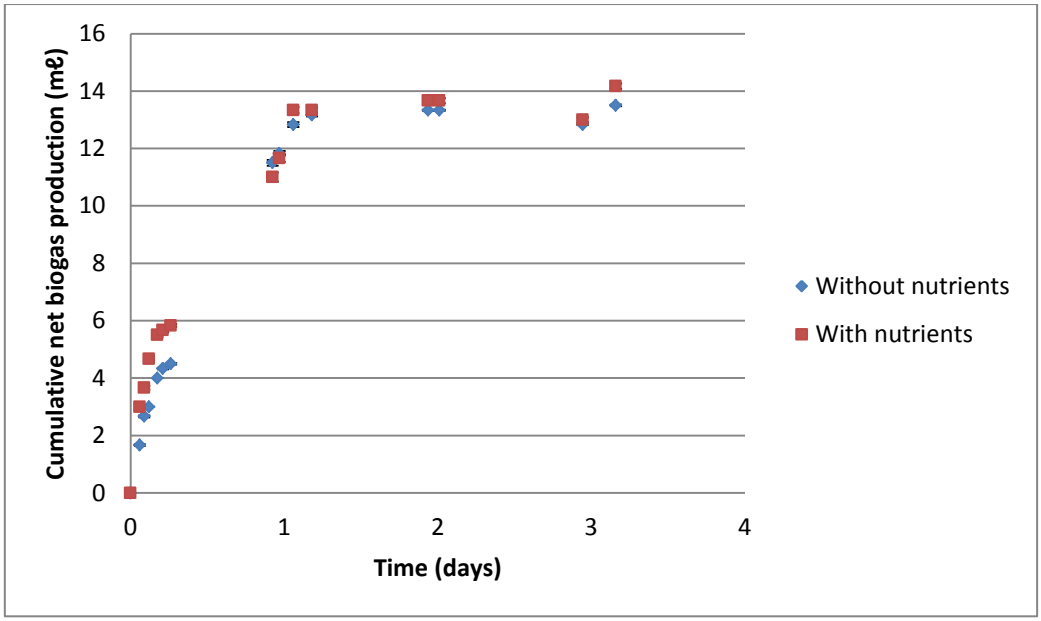

Figure 4.10: Graph showing the average cumulative net biogas production (mℓ) over time (days) with UASB 1 sludge and Fischer-Tropsch reaction water as the substrate

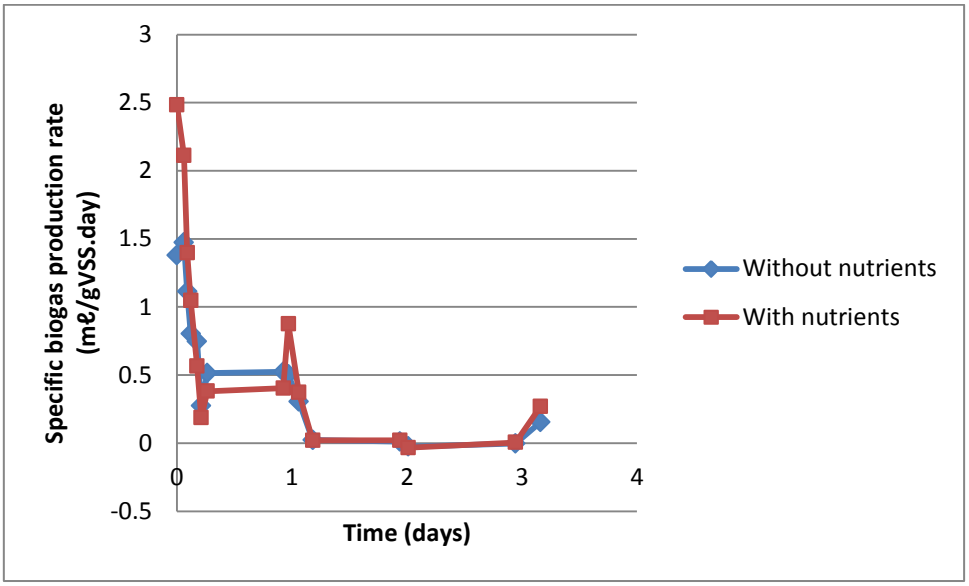

Figure 4.11: Graph showing the specific biogas production rate (mℓ/gVSS.day) over time (days) with UASB 1 sludge and Fischer-Tropsch reaction water as the substrate

The biogas production (**Figure 4.10**) and the specific biogas production rates (**Figure 4.11**) in the bottles without additional nutrients and with additional nutrients were similar. Therefore the use of additional nutrients to supplement the sludge was not necessary for experiments carried out over a short time period and additional nutrients were not used in

subsequent experiments. This may be due to the fact that the sludge was obtained from a UASB reactor which was being fed nutrients frequently, including nitrogen supplementation. In a batch test with relatively low growth requirement, the nutrients present in the UASB sludge was sufficient for additional growth that occurred. In addition, some nutrients, particularly reduced nitrogen are present in SGL. The fact that nutrients were not required for the batch test does not negate the purpose of this research to use nitrogen in SGL to provide nutrients for FTRW digestion. The batch serum bottle test with pre-conditioned sludge had sufficient nutrients for the growth requirements for the duration of the test. However, in a continuous system treating FTRW, the nutrients at the start of the continuous operation would be depleted by growth and dilution. Therefore these results do not suggest that there is no value in using SGL as a nitrogen source for the digestion of FTRW.

4.2.3. Set 4: Testing the acidified brine solution displacement method with FTRW and UASB 2 sludge

From the low volume of biogas collected in the previous experiments it was questionable whether the UASB 1 sludge was still active. Therefore the aim of the experiments in set 4 was to test the UASB 2 sludge with different food: micro-organism ratios. Three food: micro-organism ratios were tested (that is 0.03, 0.05, 0.07 g COD/g VSS), to which the sludge from UASB 2 (mixture of brewery and municipal sludge) is already acclimated. The food: micro-organism ratios corresponded to COD values of 1, 1.5 and 2 g COD/ℓ respectively. Each test was done in triplicate to check the reproducibility of the results and to ensure results were available even if there is a problem with one of the bottles. There were 3 blanks which contained only sludge and water. The biogas was collected over an acidified brine solution.

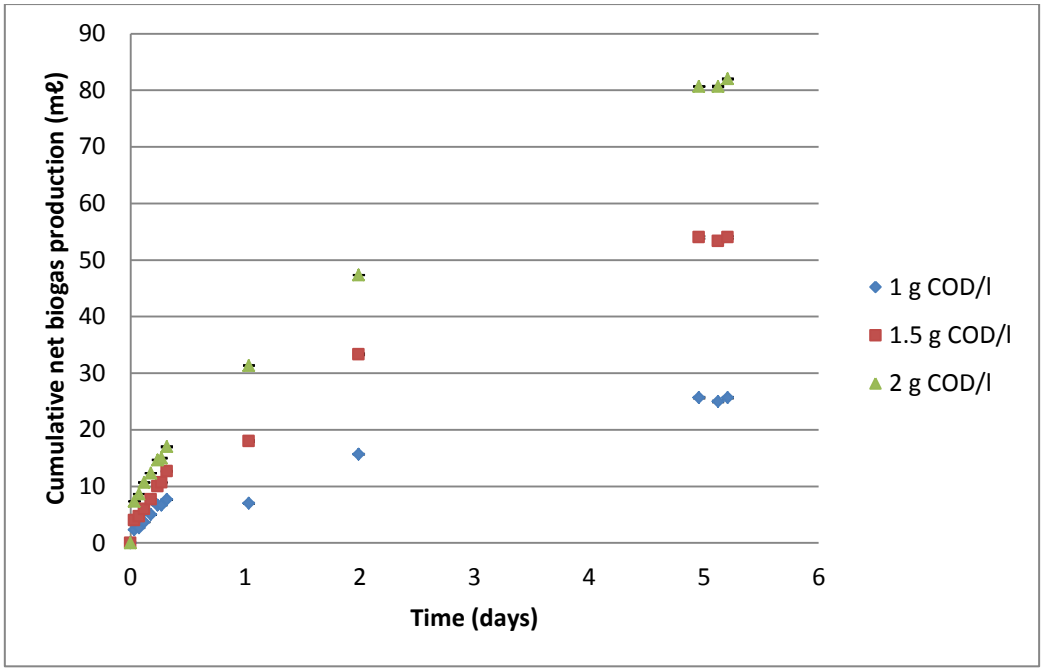

Figure 4.12: Graph showing the average cumulative net biogas production (mℓ) over time (days) with UASB 2 sludge and different concentrations of FTRW

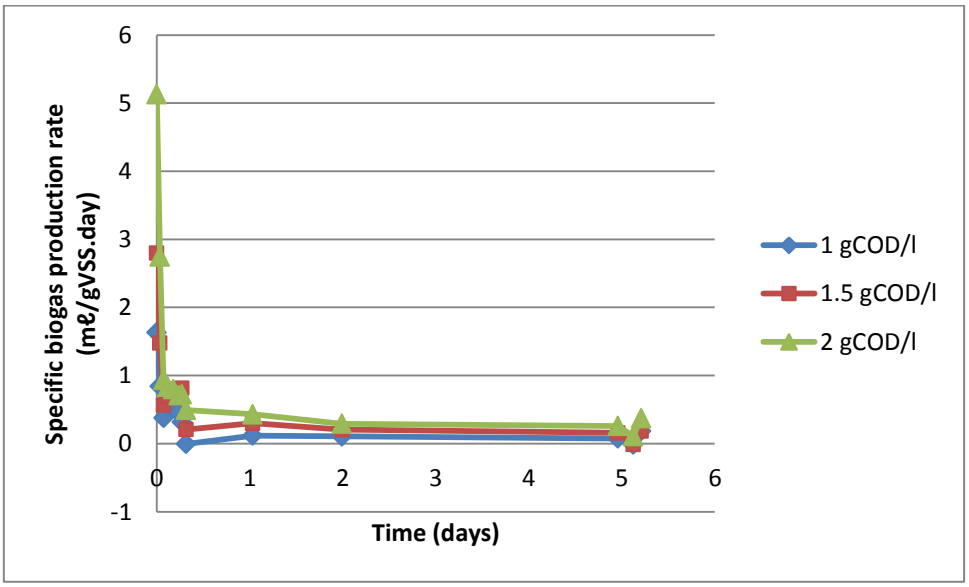

Figure 4.13: Graph showing the specific biogas production rate (mℓ/gVSS.day) over time (days) with UASB 2 sludge and different concentrations of FTRW

The total volume of biogas produced using UASB 2 sludge in set 4 was much higher than that produced by UASB 1 sludge in set 2 even with food: micro-organism ratios which are much lower than those used in the previous experiments. Therefore the use of UASB 2 sludge was continued in subsequent tests. **Figure 4.12** shows the cumulative biogas volume

produced. There is no evidence of inhibition at high substrate loading which is indicated by the fact that the cumulative volume of biogas produced at the end of the experiment was in proportion to the initial organic loading. **Figure 4.13** shows the specific biogas production rate which also appears to be in proportion to the initial loading rate. Therefore the growth rate was not saturated with respect to the substrate concentration in these experiments. The theoretical volume of biogas that could have been produced with 1 g COD/ℓ of FTRW was calculated to be about 165 mℓ (assuming that the only non-dissolvable gas was methane with a composition of 60% with regards to the total volume of biogas produced). However this set-up produced less than half of what was expected. This low gas production and the decrease of the cumulative biogas already produced can be explained by the fact that as more gas is produced in the serum bottle, the higher the concentration of carbon dioxide in the headspace which then affects the concentration of carbon dioxide in the liquid phase and consequently the pH. When the pH increases it causes carbon dioxide in the headspace to dissolve into the liquid phase causing the noticeable decrease in the cumulative volume (Parajuli, 2011). This would not be an issue if the methane composition from the gas analysis using the gas chromatograph were reliable since the methane remains in the gas phase. However technical problems with the GC analysis prevented gas composition data being collected for these experiments. Therefore a method to collect the gas and absorb the carbon dioxide, leaving only methane, was investigated, with the hope of eliminating the uncertainty caused by the carbon dioxide dynamics.

4.2.4. Advantages and disadvantages of the acidified brine solution displacement method

Advantages	Disadvantages
• Theoretically gives total biogas produced • With GC analysis can get more information about CH_4 and CO_2 composition • Can observe independent changes in specific methane production rate and specific total gas production rate	• CO_2 is partially retained in solution making gas production data difficult to interpret • GC analysis wasn't giving reliable results at the time • When equilibrating, air enters the bottle and affects the CO_2 dynamics even further

4.3. Alkaline Solution Method

This set-up consists of twelve 250 mℓ serum bottles with screw-on caps, a water bath and temperature regulator, a metal rack to place the serum bottles in, twelve more serum bottles filled with an alkaline solution, twelve 100 mℓ plastic cylinders, twelve pipes of the same length and a stand to attach the serum bottles filled with the alkaline solution and the plastic cylinders to. The method used to collect the biogas involves bubbling the biogas through the alkaline solution. The alkaline solution absorbs the carbon dioxide from the biogas leaving only methane which is collected above the alkaline solution. The volume of alkaline solution displaced is equal to the volume of methane collected. The displaced alkaline solution is collected in the plastic cylinder below the serum bottle filled with the alkaline solution and the volume can be read directly off the graduations on the plastic cylinder (Esposito et al., 2012). If the solution becomes saturated with carbon dioxide the thymol blue indicator will change the solution from blue to a purple-orange colour.

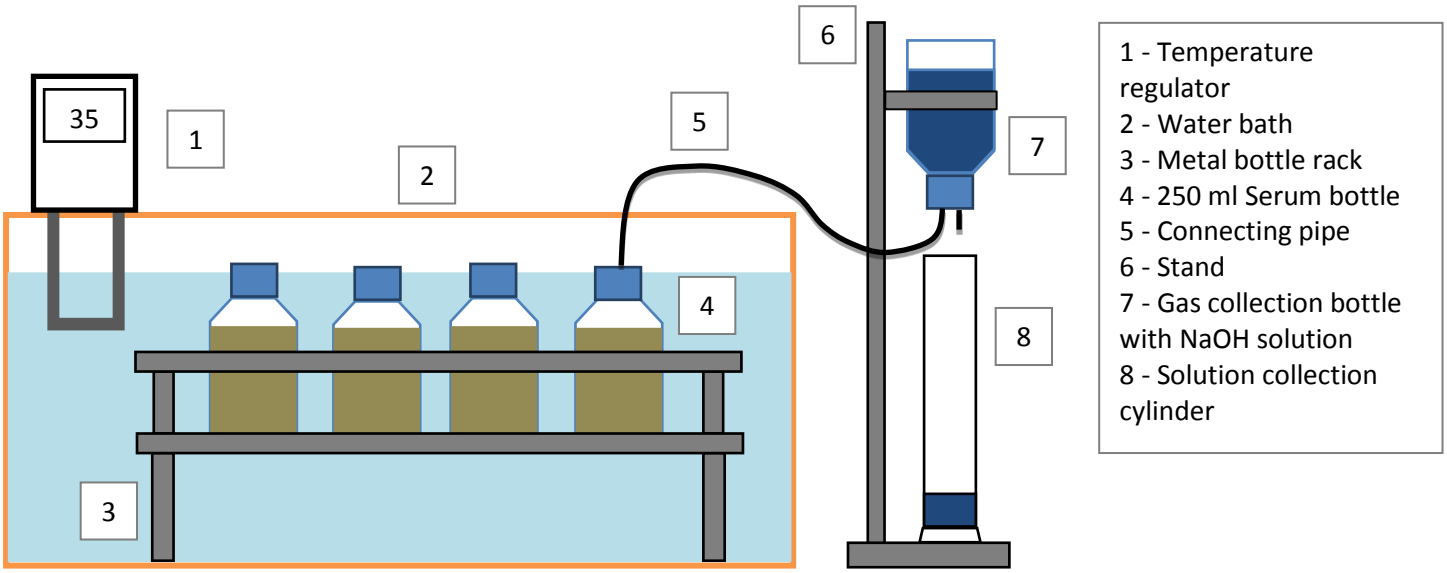

Figure 4.14: Set-up of the serum bottle test and water displacement method showing the dropper arrangement for one of the bottles

Figure 4.15: Photograph of the arrangement of the inverted bottles, filled with sodium hydroxide solution, above the plastic cylinders

Figure 4.16: Photograph of showing the attachment of the serum bottles in the water bath to the inverted bottles filled with sodium hydroxide solution

4.3.1. Set 5: Comparison of the acidified brine solution method to the alkaline solution method

In set 5 the acidified brine solution and the alkaline solution gas collection methods were compared. Six bottles were set up with the acidified brine solution displacement method. The other six bottles were set up with the alkaline solution method. The alkaline solution was prepared by adding 50 g/ℓ of sodium hydroxide to distilled water and a pinch of thymol blue indicator. The tests were done in triplicate using both methods. A food: micro-organism ratio of 0.03 g COD/g VSS was used (which corresponded to an FTRW concentration of 1 g COD/ℓ) with the UASB 2 sludge.

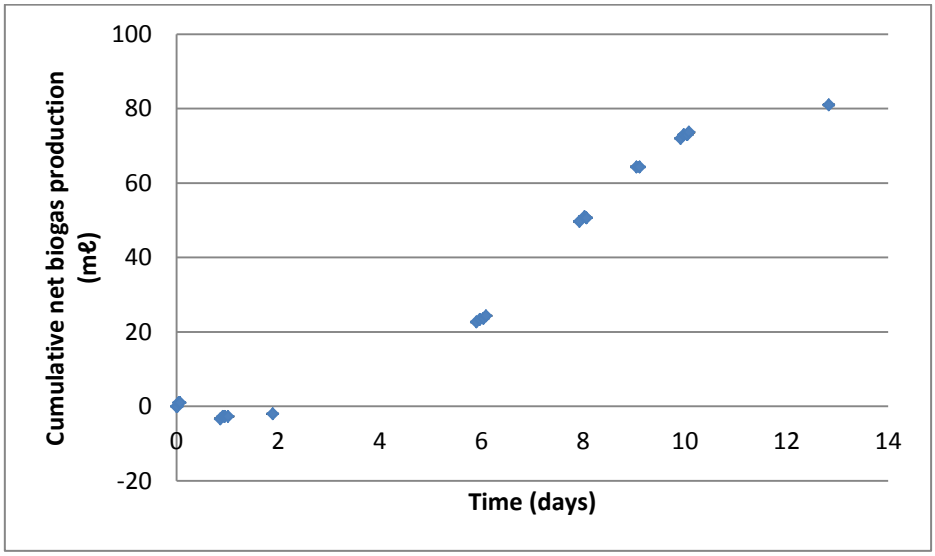

Figure 4.17: Graph showing the average cumulative net biogas production (mℓ) over time (days) with UASB 2 sludge and 1 g COD/ℓ of FTRW using the acidified brine solution method

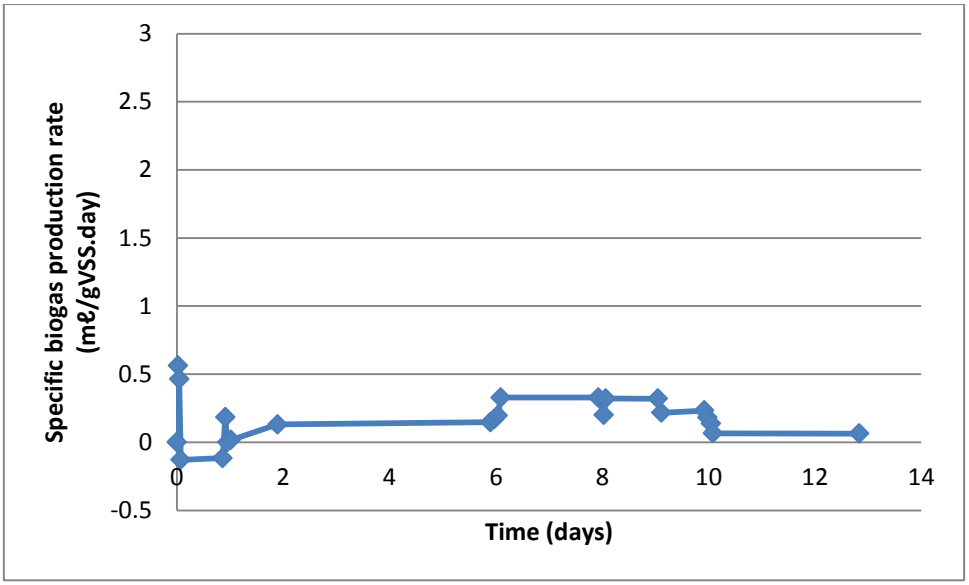

Figure 4.18: Graph showing the specific biogas production rate (mℓ/gVSS.day) over time (days) with UASB 2 sludge and 1 g COD/ℓ of FTRW using the acidified brine solution method

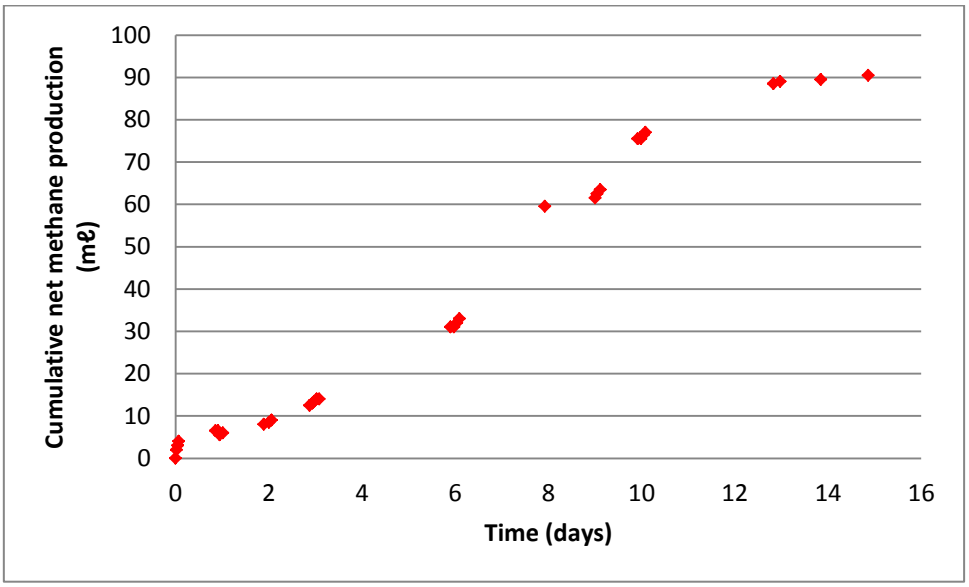

Figure 4.19: Graph showing the average cumulative net methaneproduction (mℓ) over time (days) with UASB 2 sludge and 1 g COD/ℓ of FTRW using the alkaline solution method

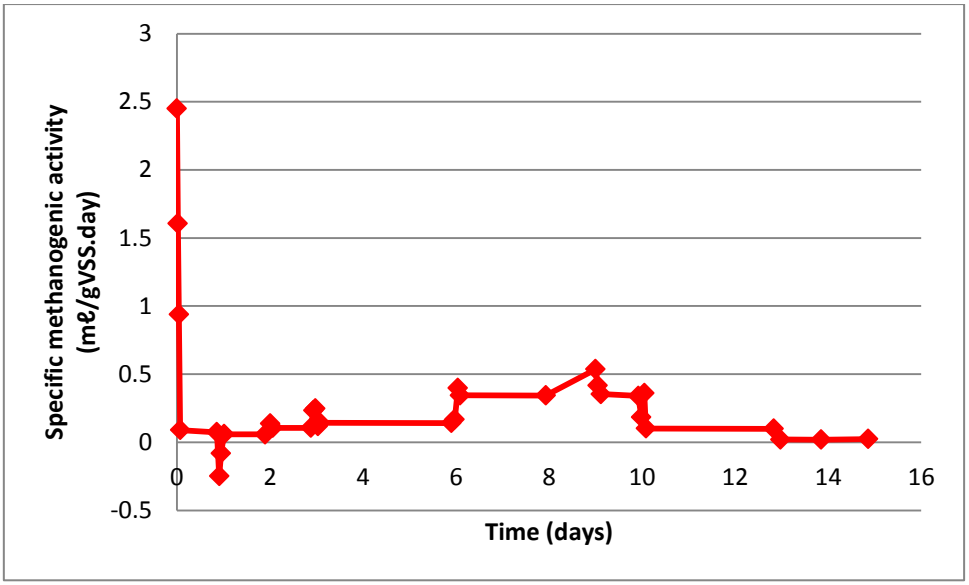

Figure 4.20: Graph showing the specific methanogenic activity (mℓ/gVSS.day) over time (days) with UASB 2 sludge and 1 g COD/ℓ of FTRW using the alkaline solution method

Figure 4.17 shows the total biogas produced and collected using the acidified brine solution method for 1 g COD/ℓ of FTRW. **Figure 4.19** shows the methane produced and collected using the alkaline solution method for 1 g COD/ℓ of FTRW. The theoretical volume of methane that could have been produced with 1 g COD/ℓ of FTRW is approximately 99 mℓ (assuming that methane is the only non-dissolvable gas produced). The theoretical volume of total biogas that could be produced with 1 g COD/ℓ of FTRW is approximately 165 mℓ (assuming that the composition of methane with regards to the total volume of biogas produced is 60%). Since **Figure 4.17** shows the results for the acidified brine solution method which collects the total biogas produced, the cumulative volume should be much higher than (almost double) that shown in **Figure 4.19**, which is only the methane production. However, **Figure 4.17** shows that only 80 mℓ of biogas was produced instead of the theoretical volume of biogas of 165 mℓ. This indicates that at least half of the biogas produced is being retained in the solution in the serum bottle giving inaccurate results. **Figure 4.19** shows that the volume of methane collected using the alkaline solution method was 90 mℓ which is very close to the theoretical volume of methane of 99 mℓ. The initial specific methanogenic activity of the sludge observed in **Figure 4.20** when using the alkaline solution method of gas collection was also higher than the initial specific biogas production rate observed in **Figure** 4.18 when the acidified brine solution method was used. Therefore the alkaline solution method is a much more reliable means of collecting the gas produced.

4.3.2. Advantages and disadvantages of the alkaline solution method

Advantages	Disadvantages
• Gives methane production only • Eliminates uncertainties arising from the CO_2 dynamics • No need for GC analysis • Direct measurement of specific methanogenic activity	• Gives no indication of the activity of the acidogenic micro-organisms

4.4. Reference Test

4.4.1. Set 6

In set 6 three concentrations of FTRW were tested (that is 1, 1.5 and 2 g COD/ℓ), to which the sludge from UASB 2 was already acclimated. The respective food: micro-organism ratios were 0.03, 0.05 and 0.07 g COD/g VSS. The biogas was bubbled through an alkaline solution so that the carbon dioxide was absorbed leaving only the methane produced. The methane was collected above the alkaline solution and displaced a volume of the solution equal to the volume of methane produced. The solution was collected in a cylinder from which the volume could be read directly. Each test was done in triplicate to check the reproducibility of the results and to ensure results were available even if there was a problem with one of the bottles.

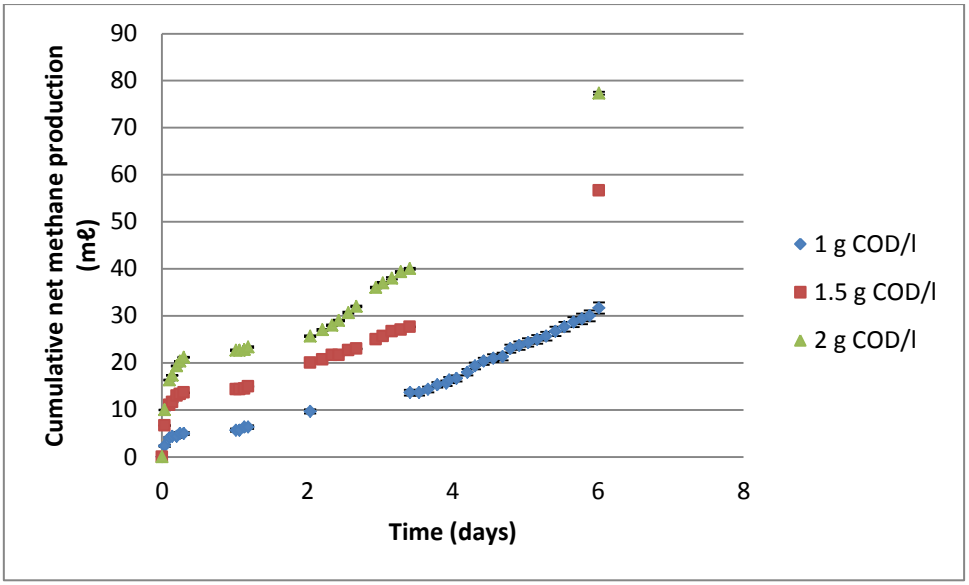

Figure 4.21: Graph showing the average cumulative net methane production (mℓ) over time (days) with UASB 2 sludge and 1, 1.5 and 2 g COD/ℓ of FTRW

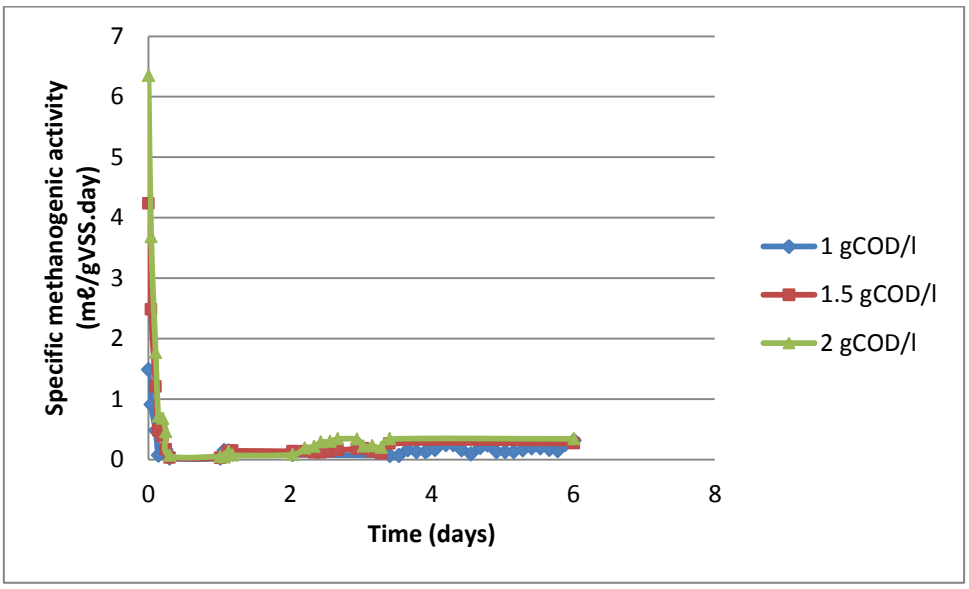

Figure 4.22: Graph showing the specific methanogenic activity (mℓ/gVSS.day) over time (days) with UASB 2 sludge and 1, 1.5 and 2 g COD/ℓ of FTRW

Figure 4.21 shows the net methane production for set 6 where 1, 1.5 and 2 g COD/ℓ of FTRW was tested. The small error bars in **Figure 4.21** indicate that the three replicates of each FTRW concentration showed identical behaviour. From **Figure 4.21** it can be observed that the bottles with the higher concentration of organics (higher COD values of FTRW) produced larger volumes of methane. **Figure 4.22** shows the specific methanogenic activity

for set 6 experiments. A paired two sample t-test indicated that the SMA for the 1 g COD/ℓ FTRW test unit was significantly lower than for the 1.5 g COD/ℓ FTRW test unit test at a 95% confidence level (P = 0.038). A paired two sample t-test also indicated that the SMA for the 1.5 g COD/ℓFTRW test unit was significantly lower than for the 2 g COD/ℓ FTRW test unit test at a 95% confidence level (P < 0.01). This suggests that an increase in the concentration of FTRW tested with this batch of sludge resulted in an increase in the methanogenic activity as expected and that there was no inhibition of this batch of sludge up to 2 g COD/ℓ of the FTRW, to which the sludge was acclimated.

4.4.2. Set 7

The aim of the experiments in set 7 was to determine which food: micro-organism ratio was the most reliable by comparing the results obtained from this experiment with the results obtained from other batches of sludge tested with the same COD values of FTRW. The same three concentrations of FTRW were tested (that is 1, 1.5 and 2 g COD/ℓ), to which the sludge from UASB 2 (mixture of brewery and municipal sludge) was already acclimated. The respective food: micro-organism ratios were 0.03, 0.05 and 0.07 g COD/g VSS. Each test was done in triplicate to check the reproducibility of the results and to ensure results were available even if there was a problem with one of the bottles. There were 3 blanks which contained only sludge and water.

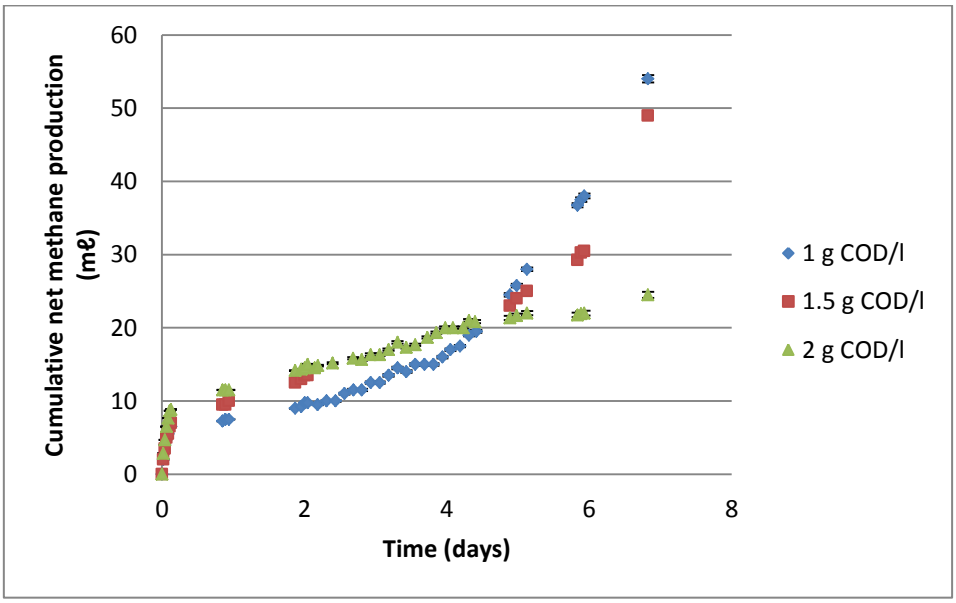

Figure 4.23: Graph showing the average cumulative net methane production (mℓ) over time (days) with UASB 2 sludge and 1, 1.5 and 2 g COD/ℓ of FTRW

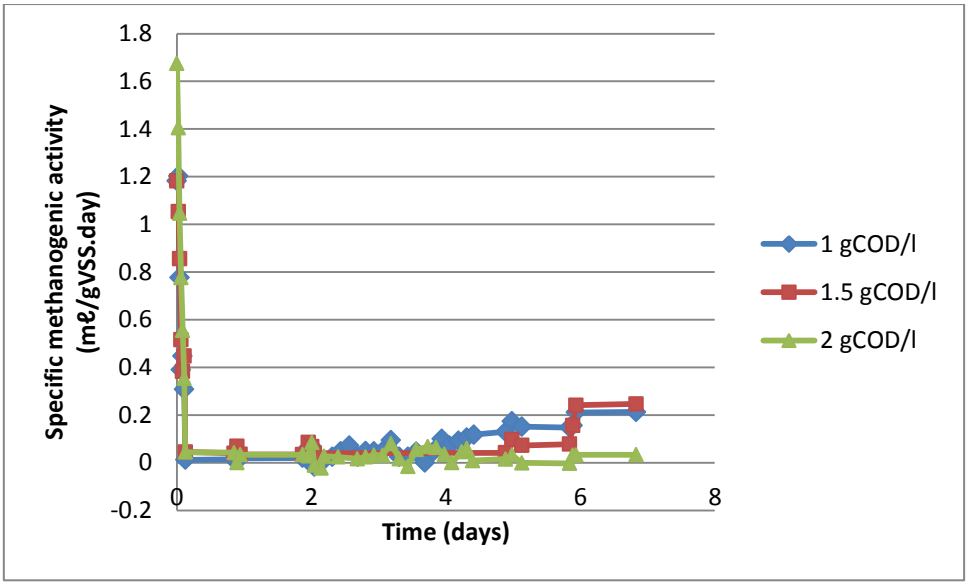

Figure 4.24: Graph showing the specific methanogenic activity (mℓ/gVSS.day) over time (days) with UASB 2 sludge and 1, 1.5 and 2 g COD/ℓ of FTRW

Figure 4.23 shows the net methane production for set 7 where 1, 1.5 and 2 g COD/ℓ of FTRW was tested. The small error bars in **Figure 4.23** indicate that the three replicates of each FTRW concentration showed identical behaviour. From **Figure 4.23** it can be observed that the bottles with the higher concentration of organics (higher COD values of FTRW) did not produce larger volumes of methane as expected. At the beginning of the experiment (over the first 4 days) the methane volumes produced by the 1.5 and 2 g COD/ℓ FTRW test units were higher than the 1 g COD/ℓ test units. However after 4 days, the bottles with the lower concentration of organics, the 1 g COD/ℓ test units, produced more methane than those bottles with the higher concentration of organics (1.5 and 2 g COD/ℓ test units). **Figure 4.24** shows the specific methanogenic activity for set 7 experiments. A paired two sample t-test indicated that the SMA for the 1 g COD/ℓ FTRW test unit was not significantly different from the 1.5 g COD/ℓ FTRW test unit test at a 95% confidence level. A paired two sample t-test indicated that the SMA for the 1 g COD/ℓ FTRW test unit was significantly lower than for the 2 g COD/ℓ FTRW test unit test at a 95% confidence level over the first 2 days ($P = 0.013$). A paired two sample t-test over the rest of the data (after 2 days) indicated that the SMA for the 2 g COD/ℓ FTRW test unit was significantly lower than for the 1 g COD/ℓ FTRW test unit test at a 95% confidence level ($P < 0.001$). This suggests that an increase in the concentration of FTRW tested with this batch of sludge did not result in an increase in the

methanogenic activity as expected and that there may be inhibition of this batch of sludge at 2 g COD/ℓ of the FTRW.

4.4.3. Comparing results from different batches of sludge

The cumulative net methane volume and the specific methanogenic activity of each FTRW concentration was compared for set 6 and set 7.

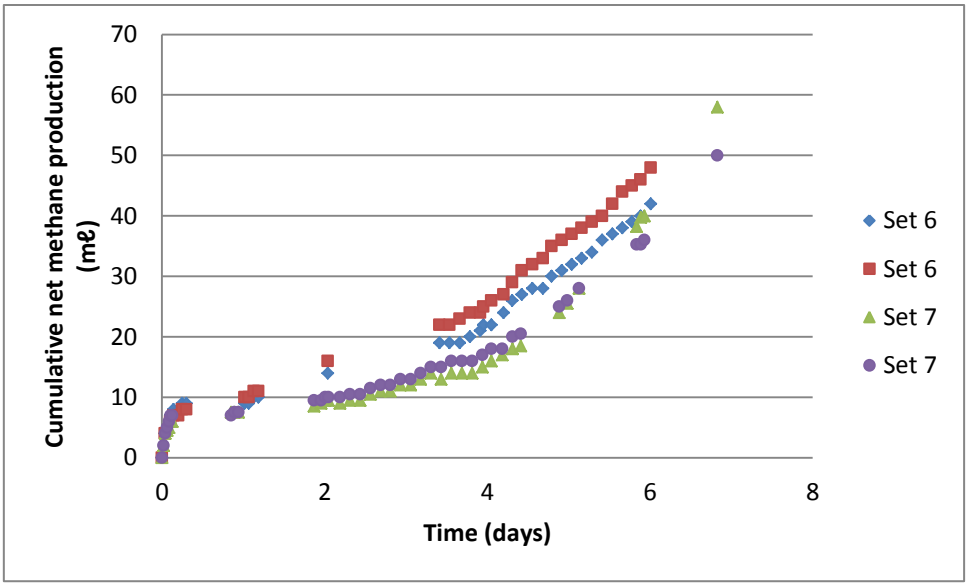

Figure 4.25: Graph showing the cumulative net methane production (mℓ) over time (days) from different batches of sludge when 1 g COD/ℓ FTRW is used

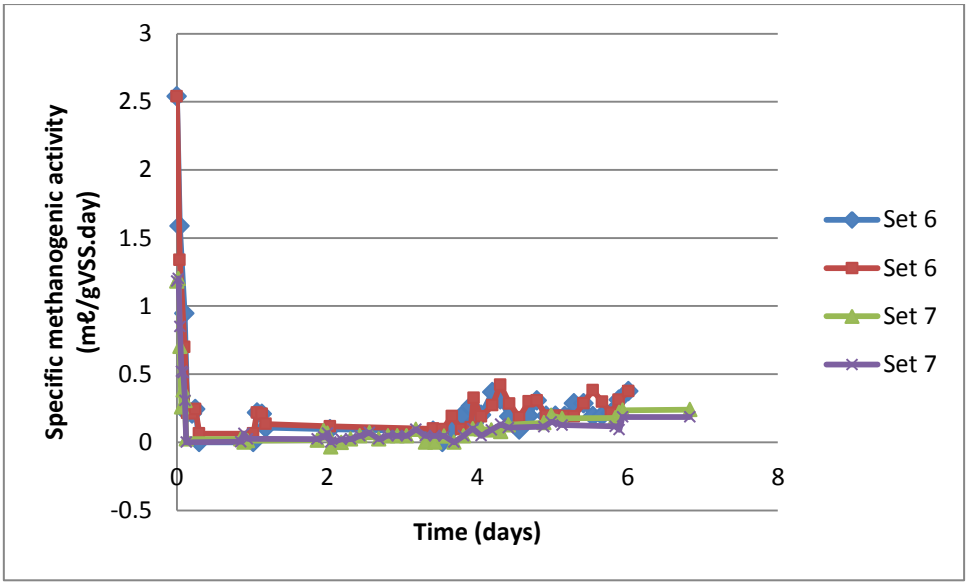

Figure 4.26: Graph showing the specific methanogenic activity (mℓ/gVSS.day) over time (days) from different batches of sludge when 1 g COD/ℓ FTRW is used

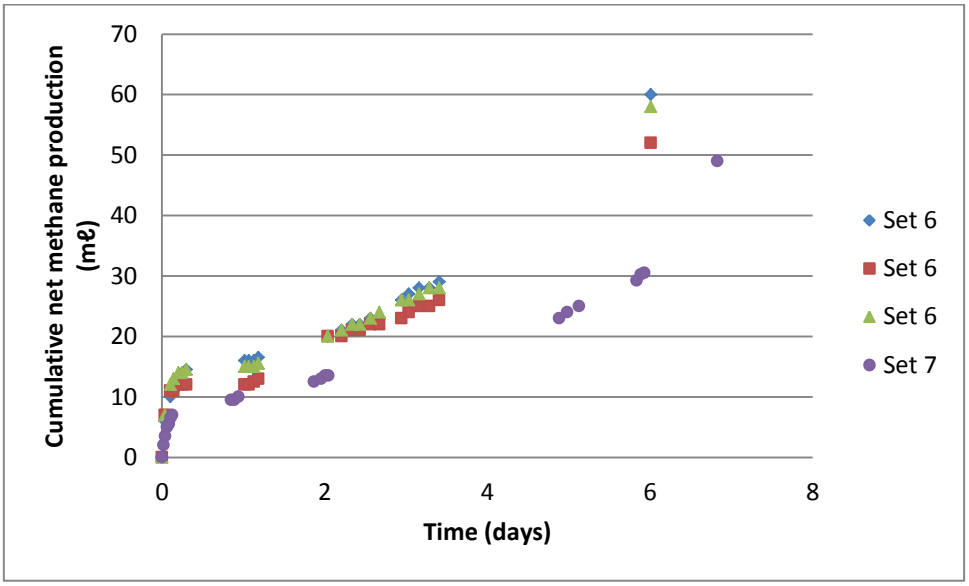

Figure 4.27: Graph showing the cumulative net methane production (mℓ) over time (days) from different batches of sludge when 1.5 g COD/ℓ FTRW is used

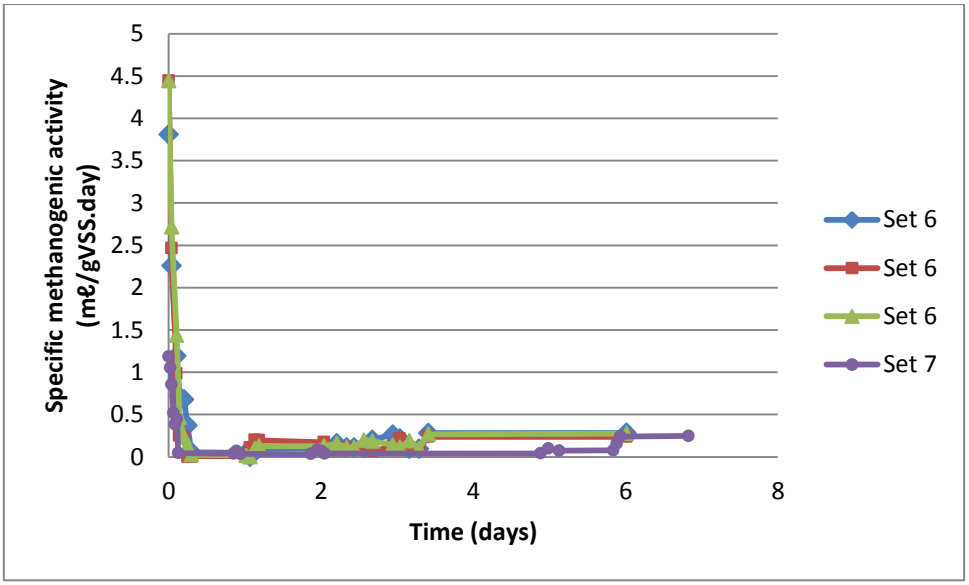

Figure 4.28: Graph showing the specific methanogenic activity (mℓ/gVSS.day) over time (days) from different batches of sludge when 1.5 g COD/ℓ FTRW is used

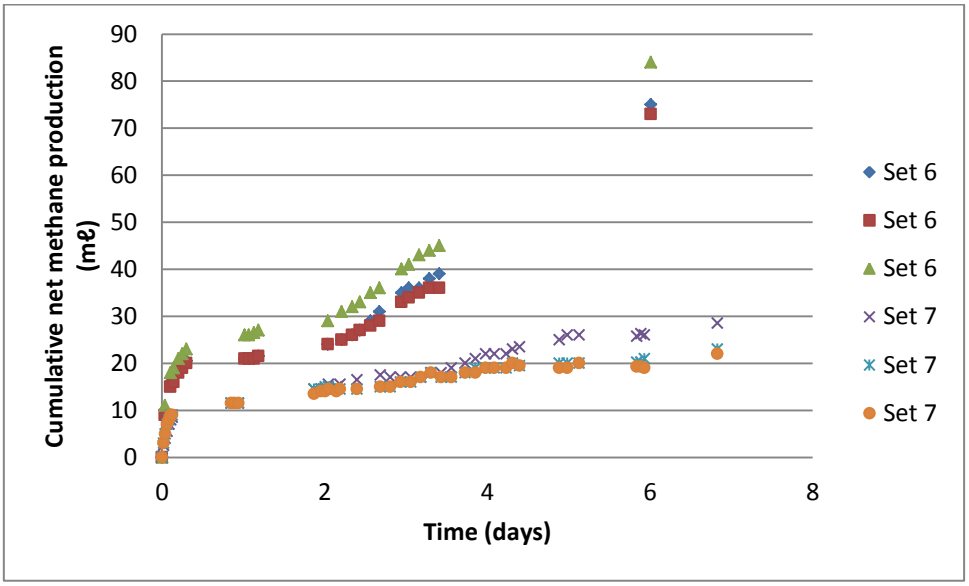

Figure 4.29: Graph showing the cumulative net methane production (mℓ) over time (days) from different batches of sludge when 2 g COD/ℓ FTRW is used

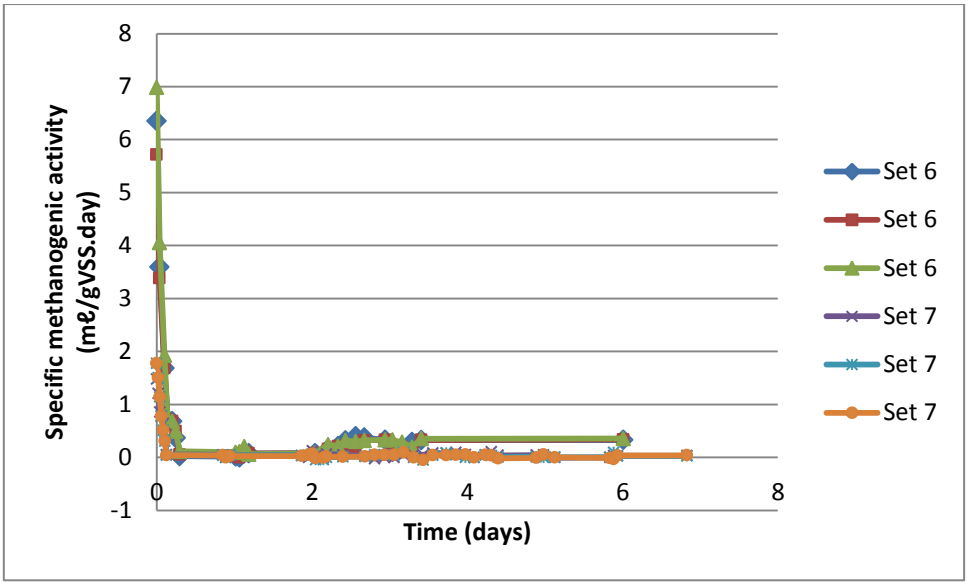

Figure 4.30: Graph showing the specific methanogenic activity (mℓ/gVSS.day) over time (days) from different batches of sludge when 2 g COD/ℓ FTRW is used

Figure 4.25 shows the comparison when 1 g COD/ℓ FTRW was used in different batches of sludge from UASB 2. It can be seen that the results were quite reproducible even when different batches of the sludge were used. **Figure 4.26** shows the specific methanogenic activity for the different batches of sludge tested with 1 g COD/ℓ of FTRW. It can be observed that there is no significant difference between the specific methanogenic activity for set 6 and that for set 7. Looking at **Figure 4.27**, which shows the comparison when 1.5 g COD/ℓ FTRW was used in different batches of sludge from UASB 2, it can be observed that although the results were reproducible for each batch there is no reproducibility when the batches of sludge vary. The net methane volume began to deviate significantly after 2 days. **Figure 4.28** shows the specific methanogenic activity for the different batches of sludge tested with 1.5 g COD/ℓ of FTRW. A paired two sample t-test indicated that the SMA for the set 6 experiments was not significantly different from the set 7 experiments test at a 95% confidence level. **Figure 4.29** shows the comparison when 2 g COD/ℓ FTRW was used in different batches of sludge from UASB 2. Again, it is obvious that the results for each batch were reproducible however there is a very large difference between the net methane volumes for the different batches of sludge. This may indicate some inhibition in set 7 at a COD loading of 2 g COD/ℓ of FTRW. **Figure 4.30** shows the specific methanogenic activity for the different batches of sludge tested with 2 g COD/ℓ of FTRW. A paired two sample t-test

indicated that the SMA for the set 7 experiments was significantly less than that for the set 6 experiments test at a 95% confidence level.

4.5. Summary

The use of additional micro-nutrients to supplement the sludge was not necessary for experiments carried out over a short period of time since it was obvious the sludge inoculum had sufficient micro-nutrients to last the experiment. Three methods of gas collection were tested, the water displacement, acidified brine solution and alkaline solution methods. With the water displacement method it was found that the gaseous carbon dioxide in the biogas may have been dissolving in or diffusing through the displacement liquid (water) causing the cumulative biogas volume to decrease. The acidified brine solution method lowered the solubility of the carbon dioxide in the displacement liquid (acidified brine solution) to a certain extent. However it was only collecting less than half of the expected biogas volume which suggests that as the biogas volume increased, the concentration of the gaseous carbon dioxide in the headspace increased and caused the carbon dioxide to dissolve back into the liquid phase. Therefore the alkaline solution method was used to absorb the carbon dioxide produced, collecting only the methane produced, which eliminated the uncertainty caused by the carbon dioxide dynamics. The volume of methane collected using the alkaline solution method was similar to the theoretical volume of methane expected. A concentration of 1 g COD/ℓ FTRW with UASB 2 sludge was chosen as the reference test due to its reproducibility of the replicates within each experiment as well as its reproducibility (in methane volume and specific methanogenic activity) across different batches of sludge.

Therefore, for the inhibition experiments, which tested the effect of SGL on the FTRW digestion, a concentration of 1 g COD/ℓ of FTRW was used together with an additional predetermined concentration of SGL. Additional micro-nutrients were not used to supplement the sludge. The alkaline solution method was used to collect the methane produced.

CHAPTER 5

5. Effect of SGL on FTRW Digestion

In chapter 5, the effect that stripped gas liquor has on the anaerobic digestion of Fischer-Tropsch reaction water is investigated. The aim is to test whether the stripped gas liquor ultimately enhances or inhibits the digestion of the Fischer-Tropsch reaction water with a sludge which is acclimated to the Fischer-Tropsch reaction water. In this chapter, it is also determined whether there is any change in the phenol concentration during digestion. In section 5.1, a constant concentration of 1 g COD/ℓ of Fischer-Tropsch reaction water is used, with the stripped gas liquor contributing an additional COD loading of 0.05 and 0.15 g COD/ℓ, respectively, to the experiment. In section 5.2, the effect when a constant total COD loading of 1 g COD/ℓ is used, with the Fischer-Tropsch reaction water contributing 85% and stripped gas liquor contributing 15% to the COD loading, is considered. Additional micro-nutrients were not used to supplement the sludge in any of the following experiments. The alkaline solution method was used to collect the methane produced.

Table 5-1: Table showing the concentration of the substrates and the type of gas measurement used in the experiments to determine the effect of SGL on FTRW digestion

Experiment	Type of gas measurement	Sodium acetate (g COD/ℓ)	FTRW (g COD/ℓ)	SGL (g COD/ℓ)	Sludge	Purpose of experiment
Set 8	Alkaline solution	0	1	0.05, 0.15	UASB 2	Effect of SGL on FTRW digestion
Set 9	Alkaline solution	0	0.85	0.15	UASB 2	Effect of SGL on FTRW digestion

Table 5-2: Table showing the initial characteristics of the SGL used in set 8 and 9

	COD (mg/ℓ)	Phenol concentration (mg/ℓ)
Set 8	2070.94	140.2
Set 9	1509.9	65.58

5.1. Set 8: Constant FTRW COD load

Set 8 tested the effect of SGL on the anaerobic digestion of FTRW. This experiment was used to determine if there is any change in the phenol concentration in the course of the test. The first three bottles were used as the reference units and contained 100 mℓ sludge and 1 g COD/ℓ FTRW only. The next three bottles were the test units and contained 100 mℓ sludge, 1 g COD/ℓ FTRW and 0.05 g COD/ℓ SGL. The last three bottles were also the test units and contained 100 mℓ sludge, 1 g COD/ℓ FTRW and 0.15 g COD/ℓ SGL. The respective food: micro-organism ratios were 0.03, 0.034 and 0.038 g COD/g VSS. Each data set was averaged over 3 replicates to give a mean and 95% confidence interval.

Table 5-3: Composition of test and reference units for constant FTRW load tests

Subset	a and b	c (5% SGL)	d (15% SGL*)
Reference	1 g COD/ℓ FTRW	1 g COD/ℓ FTRW	1 g COD/ℓ FTRW
Test	-	(1 FTRW + 0.05 SGL) g COD/ℓ	(1 FTRW + 0.15 SGL) g COD/ℓ
Duration	(a) 7 d (b) 27 d	6 d	9 d

***Note:** 15% SGL for set 8 is based on the COD of the reference test. When the percentage SGL was calculated from the total COD load of the test unit it was found to be 13%. However in the discussion, the unit test will be referred to as 15% SGL.

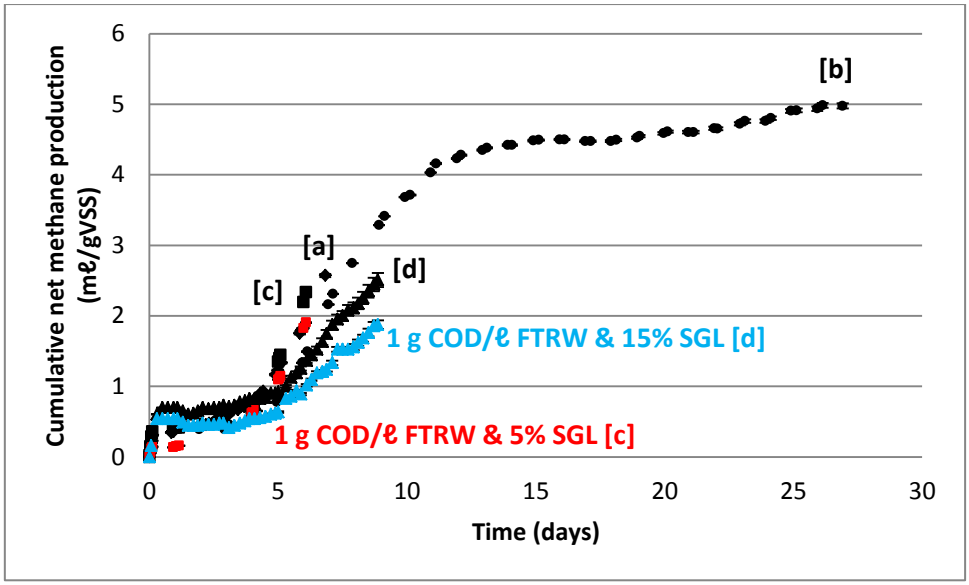

Figure 5.1: Graph showing the average cumulative net methane production (mℓ/gVSS) against time (days) when only 1 g COD/ℓ of FTRW is degraded and when 1 g COD/ℓ of FTRW is co-digested with 5% SGL and 15% SGL. Reference units are labelled according to Table 5-3

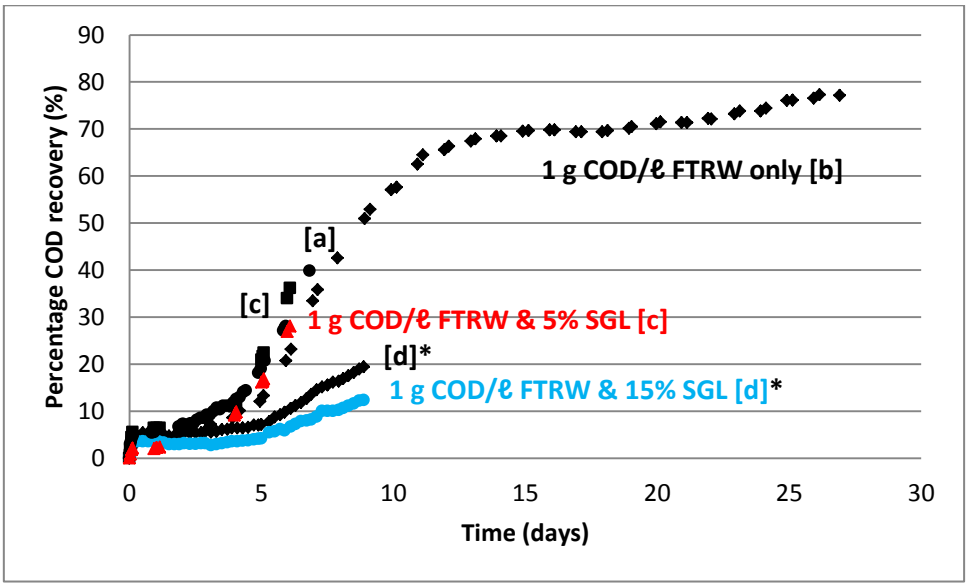

Figure 5.2: Graph showing the percentage COD recovery (%) over time (days) when only 1 g COD/ℓ of FTRW is degraded and when 1 g COD/ℓ of FTRW is co-digested with 5% SGL and 15% SGL. Reference units are labelled according to Table 5-3

***Note:** The COD recovery for the 15% SGL test [d] was much lower than the other curves due to the fact that the VSS was lower in that experiment and therefore the rate of reaction was lower for these test units

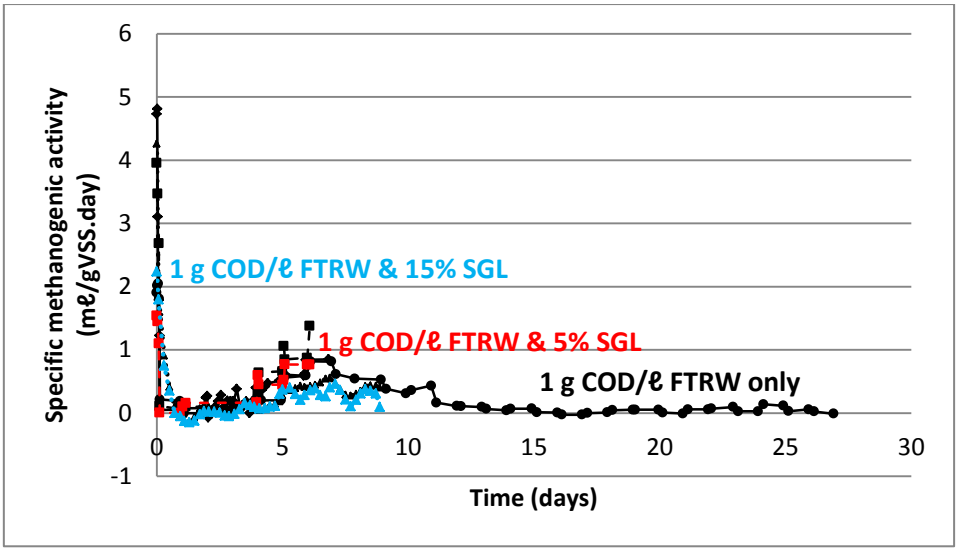

Figure 5.3: Graph showing the average specific methanogenic activity (mℓ/gVSS.day) over time (days) when only 1 g COD/ℓ of FTRW is degraded and when 1 g COD/ℓ of FTRW is co-digested with 5% SGL and 15% SGL

Figure 5.1 shows the net cumulative methane produced over time. All test units showed similar net methane production profiles. There is an initial exponential increase during the first day, after which the methane production curve seems to flatten. There is then a second increase after 5 days. Since the substrates were at room temperature before being added to the serum bottles and placed in the water bath at 35 °C, a reasonable explanation of the initial high, but variable calculated activity, would be thermal expansion brought about by a decrease in the headspace temperature through addition of cooler substrates and then reheating to the incubation temperature of 35 ºC after the units were sealed. It could also be a period of high methanogenic activity brought about by the addition of substrate but subsequently inhibited by pH reduction through acidogenesis.

The test units containing SGL produced similar amounts of methane to reference units. After 27 days at the sludge solids concentration used in subset d, 80% of COD had been recovered as methane. Methane production was still continuing.

Figure 5.3 showed the specific methanogenic activity against time. The periodic increase and decrease of the 15% test unit SMA was due to the averaging of methane volume of the replicate bottles and the calculation of the derivative of these averages. Although the bottles containing SGL had a higher net concentration of organics than those without SGL, this did not translate into an increase in methanogenic activity. A paired two sample t-test was done

to compare the 5% and 15% SGL test units to the paired reference tests. This analysis was performed only for data after the first day of the test as there may have been non-biological effects involved in the measured gas production of the first day. The t-test indicated that the SMA for the 5% SGL test unit was not significantly different from the SMA of the matching 1 g COD/ℓ FTRW reference test at a 95% confidence level (P = 0.103). The SMA for the 15% SGL test unit was significantly lower than for the matching 1 g COD/ℓ FTRW reference test at a 95% confidence level ($P = 9 \times 10^{-5}$) (refer to Appendix F for an example of the calculation). This suggests that the addition of 15% SGL quantifiably reduces the SMA. It is not possible to conclude that this is due to an inhibitory effect of SGL since the total COD load was not the same between units with SGL and those with only FTRW. From previous experiments it was found that there was no inhibition at higher COD loads in some batches of sludge (Section 4.4.1. Set 6) while other batches indicated some inhibition at 2 g COD/ℓ and above (Section 4.4.2. Set 7). However, the magnitude of the reduction in SMA suggests that at the SGL concentrations and total COD load tested, reduction of SMA due to additional COD load and the presence of SGL was not sufficiently great to indicate that co-digestion of these two substrates would not be possible. This can be seen in **Figure 5.1**, where the net methane production for the samples with SGL was reduced, but not dramatically so, compared to the paired reference units.

The colorimetric analysis returns a lumped measurement of phenolic compounds. The concentration of phenolics in the bottle with 5% SGL was measured, before the experiment was started, as 8.53 ± 7.25 mg/ℓ and again 6 days later as 7.78 ± 6.70 mg/ℓ. The wide confidence interval shows that the measurement is near the detection limit, and therefore the values have limited reliability, however, the difference between these two values is not statistically significant. The concentration of phenols in the bottle with 15% SGL was measured, before the experiment was started, as 17.53 ± 3.07 mg/ℓ and again 7 days later as 12.1 ± 1.03 mg/ℓ. There is a significant decrease in the phenol concentration for 15% SGL. This result suggests that conversion of phenolic compounds may be possible using a sludge that has been acclimated to FTRW. A modification of the standards addition technique for this analysis resulted in a smaller confidence interval in the second set of tests (Appendix E4. Phenol measurement).

The percentage SMA reduction for the 15% SGL test unit, which had a phenol concentration of 17.53 ± 3.07 mg/ℓ, was calculated by averaging the percentage SMA reduction at each time (difference in the SMA as a percentage of the reference SMA) and was found to be 31%.

The confidence interval on the percentage reduction was difficult to calculate since there was a high variance in the data, there was a high variance in the variance for different times and the SMA values were derivatives of the data. However 35 observations is a significant number of paired measurements and a confidence interval on the mean of the calculated percentage SMA reductions at each time should give an indication of the percentage inhibition experienced even if it may be different to the percentage inhibition in a continuous system. The confidence interval was calculated as 20%. Therefore the percentage SMA reduction ranged from 11 – 51%. It seems that the reduction in methanogenic activity is significant. An alternate way to calculate the percentage SMA reduction of the 15% SGL test unit was by comparing the average SMA difference to the average SMA of the reference and was found to be 24% for all data after day one. This value falls in the range of 11 – 51%. The percentage inhibition was also checked by comparing the cumulative methane produced from day 2 to day 9 in the test unit to the cumulative methane produced in the reference unit and was calculated to be 23% which also fall in the range of 11 – 51%. Calculations for the percentage SMA reduction are shown in Appendix G. Therefore, despite the noisiness of the data, several different methods of calculating the average reduction in SMA suggest that it lay between 11 and 51% lower than the paired reference test value.

Kayembe et al. (2013) observed that 1249 ± 29.59 mg/ℓ of phenol gave a percentage inhibition of 50% in a batch system and Fang et al. (1997) observed that the concentration of phenol which gave a percentage inhibition of 50% ranged from 1000 to 1750 mg/ℓ in a batch system. Assuming a linear relationship between the phenol concentration and the percentage inhibition and using the values found in literature, the theoretical percentage inhibition for the 15% SGL test unit would range from 0.51 – 0.9%. The range of these values was much lower than the range of the percentage SMA reductions calculated from the experiments. This difference may be due to the different sludge types used in literature which may contain a different mix of organisms than the sludge used in this project and thus may be able to cope better with the sudden influx of phenol.

5.2. Set 9: Constant total COD load

From the previous experiments it was not possible to definitively conclude whether the reductions in activity were due to higher COD loading or an inhibitory effect of SGL. Therefore, in set 9, all tests had the same total COD (i.e. 1 g COD/ℓ). Tests were done in triplicate. The blanks contained only sludge and tap water. A food: micro-organism ratio of 0.036 g COD/g VSS was used. A reference of 1 g COD/ℓ FTRW was used for comparison of the co-digestion tests. For the co-digestion tests, the ratio of SGL to FTRW tested was 15% SGL and 85% FTRW. The percentages were on a COD basis.

There were six extra test units set up, with the same composition as the 15% SGL test, to sacrifice at different times during the experiment to measure phenolics, COD, VSS, pH and for GC analysis. Since all of the additional bottles had the same initial composition as the 15% SGL test, analysis of the sacrificed bottles were assumed to be indicative of conditions in the replicate tests.

The results from the previous experiment were used as a guide as to the approximate time when the extra test units should be sacrificed and analysed. Looking at the results of the previous experiments it could be seen that there were 3 critical points at which a deeper understanding of the chemistry of the bottles would be desirable (circled in orange on **Figure 5.4** below) to understand the specific methanogenic activity curve. The additional test units were sacrificed at each of the points indicated and analysed for COD, solids, phenols concentration, and pH as indicated on **Figure 5.4** below. The sacrificing of the remaining 3 bottles (shown in green on **Figure 5.4** below) were distributed throughout the experiment with analysis being done more often at the beginning of the experiment and less often as the activity slows down. Analysis for solids, pH, COD and phenolics were also done at the beginning and end of the experiment.

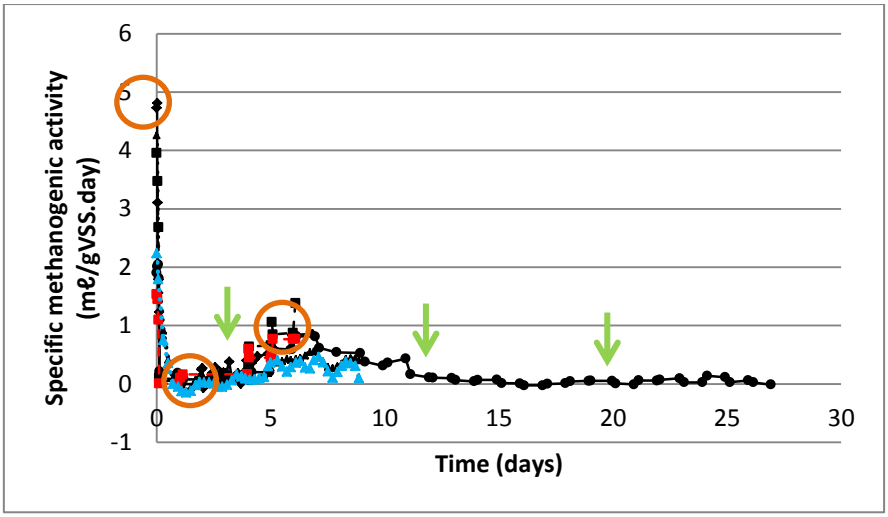

Figure 5.4: Graph showing the proposed points and dates at which the extra bottles will be sacrificed

For set 9, new sludge had to be grown since the sludge used in the previous experiments had been exhausted. Sludge was originally obtained from the South African Brewery wastewater treatment plant. This sludge was acclimated to FTRW in a UASB reactor for two months. Since the acclimation period for the new sludge was shorter than the acclimation period for the sludge used in set 1 – 8, the micro-organism consortium that established in the new sludge may have been fundamentally different to the micro-organism consortium of the previous sludge and therefore may have different biokinetic characteristics and may behave differently with the substrates.

Accurate and frequent liquid displacement data were obtained by using webcams which captured an image of the collection vessels every minute. All of the bottles (except those which were sacrificed) were run to completion (i.e. until the methane production curve flattened out and there was no further production of methane).

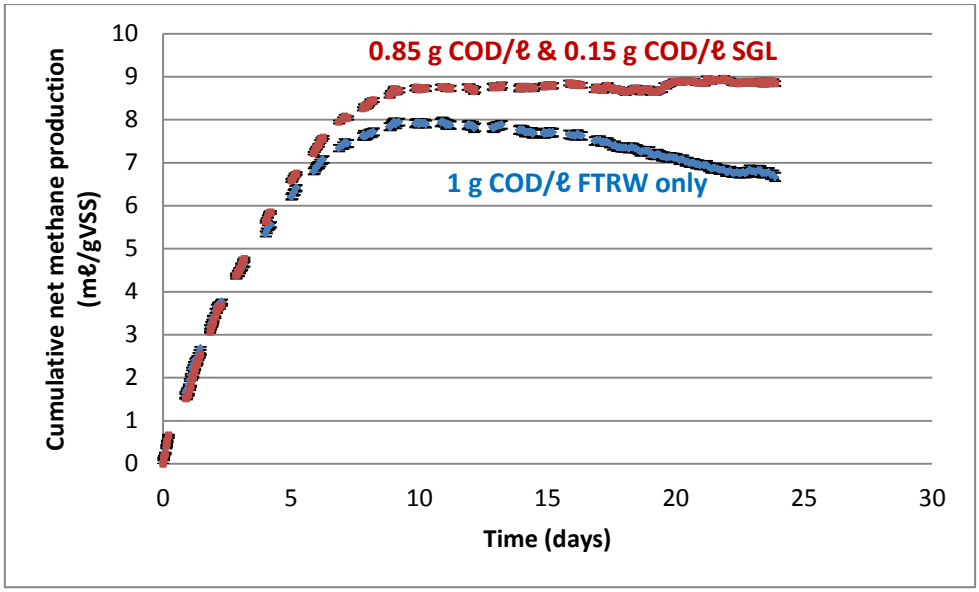

Figure 5.5: Graph showing the average cumulative net methane production (mℓ/gVSS) against time (days) when only 1 g COD/ℓ of FTRW is degraded and when 0.85 g COD/ℓ of FTRW is co-digested with 0.15 g COD/ℓ of SGL

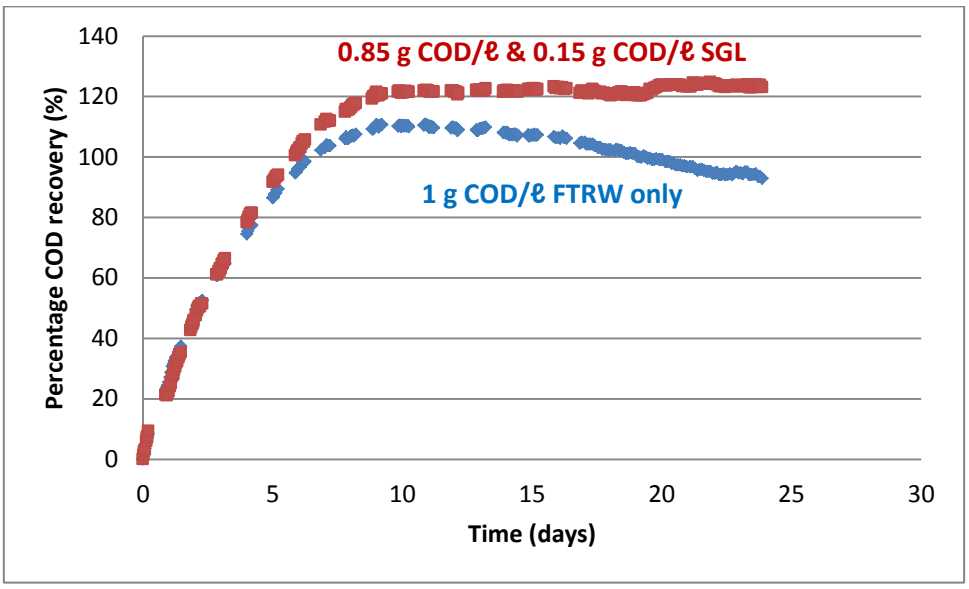

Figure 5.6: Graph showing the percentage COD recovery (%) against time (days) when only 1 g COD/ℓ of FTRW is degraded and when 0.85 g COD/ℓ of FTRW is co-digested with 0.15 g COD/ℓ of SGL

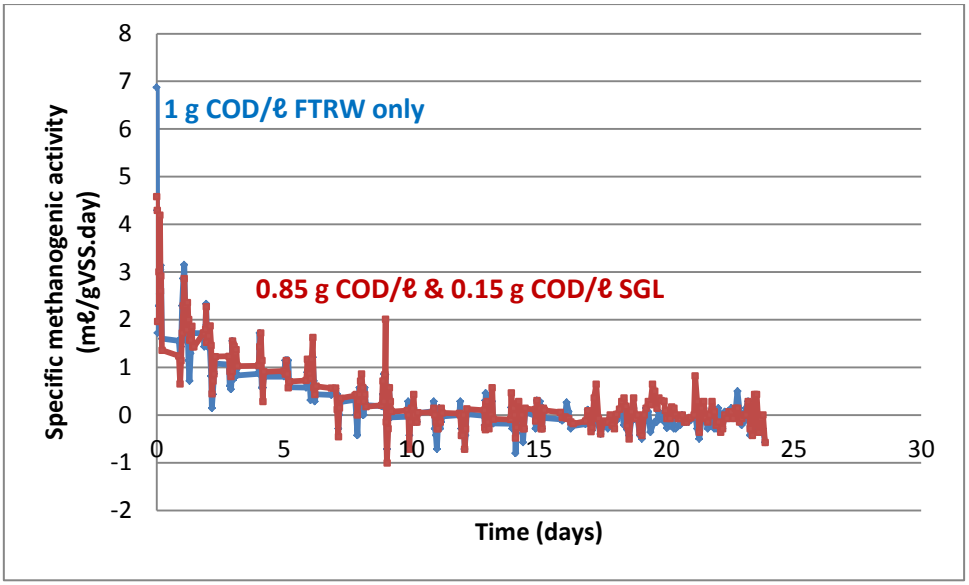

Figure 5.7: Graph showing the specific methanogenic activity (mℓ/gVSS.day) against time (days) when only 1 g COD/ℓ of FTRW is degraded and when 0.85 g COD/ℓ of FTRW is co-digested with 0.15 g COD/ℓ of SGL

Figure 5.5 shows the cumulative net methane produced over time when only 1 g COD/ℓ of FTRW was digested (reference unit) and when 0.85 g COD/ℓ of FTRW was co-digested with 0.15 g COD/ℓ of SGL (test unit). The small error bars in **Figure 5.5** indicate that the three replicates of the reference and test units showed identical behaviour. The profile of the curves in this experiment (set 9) was much smoother than that in set 8 since the temperature of the substrates were controlled by bringing the substrate temperature to the required temperature of 35 °C before adding the substrates to the serum bottle. There were no step increases like that observed in set 8 and it seemed to follow the curve of a typical Monod curve. At the beginning of the experiment, for approximately 3 – 4 days, the methane production for both the reference and test units were very similar. Thereafter, there was a significant difference in the methane volumes produced, with the test unit with SGL producing more methane. There was a decrease in the net cumulative methane curve towards the end of the reference test which may be due to endogenous respiration as the labile substrates were exhausted and the micro-organisms began to die. The decrease could also be due to the net methane curve in **Figure 5.5** being calculated from a difference between the methane production of the test unit and the methane production of the blank. The percentage COD recovery was determined by converting the volume of gas collected (assumed to be methane only) to g COD and comparing it to the initial quantity of food added to the bottle. The percentage COD recovery

for both the reference and test units were above 100% as shown in **Figure 5.6**. This may be due to sludge stimulation which caused an enhanced degradation of slowly degradable material that may have been present in the sludge from the previous use of the sludge or the degradation of products resulting from biomass death. The specific methanogenic activity is shown in **Figure 5.7** and it could be observed that the specific methanogenic activity of the reference unit was significantly lower than that of the test unit. A paired two sample t-test confirmed this difference with $P = 3.8 \times 10^{-5}$. The analysis was performed with data from day 0 to day 24. Therefore there was no evidence of SMA reduction by the addition of SGL. There may even have been some stimulation of the anaerobic activity as a result of the presence of different labile substrate constituents in the sludge. The reduction in the specific methanogenic activity of the test unit in the previous experiment (set 8) may have been due to the higher COD loading rather than an inhibitory effect of SGL. However previous experiments found that there was no inhibition at higher COD loads in some batches of sludge (Section 4.4.1. Set 6) while other batches indicated some inhibition at 2 g COD/ℓ and above (Section 4.4.2. Set 7). Therefore the suggestion that the apparent reduction in the activity in set 8 may be due to the increased COD load instead of the inhibitory effect of SGL is not strongly supported. The SGL used in set 8 and set 9 were from different batches and had different COD and phenol concentrations. The SGL used in set 8 had a higher COD and phenol concentration than that used in set 9 as shown in **Table 5-2**. The phenol concentration of the SGL used in set 9 was 65.58 mg/ℓ while the phenol concentration of the SGL used in set 8 was 140.2 mg/ℓ, which was more than twice the concentration of the set 9 SGL. Therefore it was concluded the addition of SGL potentially reduced the SMA and that this could be an inhibitory effect, but that any inhibition would be a function of the concentration of potentially inhibitory substances in SGL and that these concentrations vary from batch to batch. However, even if the SGL is inhibitory at the 15% SGL level with a total COD load of 1.15 g COD/ℓ, the degree of inhibition is fairly low and would not prevent co-digestion of the two streams at the concentrations tested. The mechanism of inhibition is unknown at this stage and determining the mechanism of inhibition would require much more complex, expensive and time-consuming experiments that were outside the scope of the project.

The pH, VSS, COD and phenol concentration of the test units (0.85 g COD/ℓ FTRW and 0.15 g COD/ℓ SGL) were measured during the experiment.

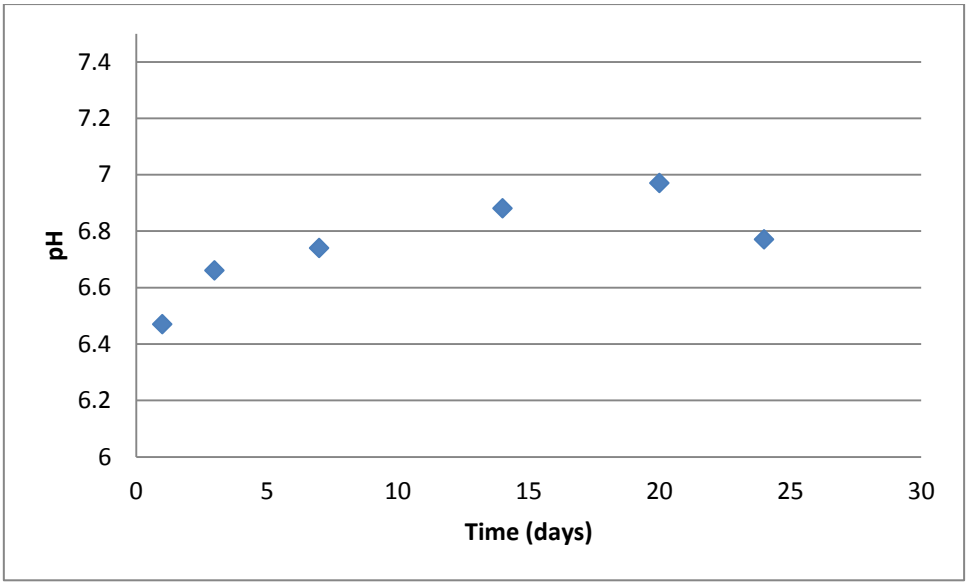

Figure 5.8: Graph showing the pH value against time (days) for the test unit with 0.85 g COD/ℓ of FTRW co-digested with 0.15 g COD/ℓ of SGL

Figure 5.8 showed the pH of the test unit at intervals during the experiment. All measurements were made from different but identically constituted bottles. The pH remained between 6.5 and 7.5 which is the optimum pH range for anaerobic digestion (Liu et al., 2007).

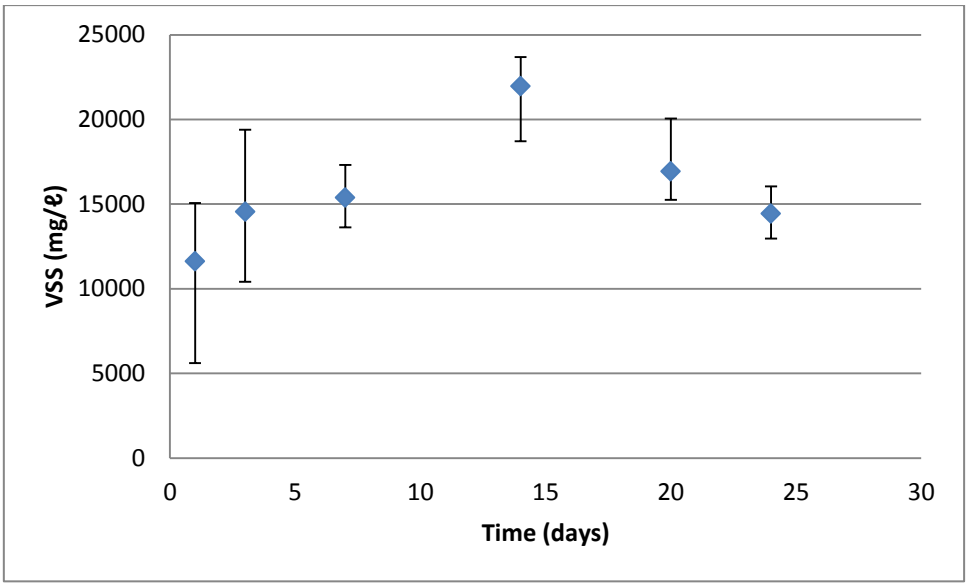

Figure 5.9: Graph showing the volatile suspended solids (mg/ℓ) against time (days) for the test unit with 0.85 g COD/ℓ of FTRW co-digested with 0.15 g COD/ℓ of SGL

The change in the volatile suspended solids (VSS) over time is shown in **Figure 5.9**. There was an increase in the VSS over the first 14 days of the experiment, and then the VSS began to decrease. The VSS increased at the beginning as the biomass concentration increased through growth by digesting the labile substrate. After 14 days the VSS had started to decrease due to endogenous respiration as the labile substrate may have been exhausted and the micro-organisms were beginning to die. Each point on the graph represents the average of triplicate measurements of the same sample as one bottle was sacrificed at each point. Therefore it does not account for any variation that may be expected between test units. The trend in the VSS plot matches the trend in the net methane production curve shown in **Figure** 5.5**.**

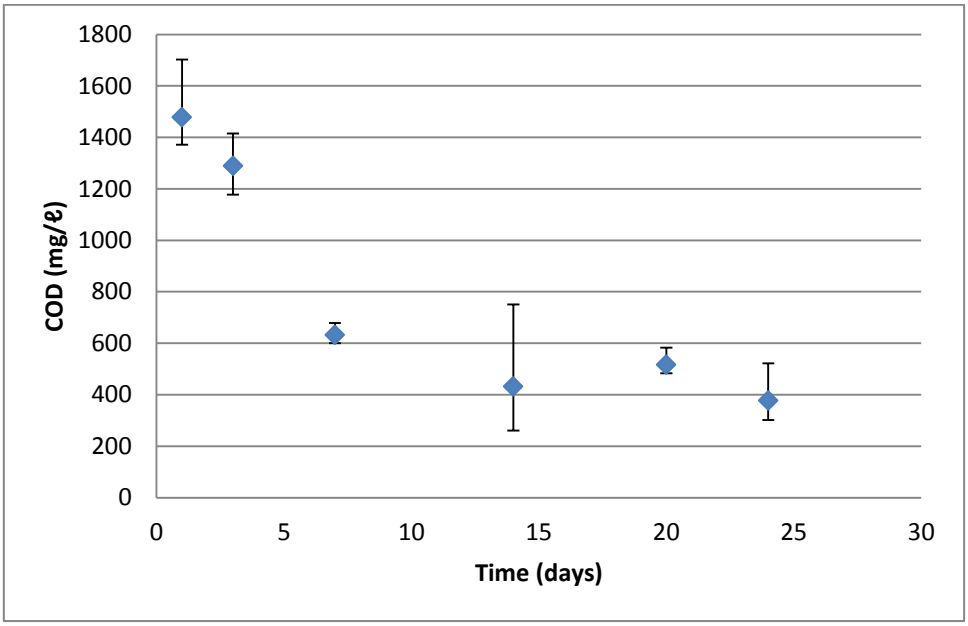

Figure 5.10: Graph showing the COD (mg/ℓ) against time (days) for the test unit with 0.85 g COD/ℓ of FTRW co-digested with 0.15 g COD/ℓ of SGL

Figure 5.10 shows the COD concentration with time as determined by the COD test using the closed reflux method. The COD decreased as COD was removed from the solution and converted to methane gas. From this, the percentage of COD removed could be calculated and was found to be 74.5 ± 7.8%. The percentage COD was calculated using data from day 1 to the end and not from day 0. It is possible that the percentage of COD actually removed may be higher. Each point on the graph represents the average of triplicate measurements of

the same sample as one bottle was sacrificed at each point. Therefore it does not account for any variation that may be expected between test units.

The phenols were analysed by the colorimetric method and the gas chromatograph.

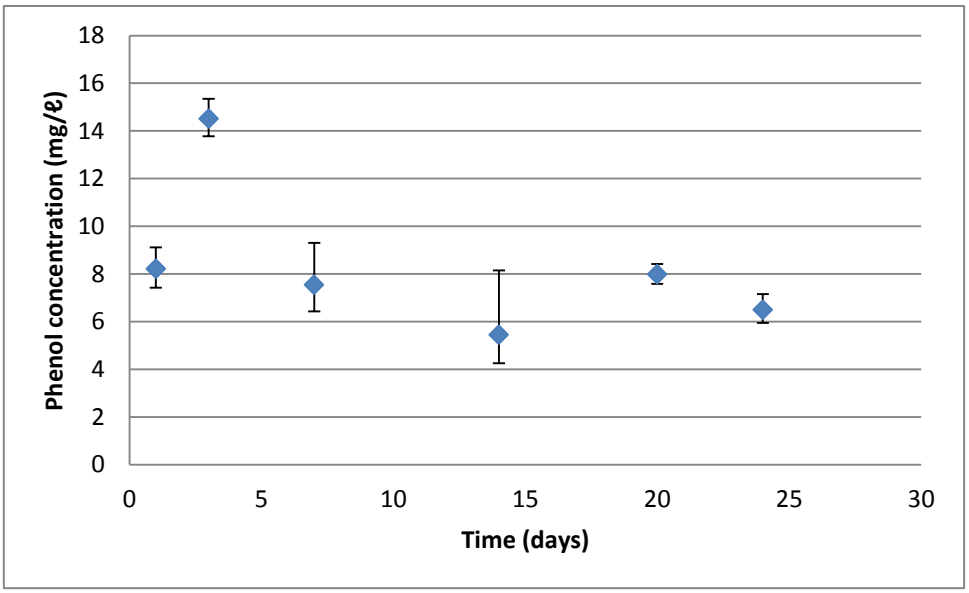

Figure 5.11: Graph showing the phenol concentration (mg/ℓ) against time (days) for the test unit with 0.85 g COD/ℓ of FTRW co-digested with 0.15 g COD/ℓ of SGL

Figure 5.11 shows the phenol concentration results from the colorimetric method. At the beginning of the experiment the phenol concentration was 8.21 ± 0.91 mg/ℓ and at the end of the experiment it was 6.49 ± 0.67 mg/ℓ. There was a decrease in the concentration of the phenols in the test unit from the beginning of the experiment to the end and the two values were significantly different, but only by a very small amount. While some removal may occur, the percentage phenol removal appears to be a lot lower than the percentage COD removal, suggesting that even if phenols are degraded, the sludge is not effective for phenol removal.

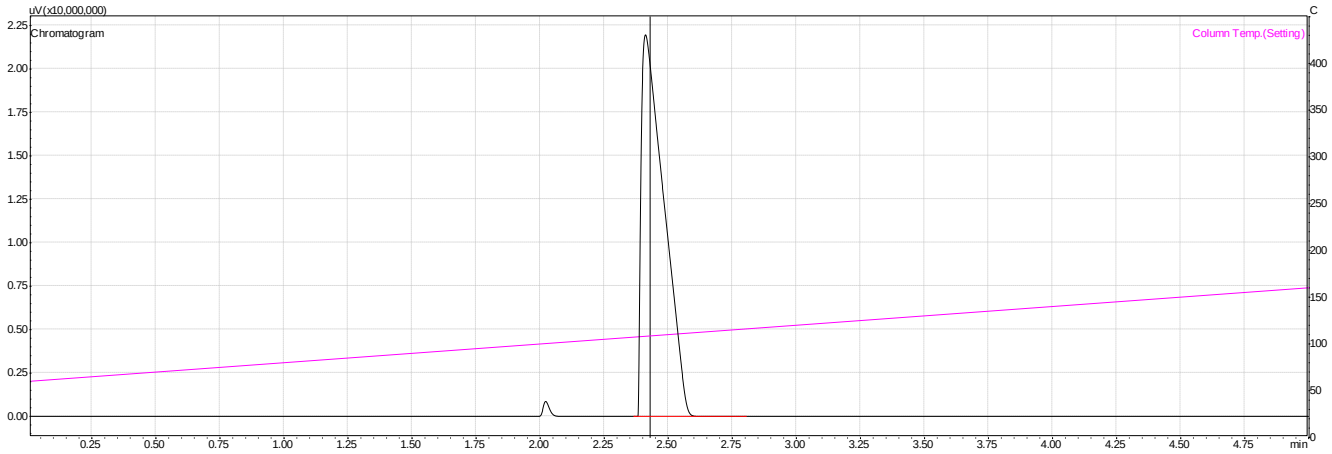

Figure 5.12: Gas chromatograph profile of the test unit showing the phenol peak at the beginning of the experiment (day 1)

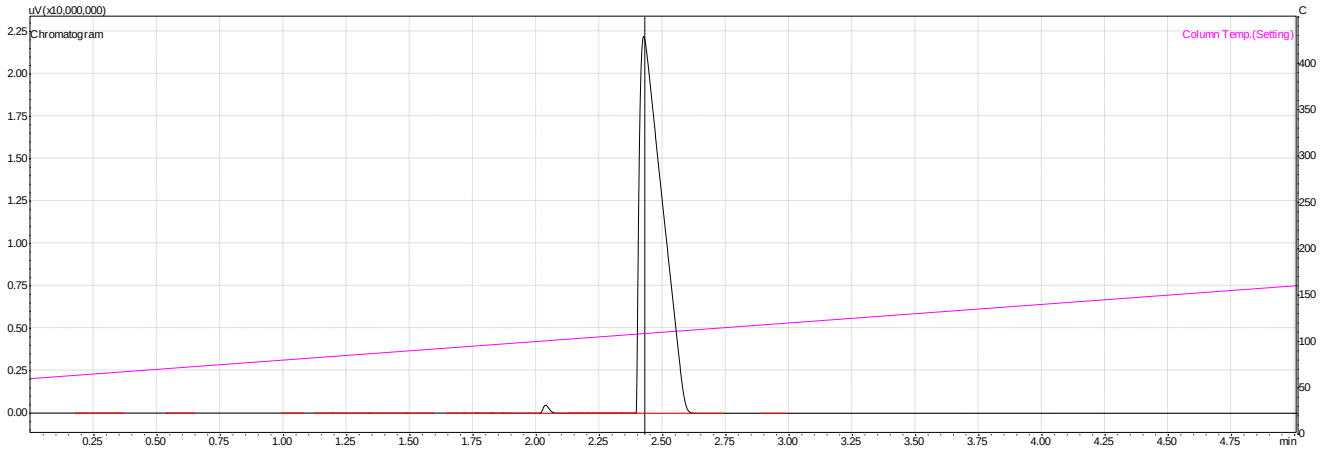

Figure 5.13: Gas chromatograph profile of the test unit showing the phenol peak at the end of the experiment (day 23)

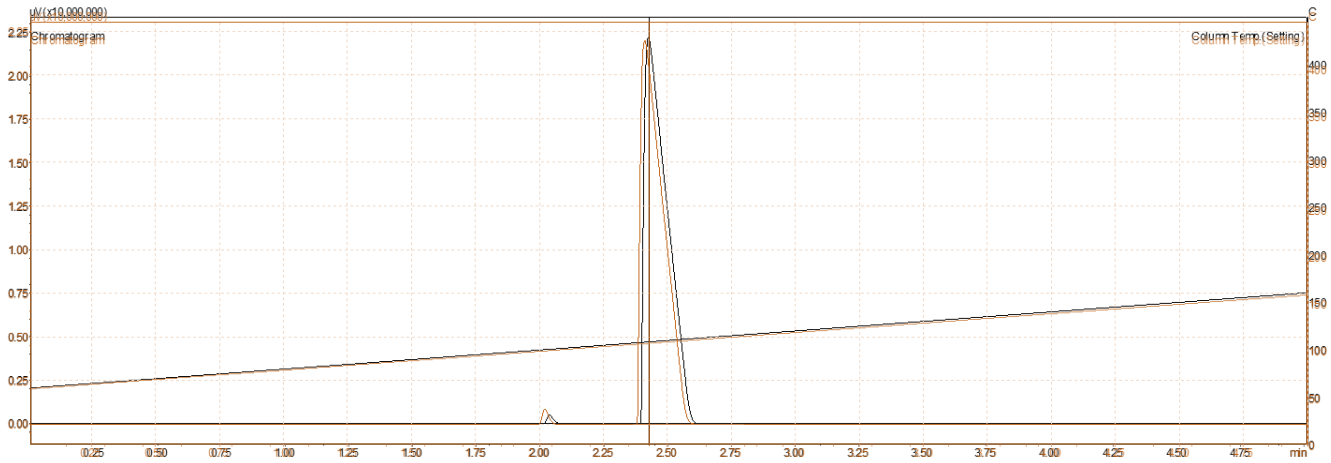

Figure 5.14: Overlap of the gas chromatograph profiles of the test unit at the beginning (orange) and end of the experiment (black)

The concentration of the phenol in the test unit test was also analysed using a gas chromatograph. A solid phase extraction was performed before GC analysis. During the solid phase extraction there were a number of steps where uncertainty may have been introduced and with the heterogeneous nature of the samples it was not possible to obtain a high enough precision on the standard samples to be confident in the calibration curve. Therefore the chromatograph profiles have been used for a qualitative validation of the results obtained from the colorimetric method. An analysis of a pure phenol solution showed that the retention time for the phenol peak was around 2.4 minutes. **Figure 5.12** and **Figure 5.13** show the gas chromatograph profiles of the test unit at the beginning and end of the experiment, respectively. An overlay of the gas chromatograph profiles (**Figure 5.14**) showed that there was no significant difference in the phenol peaks and therefore little change in the phenol concentration during the experiment.

CHAPTER 6

6. Discussion

The purpose of this study was to determine the influence of stripped gas liquor on the specific methanogenic activity of Fischer-Tropsch reaction water acclimated sludge and to investigate the fate of indicator compounds for potentially toxic higher molecular weight organics in stripped gas liquor, such as phenols, in batch anaerobic co-digestion experiments. In order to determine this, serum bottle tests were performed under mesophilic conditions to measure the methanogenic activity when Fischer-Tropsch reaction water and stripped gas liquor are anaerobically co-digested. The biogas produced was collected and monitored during the experiments. This chapter discusses the experimental results obtained with regards to the research outcomes.

6.1. Method development

The method development chapter investigated whether the use of additional nutrients was necessary and evaluated techniques to collect and measure the biogas produced. Different COD loads of the FTRW were tested to determine what the base experiment should look like and a reference test was chosen.

6.1.1. The necessity of additional nutrients

It was found early in the method development that the use of additional micro-nutrients to supplement the sludge was not necessary for experiments carried out over a short period of time since the biogas produced was similar in the test units with and without additional nutrients. It was obvious the sludge inoculum had sufficient micro-nutrients to last the experiment. Therefore nutrients were not added to subsequent serum bottle tests.

6.1.2. Gas collection techniques

Three methods of gas collection were tested, the water displacement, acidified brine solution and alkaline solution methods. The water displacement method consisted of a serum bottle, filled with sludge and substrates, attached to a gas collection dropper which displaced water into an overflow chamber as biogas was produced. With the water displacement method it was found that the gaseous carbon dioxide in the biogas may have been dissolving in or diffusing through the displacement liquid (tap water) causing the cumulative biogas volume to decrease. The tap water in the gas collection chamber was then replaced with an acidified brine solution. Although the acidified brine solution method lowered the solubility of the carbon dioxide in the displacement liquid (acidified brine solution) to a certain extent, it was only collecting less than half of the expected biogas volume. This suggests that as the biogas volume increased, the concentration of the gaseous carbon dioxide in the headspace increased and caused the carbon dioxide to dissolve back into the liquid phase. Finally the alkaline solution method was tested. The alkaline solution method consisted of a serum bottle, filled with sludge and substrates, attached to an inverted bottle filled with sodium hydroxide solution. The sodium hydroxide solution absorbed the carbon dioxide produced, collecting only the methane produced, which eliminated the uncertainty caused by the carbon dioxide dynamics. The volume of methane collected using the alkaline solution method was similar to the theoretical volume of methane expected.

6.1.3. Reference test

Before any of the serum bottle tests were undertaken the pH of the sludge, FTRW and SGL, VSS of the sludge and COD of FTRW and SGL were measured.

In order to select the reference test, different COD loads of the FTRW only was tested. Three concentrations of FTRW were tested (1, 1.5 and 2 g COD/ℓ). 1 g COD/ℓ of FTRW with UASB 2 sludge was reproducible in methane volume and specific methanogenic activity within each experiment as well as across different batches of sludge. The results of 1.5 and 2 g COD/ℓ of FTRW with UASB 2 were found to be reproducible for each batch of sludge; however there was no reproducibility when the batches of sludge varied. In set 6, an increase in the COD loading, and therefore an increase in the concentration of organics, resulted in an increase in the specific methanogenic activity. However, in set 7, the volume of methane

produced in the bottles testing 2 g COD/ℓ of FTRW was much lower than that produced in the bottles with 1 and 1.5 g COD/ℓ of FTRW. This may indicate some inhibition in this batch of sludge with a COD loading of 2 g COD/ℓ of FTRW.

Therefore, for the inhibition experiments, which tested the effect of SGL on the FTRW digestion, a concentration of 1 g COD/ℓ of FTRW was used together with an additional predetermined concentration of SGL. Additional micro-nutrients were not used to supplement the sludge. The alkaline solution method was used to collect the methane produced.

6.2. Effect of SGL on FTRW digestion

Chapter 5 investigated whether the stripped gas liquor would enhance or inhibit the digestion of the Fischer-Tropsch reaction water with a sludge which was acclimated to the Fischer-Tropsch reaction water and whether there would be any change in the phenol concentration during digestion.

6.2.1. Constant FTRW COD load (Set 8)

A concentration of 1 g COD/ℓ of FTRW only was used as the reference unit. For the test units, a constant concentration of 1 g COD/ℓ of FTRW was used, with the SGL contributing an additional COD loading of 0.05 g COD/ℓ (5% SGL) and 0.15 g COD/ℓ (15% SGL), respectively, to the experiment. This gave the test units total COD loadings of 1.05 g COD/ℓ and 1.15 g COD/ℓ, respectively. The SMA for the 5% SGL test unit was not significantly different from the SMA of the matching 1 g COD/ℓ FTRW reference test. The SMA for the 15% SGL test unit was significantly lower than for the matching 1 g COD/ℓ FTRW reference test. This suggested that the addition of 15% SGL quantifiably reduced the SMA. However it was not possible to conclude whether this was due to an inhibitory effect of SGL or a higher COD loading since the total COD load was not the same between the test units with SGL and the reference unit with only FTRW. Previous experiments showed that there was no inhibition at higher COD loads in some batches of sludge (Section 4.4.1. Set 6) while other batches indicated some inhibition at 2 g COD/ℓ and above (Section 4.4.2. Set 7).

The colorimetric analysis was performed at the beginning and end of the experiment to determine whether the phenol concentration had decreased during the anaerobic digestion of

FTRW and SGL. For the 5% SGL test unit, the difference between the two values was not statistically significant. There was a significant but small decrease in the phenol concentration for the 15% SGL test unit. This result suggested that conversion of phenolic compounds may be possible using a sludge that has been acclimated to FTRW. However the reduction of phenol was lower than the reduction of COD.

6.2.2. Constant total COD load (Set 9)

In this experiment the total COD load was kept constant at 1 g COD/ℓ. The reference test was 1 g COD/ℓ FTRW only. The test unit consisted of 85% FTRW (0.85 g COD/ℓ) and 15% SGL (0.15 g COD/ℓ). This was to determine whether the reduction in the SMA in the previous experiment was due to an inhibitory effect of SGL or an increase in the COD load. Although in set 9 the total COD load was kept constant in order to make comparison of results simpler, it is not the only option. The acetate concentration could be kept constant by calculating the acetate contribution from SGL and FTRW and ensuring that each of the replicates added up to a constant value. A constant low molecular weight organics concentration can be calculated in a similar manner.

It was found that the SMA of the test unit was higher than that of the reference unit. Therefore there seemed to be no inhibition by SGL when the COD load was kept constant. This suggested that the reduction in SMA in the test unit in the previous experiment may have been due to an increase in the COD load when SGL was added over and above the 1 g COD/ℓ of FTRW. However, it could also have been due to a fundamental difference between the two batches of SGL since they had different COD and phenol concentrations. The SGL used in set 8 had a higher COD and phenol concentration than that used in set 9. The phenol concentration of the SGL used in set 8 was more than twice the phenol concentration of the set 9 SGL.

The pH, VSS, COD and phenol concentration of the test units were measured regularly during the experiment. The pH remained within the optimum pH range of 6.5 and 7.5. The VSS increased as more biomass was produced. Then after 14 days the VSS began to decrease due to endogenous respiration as the labile substrate was exhausted and the micro-organisms began to die. The COD decreased as it was removed from solution and converted to methane gas. 74.5 ± 7.8% of COD was removed between day 1 and the end of the test. The overall

percentage COD removed between day 0 and the end of the test would be higher. The colorimetric method and the gas chromatograph profiles showed that there was little decrease in the phenol concentration during the test.

6.3. Hypotheses

The first hypothesis was that the anaerobic sludge, which was acclimated to Fischer-Tropsch reaction water only, would be inhibited by the higher molecular weight organics such as phenols in stripped gas liquor. The results from Chapter 5 (Effect of SGL on FTRW Digestion) were ambiguous in terms of the first hypothesis. This may be due to the differences in the SGL batches between the two sets of experiments (set 8 and 9). Set 8 supports the first hypothesis, but not conclusively since the COD load was varying between the reference and test units. Set 9 does not support the first hypothesis since there was no evidence of inhibition by the SGL. Therefore it has not been proven that SGL will not be inhibitory, but it has been shown that FTRW digestion can occur in the presence of SGL and that certain components of the SGL can be degraded (as shown by the higher methanogenic activity in the test units in set 9). The percentage reduction in methanogenic activity of the 15% SGL test units ranged from 11% to 51% for set 8 and there was no inhibition in set 9. This suggests that any reduction in SMA due to additional SGL at concentrations and SGL:FTRW ratios tested was between 0 and 51%. However, the phenolic compounds in the SGL used in this project had a larger range of SMA reduction percentages than that found in literature. This difference may be due to the different sludge types used in literature which may contain a different mix of organisms than the sludge used in this project and thus may be able to cope better with a sudden influx of phenol.

The second hypothesis was that the Fischer-Tropsch reaction water acclimated sludge would not be able to degrade the phenolics in stripped gas liquor. Set 8 does not seem to support the second hypothesis since a significant decrease in the phenol concentration was observed after 7 days of incubation with the FTRW-acclimated sludge. However, percentage wise, the phenol reduction was lower than the COD removal. In set 9, the second hypothesis seems to be supported in that there was very little change in the measured phenol concentration after 24 days of incubation with the FTRW-acclimated sludge. However, the results are again not conclusive because of the high variance between values. However again, the percentage decrease in the phenol concentration is small compared to the percentage decrease in COD.

By looking at the variance between the samples in set 9, it could be seen that there can be quite a large difference between samples, but no evidence overall that the phenol concentration was decreasing.

At the COD blend ratios tested during this project, anaerobic digestion proceeds adequately. However, there is evidence that certain components present in SGL cannot be degraded or are degraded slowly. The sludge will have to be acclimated to SGL if these constituents are to be degraded in the co-digester. In a continuous digester which is appropriately seeded and acclimated to SGL, much higher rates of phenol removal might be observed.

The experimental method used in this project has certain limitations. The serum bottle technique used was only a batch process and could not accurately represent the behaviour of the micro-organisms in a continuous process. The serum bottles were small and could only accommodate a limited volume of biomass and substrates. The NaOH collection system also had limitations as it had to be emptied while the reaction was still continuing whenever it filled up too much. However, the experimental method was sufficient for the requirements of this project as it is just the first step in the broader project of FTRW and SGL co-digestion. The experiments in this project gave an indication of the inhibition of the FTRW digestion by SGL. The results from this project can be used to design, model and select possible continuous experiments at 15% SGL and higher since it is impossible to physically try every option. The inhibition for the continuous experiments can then be measured. The "worst case scenario" of percentage SMA reduction values can be used in a model for a conservative experiment design. It is recommended that a sensitivity analysis be performed using a range of SMA reduction values to observe how differences in this value influence the possible range of operating conditions of a continuous co-digester.

CHAPTER 7

7. Conclusions and Recommendations

This project was set out to investigate the effect that stripped gas liquor had on the Fischer-Tropsch reaction water anaerobic digestion by determining the influence of stripped gas liquor on the specific methanogenic activity of Fischer-Tropsch reaction water acclimated sludge and by evaluating the fate of indicator compounds for potentially toxic higher molecular weight organics in stripped gas liquor, such as phenols, in batch anaerobic co-digestion experiments. The serum bottle was used as a batch reactor and the biogas production was monitored to measure the extent of the degradation.

It was hypothesised that (i) the anaerobic sludge, which was acclimated to Fischer-Tropsch reaction water only, would be inhibited by the higher molecular weight organics such as phenols in stripped gas liquor; and (ii) the Fischer-Tropsch reaction water acclimated sludge would not be able to degrade the phenolics in stripped gas liquor.

This chapter presents the conclusions relating to the experiment design and the hypotheses. It also gives recommendations that may be useful in future research.

7.1. Conclusions

- The use of additional nutrients to supplement the sludge was not necessary for experiments carried out over a short time period.
- A method was investigated to successfully collect the biogas produced. The alkaline solution displacement method, which absorbed the carbon dioxide produced and collected only the methane, was selected to collect and measure the biogas produced since it eliminated the uncertainties arising from the carbon dioxide dynamics.
- A concentration of 1 g COD/ℓ FTRW was chosen as the reference test due to its reproducibility of the replicates within each experiment as well as its reproducibility

(in methane volume and specific methanogenic activity) across different batches of sludge.

- From the results of the co-digestion it was concluded that the addition of SGL potentially reduced the SMA and that this could be an inhibitory effect, but that any inhibition would be a function of the concentration of potentially inhibitory substances in SGL and that these concentrations vary from batch to batch. However, even if the SGL is inhibitory at the 15% SGL level with a total COD load of 1.15 g COD/ℓ, the degree of inhibition is fairly low and would not prevent co-digestion of the two streams at the concentrations tested.
- There was a significant but small decrease in the phenol concentration. Therefore it was concluded that the Fischer-Tropsch reaction water acclimated sludge may be able to degrade some of the phenolics in stripped gas liquor but the measured percentage removal of phenol was lower than the measure percentage COD removal.
- In terms of the first hypothesis (the anaerobic sludge, which is acclimated to Fischer-Tropsch reaction water only, will be inhibited by the higher molecular weight organics such as phenols in stripped gas liquor) it has not been proven that SGL will not be inhibitory, but it has been shown that FTRW anaerobic digestion can proceed adequately in the presence of SGL. The percentage SMA reduction observed ranged from 0 – 51% for the 15% SGL test units, however it is anticipated that much lower ranges would be observed in a continuous system. Literature and set 9 suggest that the actual SMA reduction due to potentially inhibitory organic compounds in the SGL may be negligible.
- In terms of the second hypothesis (the Fischer-Tropsch reaction water acclimated sludge will not be able to degrade the phenolics in stripped gas liquor) there was some evidence that phenolics were degraded but at a much slower rate than COD.

7.2. Recommendations

In future research it is recommended that some way be developed to take samples of the gas collected above the alkaline solution method in the inverted bottle during the experiment. The samples can then be analysed in a gas chromatograph to ensure that only methane is present to eliminate the possibility that air may diffuse into the inverted bottle and cause incorrect gas measurements.

The results from this project can be incorporated into a simple co-digestion model which could later be used to design continuous experiments at a range of FTRW:SGL ratios. The inhibition can then be measured for the continuous experiments. It is recommended that a sensitivity analysis be performed using a range of SMA reduction values to observe how differences in this value influence the possible range of operating conditions of a continuous co-digester.

Experiments can be conducted to determine the mechanism of inhibition.

The experiments in this project found that there was a very small decrease in the phenol concentration when stripped gas liquor was anaerobically digested by Fischer-Tropsch reaction water acclimated sludge. Therefore it may be useful to acclimatise the sludge to stripped gas liquor instead (Chen et al., 2007), by using a gradual start-up and slowly introducing the higher molecular weight organics so that the micro-organisms that are capable of metabolising these organics can become well-established.

This project is only the first step of the development of a co-digestion model relating organic loading rate, SGL**:** FTRW feed ratio to methane recovery and extent of biodegradation of phenol for use in the design and optimization of a co-digestion system. There is some indication of how SGL and the higher molecular weight organics, such as phenols, in SGL may affect FTRW digestion and an indication of percentage SMA reduction that may be experienced. The results from this project can be incorporated into a simple co-digestion model, together with results from future projects investigating the digestion of SGL, to design and model continuous experiments from which the inhibition can be measured. This will contribute to the investigation of the co-digestion of stripped gas liquor and Fischer-Tropsch reaction water.

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Appendices

A. Physicochemical characteristics of substrates and sludge

Sludge and substrates used in sets 1 - 8

Table A 1: Table showing the characteristics of SGL, FTRW and sludge

pH of SGL	7.00 - 7.03
pH of FTRW	2.90 – 2.94
Alkalinity of SGL (mg $CaCO_3$/ℓ)	300.67
Total solids of SGL (mg/ℓ)	987.78
Total solids of FTRW (mg/ℓ)	693.33
Total suspended solids of sludge (mg/ℓ)	98470
Volatile suspended solids of sludge (mg/ℓ)	92893.33
COD of SGL (mg/ℓ)	2070.94
COD of FTRW (mg/ℓ)	12963.94

Sludge and substrates used in set 9

Table A 2: Table showing the characteristics of SGL, FTRW and sludge used in set 9

pH of FTRW	3.01
pH of SGL	7.81
pH of sludge	7.52
COD of FTRW (mg/ℓ)	22624.35
COD of SGL (mg/ℓ)	1509.9
Volatile suspended solids of sludge (mg/ℓ)	69853.33

B. Gas chromatograph calibration curves for biogas testing

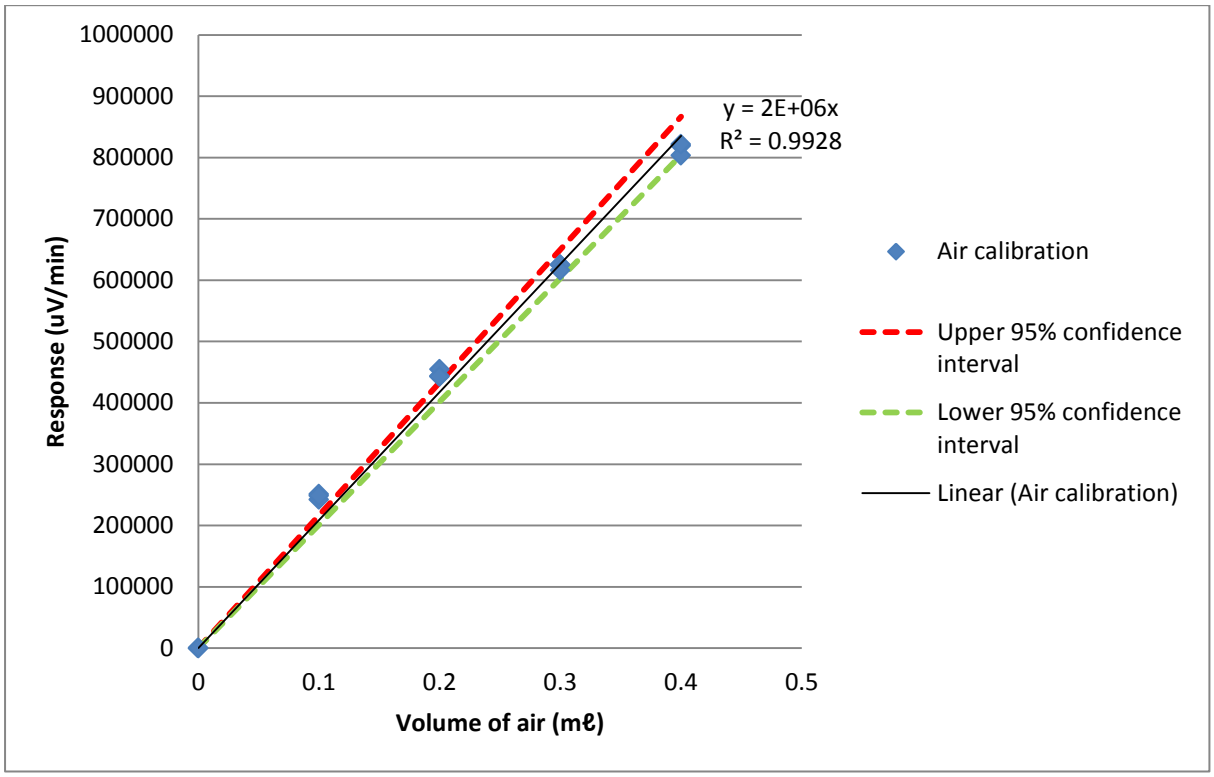

Figure B 1: Graph showing the response of air (mV/s) when different volumes of air (mℓ) are injected into a gas chromatograph

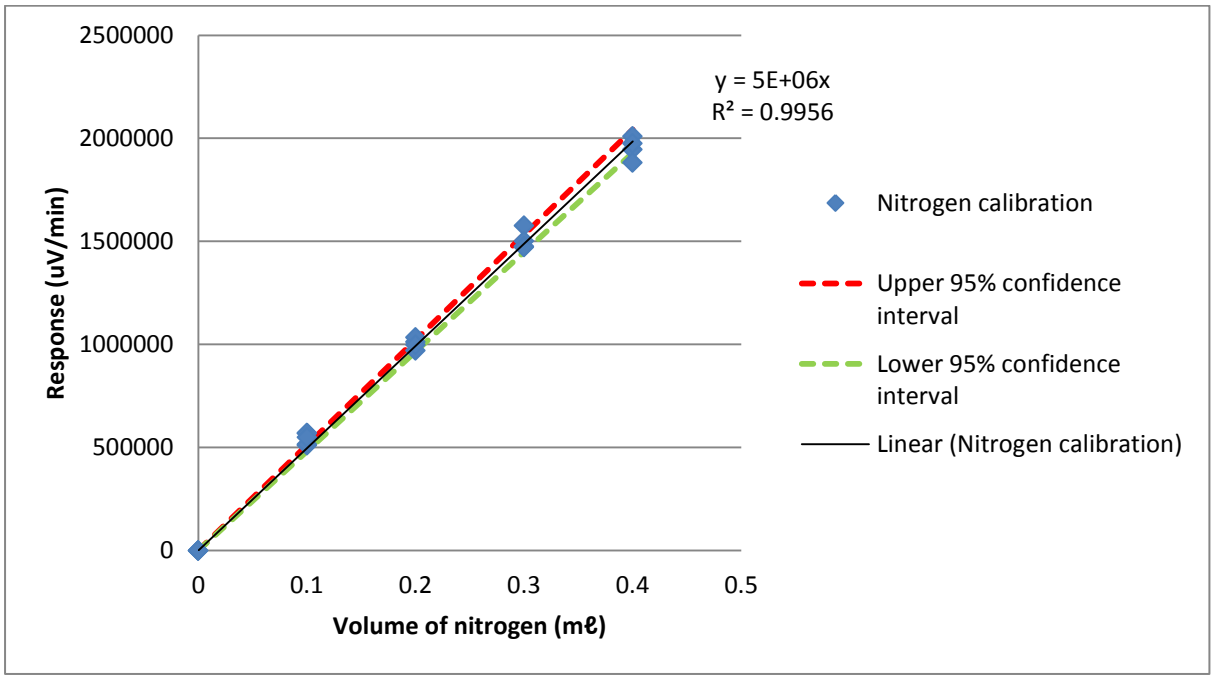

Figure B 2: Graph showing the response of nitrogen (mV/s) when different volumes of nitrogen (mℓ) are injected into a gas chromatograph

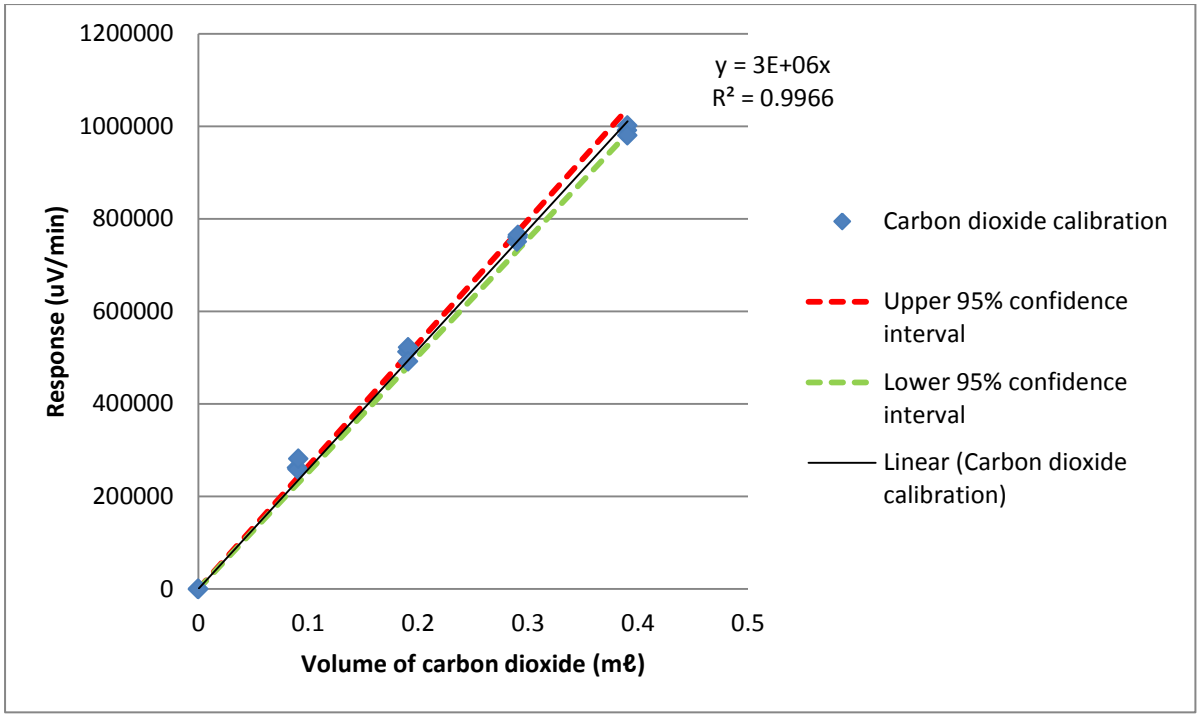

Figure B 3: Graph showing the response of carbon dioxide (mV/s) when different volumes of carbon dioxide (mℓ) are injected into a gas chromatograph

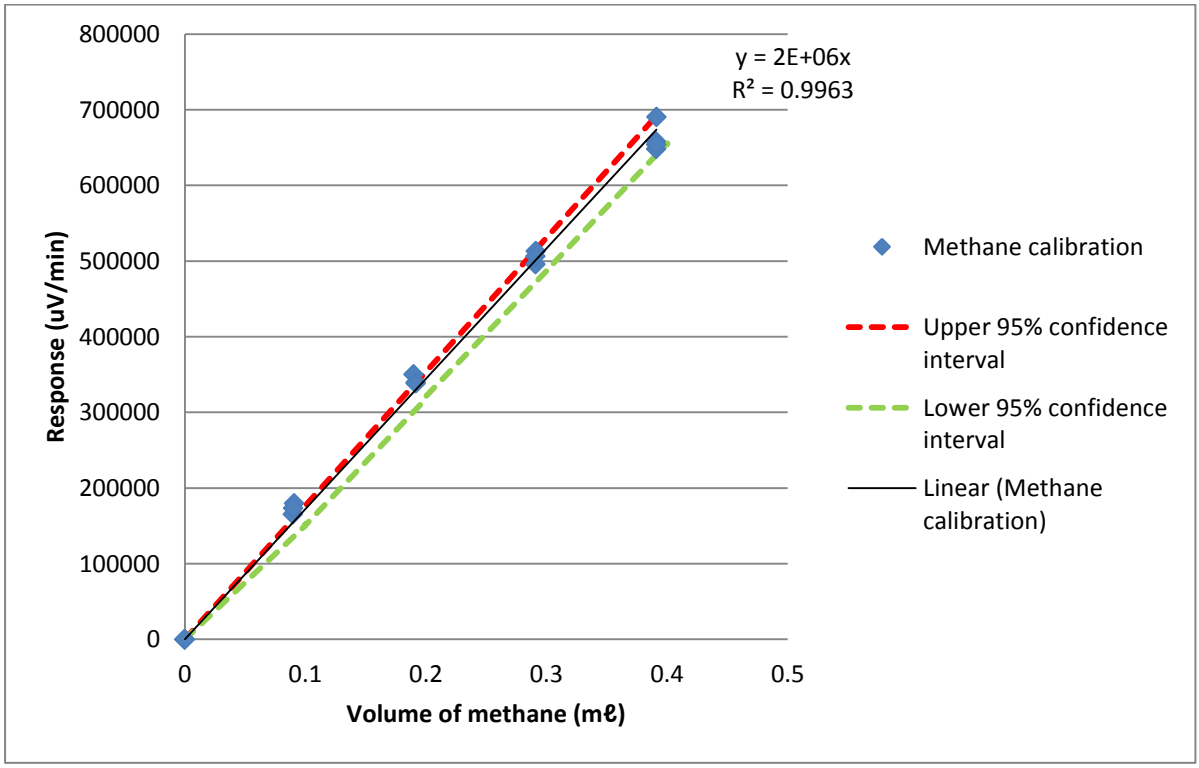

Figure B 4: Graph showing the response of methane (mV/s) when different volumes of methane (mℓ) are injected into a gas chromatograph

C. Proof of concept of water displacement method

Preparation of stock solution

Using acetate:

Molar mass = 60 g/mol

COD = 1.07 g COD/g acetate

Want a COD of 2.5 g/ℓ

∴ for a 250 mℓ bottle: $\text{COD} = \frac{2.5}{4} = 0.625 \text{ g COD}$

$$\text{Mass of acetate per bottle} = 0.625 \text{ g COD} \times \frac{1 \text{ g Acetate}}{1.07 \text{ g COD}} = 0.584 \text{ g acetate}$$

(diluted in 5 mℓ water)

To make up a stock solution of 100 mℓ:

$$\text{Mass of acetate for stock solution} = 0.584 \text{ g} \times \frac{100 \text{ m}\ell}{5 \text{ m}\ell} = 11.68 \text{ g acetate}$$

Using sodium acetate:

Molar mass of sodium = 82.03 g/mol

Molar mass of acetate = 60 g/mol

$$\text{Mass of sodium acetate} = 0.584 \text{ g acetate} \times \frac{82.03 \frac{\text{g}}{\text{mol}}}{60 \frac{\text{g}}{\text{mol}}}$$

$$= 0.798 \text{ g sodium acetate per bottle}$$

To make up a stock solution of 100 mℓ:

$$\text{Mass of sodium acetate for stock solution} = 0.798\ \text{g} \times \frac{100\ \text{m}\ell}{5\ \text{m}\ell}$$
$$= 15.96\ \text{g sodium acetate}$$

Theoretical amount of methane and carbon dioxide produced

$$CH_3COOH \rightarrow CH_4 + CO_2$$

0.584 g acetate is used per 250 mℓ bottle

$$\text{Moles of acetate} = 0.584\ \text{g} \times \frac{1\ \text{mol}}{60\ \text{g}} = 0.0097\ \text{mol acetate}$$

$$\therefore 0.0097\ \text{mol}\ CH_3COOH \text{ gives } 0.0097\ \text{mol}\ CH_4 \text{ and } 0.0097\ \text{mol}\ CO_2$$

For an ideal gas 1 mol = 22.4 ℓ

$$\text{Volume of methane} = 0.0097\ \text{mol} \times \frac{22.4\ \ell}{1\ \text{mol}} = 0.218\ \ell \text{ per bottle}$$

$$\text{Volume of carbon dioxide} = 0.0097\ \text{mol} \times \frac{22.4\ \ell}{1\ \text{mol}} = 0.218\ \ell \text{ per bottle}$$

D. Volumes of sludge, nutrients and substrate used in experiments

Set 1: Testing the water displacement method with FTRW and UASB 1 sludge

Table D 1: Table showing the volumes of sludge, FTRW and nutrients used for set 1

FTRW:Sludge	0	0.2	0.25	0.3
Percentage sludge	25	25	25	25
Volume of sludge (mℓ)	75	75	75	75
mg VSS of sludge	1500	1500	1500	1500
mg COD of FTRW	0	300	375	450
Volume of FTRW (mℓ)	0	23.14	28.93	34.71
Volume of nutrients (mℓ)	75	75	75	75
Volume of liquid (mℓ)	150	173.14	178.93	184.71
Headspace (mℓ)	150	126.86	121.07	115.29
Headspace: total volume	0.5	0.42	0.40	0.38

Set 2: Testing the acidified brine solution displacement method

Table D 2: Table showing the volumes of sludge, FTRW and nutrients used for set 2

FTRW:Sludge	0	0.25	0	0.25
Percentage sludge	50	50	30	30
Volume of sludge (mℓ)	150	150	90	90
mg VSS of sludge	3000	3000	1800	1800
mg VSS/ℓ	20000	14433.29	10000	8383.34
mg COD of FTRW	0	750	0	450
Volume of FTRW (mℓ)	0	57.85	0	34.71
g COD/ℓ	0	3	0	1.8
Volume of nutrients (mℓ)			90	90
Volume of liquid (mℓ)	150	207.85	180	214.71
Headspace (mℓ)	150	92.15	120	85.29
Headspace: total volume	0.5	0.31	0.4	0.28

Set 3: Testing the necessity of nutrients

Table D 3: Table showing the volumes of sludge, FTRW and nutrients used for set 3

FTRW: Sludge	0	0.25	0	0.25
Percentage sludge	50	50	30	30
Volume of sludge (mℓ)	48.44	48.44	48.44	48.44
mg VSS of sludge	4500	4500	4500	4500
mg VSS/ℓ	20000	20000	20000	20000
mg COD of FTRW	0	1125	0	1125
Volume of FTRW (mℓ)	0	86.78	0	86.78
g COD/ℓ	0	4.5	0	4.5
Volume of nutrients (mℓ)			48.44	48.44
Volume of water (mℓ)	176.56	89.78	128.11	41.34
Volume of liquid (mℓ)	225	225	225	225
Headspace (mℓ)	75	75	75	75
Headspace: total volume	0.25	0.25	0.25	0.25

Set 4: Testing the acidified brine solution displacement method with FTRW and UASB 2 sludge

Table D 4: Table showing the volumes of sludge and FTRW used for set 4

Percentage sludge	17	17	17	17
Volume of sludge (mℓ)	50	50	50	50
mg VSS of sludge	6591	6591	6591	6591
mg VSS/ℓ	26364	26364	26364	26364
mg COD of FTRW	0	259.28	388.92	518.56
Volume of FTRW (mℓ)	0	20	30	40
g COD/ℓ	0	1.04	1.56	2.07
Volume of water (mℓ)	200	180	170	160
Volume of liquid (mℓ)	250	250	250	250
Headspace (mℓ)	50	50	50	50
Headspace: total volume	0.17	0.17	0.17	0.17

Set 5: Comparison of the acidified brine solution method to the alkaline solution method

Table D 5: Table showing the volumes of sludge and FTRW used for set 5

	Water displacement method		Alkaline solution method	
Percentage sludge	0.17	0.17	0.17	0.17
Volume of sludge (mℓ)	50	50	50	50
mg VSS of sludge	10517.08	10517.08	10517.08	10517.08
mg VSS/ℓ	42068.33	42068.33	42068.33	42068.33
mg COD of FTRW	0	343.12	0	343.12
Volume of FTRW (mℓ)	0	20	0	20
g COD/ℓ	0	1.37	0	1.37
Volume of water (mℓ)	200	180	200	180
Volume of liquid (mℓ)	250	250	250	250
Headspace (mℓ)	50	50	50	50
Headspace:total volume	0.17	0.17	0.17	0.17

Set 6: COD values of 1, 1.5 and 2 g COD/ℓ

Table D 6: Table showing the volumes of sludge and FTRW used for set 6

Percentage sludge	0.17	0.17	0.17	0.17
Volume of sludge (mℓ)	50	50	50	50
mg VSS of sludge	10517.08	10517.08	10517.08	10517.08
mg VSS/ℓ	42068.33	42068.33	42068.33	42068.33
mg COD of FTRW	0	343.12	514.68	686.25
Volume of FTRW (mℓ)	0	20	30	40
g COD/ℓ	0	1.37	2.06	2.74
Volume of water (mℓ)	200	180	170	160
Volume of liquid (mℓ)	250	250	250	250
Headspace (mℓ)	50	50	50	50
Headspace: total volume	0.17	0.17	0.17	0.17

Set 7: COD values of 1, 1.5 and 2 g COD/ℓ

Table D 7: Table showing the volumes of sludge and FTRW used for set 7

Percentage sludge	0.33	0.33	0.33	0.33
Volume of sludge (mℓ)	100	100	100	100
mg VSS of sludge	21034.17	21034.17	21034.17	21034.17
mg VSS/ℓ	84136.67	84136.67	84136.67	84136.67
mg COD of FTRW	0	343.12	514.68	686.25
Volume of FTRW (mℓ)	0	20	30	40
g COD/ℓ	0	1.37	2.06	2.74
Volume of water (mℓ)	150	130	120	110
Volume of liquid (mℓ)	250	250	250	250
Headspace (mℓ)	50	50	50	50
Headspace: total volume	0.17	0.17	0.17	0.17

Set 8: Constant FTRW COD load

Table D 8: Table showing the volumes of sludge and FTRW used for set 8

Percentage sludge	0.33	0.33	0.33	0.33
Volume of sludge (mℓ)	100	100	100	100
mg VSS of sludge	21034.17	21034.17	21034.17	21034.17
mg VSS/ℓ	84136.67	84136.67	84136.67	84136.67
mg COD of food	0	361.18	361.18	361.18
g COD/ℓ of food	0	1.44	1.44	1.44
mg COD of FTRW	0	343.12	343.12	343.12
Volume of FTRW (mℓ)	0	20	20	20
g COD/ℓ of FTRW	0	1.37	1.37	1.37
Percentage SGL	0	0.05	0.05	0.05
mg COD of SGL	0	18.06	18.06	18.06
Volume of SGL (mℓ)	0	7.65	7.65	7.65
g COD/ℓ of SGL	0	0.07	0.07	0.07
Volume of water (mℓ)	150	122.35	122.35	122.35
Volume of liquid (mℓ)	250	250	250	250
Headspace (mℓ)	50	50	50	50
Headspace:total volume	0.17	0.17	0.17	0.17

Set 9: Constant total COD load

Table D 9: Table showing the volumes of sludge and FTRW used for set 9

Percentage sludge	0.17	0.17	0.17	0.17	0.17
Volume of sludge (mℓ)	50	50	50	50	50
mg VSS of sludge	1117.33	1117.33	1117.33	1117.33	1117.33
mg VSS/ℓ	4469.33	4469.33	4469.33	4469.33	4469.33
mg COD of food	0	257.39	257.39	257.39	257.39
g COD/ℓ of food	0	1.03	1.03	1.03	1.03
mg COD of FTRW	0	257.39	244.52	231.65	218.78
Volume of FTRW (mℓ)	0	8	7.6	7.2	6.8
g COD/ℓ of FTRW	0	1.03	0.98	0.93	0.88
Percentage SGL	0	0	0.05	0.1	0.15
mg COD of SGL	0	0	12.87	25.74	38.61
Volume of SGL (mℓ)	0	0	5.51	11.02	16.52
g COD/ℓ of SGL	0	0	0.05	0.1	0.15
Volume of water (mℓ)	200	192	186.89	181.78	176.68
Volume of liquid (mℓ)	250	250	250	250	250
Headspace (mℓ)	50	50	50	50	50
Headspace:total volume	0.17	0.17	0.17	0.17	0.17

E. Standard operating procedures

E1. Determination of characteristics of stripped gas liquor, Fischer-Tropsch reaction water and UASB 1 sludge

- **pH test**

Chemicals

- Distilled water
- Buffer solutions of pH 4.01, 7.01 and 10.01
- Stripped gas liquor
- Fischer-Tropsch reaction water

Equipment

- Hanna pH meter (HI 255 combined meter) with pH probe (blue probe)
- Temperature probe (silver probe)
- Small beaker

Calibration procedure

1. The pH probe was washed with distilled water and wiped dry
2. The *cal* button on pH meter was pressed to begin calibration
3. The pH probe was placed in the buffer solution of the same pH as that displayed on the right-hand side of the screen (eg. pH 7.01)
4. Waited until the reading was close to pH of buffer solution
5. When the screen displayed *ready*, the *CFM/GLP* button was pressed to confirm the value
6. The pH probe was removed, washed with distilled water and wiped dry
7. The procedure was repeated for buffer solutions of pH 4.01 and 10.01 as prompted

pH measurement procedure

1. The pH and temperature probes were washed with distilled water and wiped dry
2. The beaker was washed with distilled water
3. A sample (i.e. stripped gas liquor or Fischer-Tropsch reaction water) was poured into the beaker with a volume large enough for the tip of the pH probe to be completely submerged
4. The pH and temperature probes were placed into the sample
5. The pH and temperature readings on pH meter were recorded when it became relatively stable

6. The procedure was followed for three samples each for the stripped gas liquor and Fischer-Tropsch reaction water
7. The probes were washed and dried after each time it was placed into a solution

- **Alkalinity test**

Chemicals

- Distilled water
- 0.1 N sulphuric acid
- Indicator: 0.2 g bromocresol green and 0.04 g methyl red in 100 mℓ 95% ethyl alcohol
- Stripped gas liquor

Equipment

- Digital burette
- Small beaker
- Hanna pH meter with pH probe (blue probe)
- 25 mℓ volumetric flask

Alkalinity measurement procedure

1. The beaker and volumetric flask were washed with distilled water
2. The pH probe was washed with distilled water and dried
3. 25 mℓ of the stripped gas liquor was measured using the volumetric flask and poured into the small beaker
4. Two drops of the indicator were added to the sample
5. The air bubbles in the digital burette were removed and the reading on the digital burette was zeroed
6. The sample was titrated slowly and the pH was checked after every few drops of sulphuric acid on the pH meter since the strip gas liquor was brown which made it difficult to observe the colour change
7. The titration was continued and the pH checked until a pH of 4.5 was reached
8. The alkalinity was then calculated according the following equation:

$$Alkalinity\ \left(\frac{mgCaCO_3}{\ell}\right) = \frac{A \times N \times 50000}{V}$$

Where:

A = Volume of sulphuric acid used (mℓ)

N = Normality of standard acid

V = Volume of sample (mℓ)

Table E 1: Table showing the alkalinity of SGL in $mgCaCO_3/\ell$

Sample	Normality of acid (N)	Volume of sample (mℓ)	Volume of acid used (mℓ)	Alkalinity ($mgCaCO_3/\ell$)
SGL 1	0.1	25	1.51	302
SGL 2	0.1	25	1.51	302
SGL 3	0.1	25	1.49	298
			Average	300.67
			SD	2.31
			%RSD	0.77

Sample calculation: $Alkalinity = \frac{1.51 \times 0.1 \times 50000}{25} = 302\ mgCaCO_3/\ell$

- **Total solids test**

Chemicals

- Distilled water
- Stripped gas liquor
- Fischer-Tropsch reaction water

Equipment

- Crucibles
- Oven
- Desiccator
- Mass balance

Total solids measurement procedure

1. The crucibles were washed with distilled water
2. The crucibles were then placed in the oven at about 103 - 105°C for 2 hours to dry
3. Thereafter the crucibles were placed in the desiccator for 15 minutes to cool

4. Once cool, the crucibles were labelled (from 1 to 6) and weighed
5. 30 mℓ each of stripped gas liquor was added to the first three crucibles (1 to 3)
6. 30 mℓ each of Fischer-Tropsch reaction water was added to the other three crucibles (4 to 6)
7. The crucibles were left in the oven overnight
8. The crucibles were then placed in the desiccator for 15 minutes to cool
9. The weight of the crucibles and the residue were measured
10. The total solids was calculated according to the following equation:

$$Total\ solids\ \left(\frac{mg}{\ell}\right) = \frac{(W_2 - W_1) \times 1\ 000\ 000}{V}$$

Where:

W_1 = Weight of crucible (g)

W_2 = Weight of crucible + residue (g)

V = Volume of sample (mℓ)

Table E 2: Table showing the total solids (mg/ℓ) of SGL and FTRW

Sample	Weight of crucible only (g)	Volume of sample (mℓ)	Weight of crucible + residue (g)	Weight of residue only (g)	Total solids (mg/ℓ)
SGL 1	30.8842	30	30.9124	0.0282	940
SGL 2	33.0619	30	33.0900	0.0281	936.7
SGL 3	27.0261	30	27.0587	0.0326	1086.7
				Average	**987.78**
				SD	**85.66**
				%RSD	**8.67**
FTRW 1	29.7381	30	29.7621	0.0240	800
FTRW 2	30.1774	30	30.1950	0.0176	586.7
				Average	**693.33**
				SD	**75.43**
				%RSD	**10.88**

Sample calculation: $Total\ solids = \frac{(30.91-30.88)\times 1\ 000\ 000}{30} = 940\ mg/\ell$

- **Total suspended solids test**

Chemicals

- Distilled water
- Stripped gas liquor
- Fischer-Tropsch reaction water

Equipment

- Whatman glass microfiber filters (90 mm diameter)
- Desiccator
- Mass balance
- Funnel and flask
- Vacuum pump
- Crucibles
- Oven

Total suspended solids measurement procedure

1. The filter papers were placed in the oven at about 103 - 105°C for 30 minutes to dry
2. The filter papers were then placed in the desiccator for 20 minutes to cool
3. The filter papers were labelled, weighed and the weights were recorded
4. A filter paper was placed in the funnel and the vacuum pump was switched on
5. The filter paper was wet with distilled water to seal it to the funnel surface
6. 100 mℓ of the sample (Fischer-Tropsch reaction water or stripped gas liquor) was poured into the funnel onto the middle of the filter paper
7. After filtration, the filter paper was removed, folded in half and placed into a labelled crucible
8. The crucible was then placed into the oven at 103 - 105°C for 1 hour
9. This was done 3 times for the Fischer-Tropsch reaction water and 3 times for the stripped gas liquor
10. Two blanks were prepared by using the same volume (100 mℓ) of distilled water as the sample and following the above procedure
11. After 1 hour all of the crucibles were removed from the oven and placed into the desiccator for 20 minutes to cool
12. The filter papers with the solid residue were weighed and the suspended solids was calculated according to the following equation:

$$Suspended\ solids\ \left(\frac{mg}{\ell}\right) = \frac{[(W_2 - W_1) + WB] \times 1\ 000\ 000}{V}$$

Where:

W_1 = Weight of filter paper (g)

W_2 = Weight of filter paper + residue (g)

WB = Weight of blank (g)

V = Volume of sample (mℓ)

Table E 3: Table showing the total, volatile and fixed suspended solids (mg/ℓ) of the sludge

Sample	Weight of filter paper only (g)	Volume of sample (mℓ)	Weight of filter paper + residue before ignition (g)	Weight of filter paper + residue after ignition (g)	Total suspended solids (mg/ℓ)	Volatile solids (mg/ℓ)	Fixed solids (mg/ℓ)
Sludge 1	0.3983	10	0.4664	0.4151	6810	5130	1680
Sludge 2	0.3909	10	1.0349	0.4517	64400	58320	6080
Sludge 3	0.3968	10	2.6388	0.4865	224200	215230	8970
				Average	98470	92893.33	5576.67
				SD	112628.49	109233.64	3670.97
				%RSD	114.38	117.59	65.83

Sample calculation: $Suspended\ solids = \frac{(0.4664-0.3983)\times 1\ 000\ 000}{10} = 6810\ mg/\ell$

- **Volatile and fixed solids test**

(Follows on from total suspended solids test)

Chemicals

- As above

Equipment

- Furnace
- As above

Volatile and fixed solids measurement procedure

1. The filter paper and residue from total suspended solids test were placed into the furnace at 550°C for 30 minutes to ignite to constant weight
2. After 1 hour, the samples were removed and cooled partially in air
3. The samples were then cooled in the desiccator
4. The filter papers and the residue were weighed and recorded
5. The volatile solids and fixed solids were calculated according to the following equations:

$$Volatile\ solids\ \left(\frac{mg}{\ell}\right) = \frac{(W_2 - W_3) \times 1\ 000\ 000}{V}$$

$$Fixed\ solids\ \left(\frac{mg}{\ell}\right) = \frac{(W_3 - W_1) \times 1\ 000\ 000}{V}$$

Where:

W_1 = Weight of filter paper (g)

W_2 = Weight of filter paper + residue before ignition (g)

W_3 = Weight of filter paper + residue after ignition (g)

V = Volume of sample (mℓ)

Sample calculation (from Table E3): $Volatile\ solids\ = \frac{(0.4664-0.4151)\times 1\ 000\ 000}{10} = 5130\ mg/\ell$

$$Fixed\ solids\ = \frac{(0.4151-0.3983)\times 1\ 000\ 000}{10} = 1680\ mg/\ell$$

- **Chemical oxygen demand test**

Chemicals

- Potassium dichromate ($K_2Cr_2O_7$)
- Silver sulphate (Ag_2SO_4)
- Sulphuric acid (H_2SO_4)
- 1,10-Phenanthroline monohydrate ($C_{12}H_8N_2.H_2O$)
- Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$)
- Ammonium iron (II) sulphate hexahydrate ($Fe(NH_4)_2(SO_4)_2.6H_2O$)
- Distilled water

- Stripped gas liquor
- Fischer-Tropsch reaction water

Equipment

- ETHOS One Microwave
- Rotor that houses 10 teflon vessels
- 100 mℓ Erlenmeyer flasks
- 5 mℓ pipette
- 10 mℓ and 5 mℓ automatic bottle top dispensers

Reagent preparation

- Standard potassium dichromate digestion solution
  4.913 g of $K_2Cr_2O_7$ was dried at 105°C for 2 hours and then cooled in a desiccator. The $K_2Cr_2O_7$ was then dissolved in distilled water and made up to 500 mℓ. 167 mℓ concentrated H_2SO_4 and 13.3 g $HgSO_4$ was added to the solution and cooled to room temperature. Finally the solution was diluted to 1000 mℓ.

- Sulphuric acid reagent (COD reagent)
  26 g of silver sulphate (Ag_2SO_4) was dissolved in 2500ml of concentrated (>98%) H_2SO_4 using a magnetic stirrer. Dissolution takes about 2 days.

- Ferroin indicator solution
  1.485 g of 1.10-phenanthroline monohydrate and 0.695 g of $FeSO_4.7H_2O$ was dissolved in distilled water and diluted to 100mℓ.

- Standard ferrous ammonium sulphate (FAS)
  39.2 g of $Fe(NH_4)_2(SO_4)_2.6H_2O$ was dissolved in distilled water using a 1000 mℓ volumetric flask. 20 mℓ of concentrated H_2SO_4 was added and the mixture was made up to 1000 mℓ.

Testing the molarity of FAS

1. 3 mℓ of $K_2Cr_2O_7$ digestion solution was added to an Erlenmeyer flask and was diluted with 5 mℓ distilled water
2. 7 mℓ of the COD reagent was added
3. 2 drops of ferroin indicator was then added before the solution was titrated with FAS until reddish brown

4. The volume of FAS used was recorded and the molarity of FAS was calculated according to the following equation:

$$Molarity\ of\ FAS = \frac{Volume\ of\ 0.0167\ M\ K_2Cr_2O_7\ solution\ titrated\ (m\ell) \times 0.10}{Volume\ of\ FAS\ used\ in\ titration\ (m\ell)}$$

Table E 4: Table showing the molarity of ferrous ammonium sulphate

Sample	Volume of $K_2Cr_2O_7$ (mℓ)	Volume of distilled water (mℓ)	Volume of COD reagent (mℓ)	Drops of ferroin indicator	Volume of FAS used in titration (mℓ)	Molarity of FAS
1	3	5	7	2	3.28	0.091
2	3	5	7	2	3.22	0.093
3	3	5	7	2	3.22	0.093
					Average FAS molarity	**0.093**
					SD	**0.001**
					%RSD	**1.06**

Sample calculation: $Molarity\ of\ FAS = \frac{3 \times 0.10}{3.28} = 0.091$

Chemical oxygen demand measurement procedure (closed reflux)

1. For the SGL samples, 7.5 mℓ was added to a 100 mℓ flask and diluted to 100 mℓ with distilled water; 5 mℓ each of this mixture was added to the first four Teflon vessels
2. For the FTRW samples, 0.4 mℓ was added to a 100 mℓ flask and diluted to 100 mℓ with distilled water; 5 mℓ each of this mixture was added to the next three Teflon vessels
3. 5 mℓ each of distilled water was added to the last three Teflon vessels
4. 3 mℓ of $K_2Cr_2O_7$ digestion solution and 7 mℓ of the COD reagent was then added to each of the vessels
5. The COD reagent was poured down the wall of the vessel to prevent splashing
6. The vessels were into the rotor and the temperature probe was inserted into vessel 1
7. The microwave was switched on
8. The COD method was selected which heats to 150 °C in 15 minutes, digests for 30 minutes and finally cools down to 50 °C in 1 hour
9. The vessels were then removed and the contents were transferred to Erlenmeyer flasks
10. 2 drops of ferroin indicator was added to each
11. The mixtures were titrated to a reddish-brown colour using FAS
12. The volume used for the titrations were recorded and the COD was calculated according to the following equation:

$$COD\ \left(mg\frac{O_2}{\ell}\right) = \left[\frac{(blank - titration) \times molarity\ of\ FAS \times 8000}{Volume\ of\ sample\ (m\ell)}\right] \times dilution\ factor$$

Where:

8000 = milliequivalent weight of O_2 × 1000 mℓ/ℓ

Table E 5: Table showing the chemical oxygen demand (mgO_2/ℓ) of SGL and FTRW

Sample	Volume of sample (mℓ)	Volume of $K_2Cr_2O_7$ (mℓ)	Volume of COD reagent (mℓ)	Drops of ferroin indicator	Volume of FAS used in titration (mℓ)	COD (mgO_2/ℓ)
Blank 1	5	3	7	2	3.2	
Blank 2	5	3	7	2	3.17	
Blank 3	5	3	7	2	3.09	
				Average	3.15	
				SD	0.057	
				%RSD	1.80	
SGL 1	5 (DF = 40/3)	3	7	2	2.03	2219.1
SGL 2	5 (DF = 40/3)	3	7	2	2.17	1942.53
SGL 3	5 (DF = 40/3)	3	7	2	2.07	2140.08
				Average	2.15	1982.04
				SD	2.11	2070.94
				%RSD	0.066	
					3.14	
FTRW 1	5 (DF = 250)	3	7	2	2.86	10865.02
FTRW 2	5 (DF = 250)	3	7	2	2.78	13828.21
FTRW 3	5 (DF = 250)	3	7	2	2.77	14198.61
				Average	2.80	12963.94
				SD	0.049	
				%RSD	1.76	

Sample calculation: $COD = \left[\frac{(3.15-2.03)\times 0.093\times 8000}{5}\right] \times \frac{40}{3} = 2219.1$

E2. Serum bottle test procedure

- **Serum bottle test procedure with water displacement method or acidified brine solution method**

Chemicals

- Sludge
- Stripped gas liquor
- Fischer-Tropsch reaction water
- Water
- Sodium chloride
- Sulphuric acid

Apparatus

- Water bath
- Temperature regulator
- Dropper stand
- Twelve 250 mℓ serum bottles
- Serum bottle metal holder
- 24 droppers

Procedure

1. Clamp droppers to stand (12 at the bottom – for gas collection and 12 at the top – for water displacement)
2. Attach a pipe from the bottom dropper to the top dropper so that there are 12 pairs
3. Ensure that water bath is filled to the correct level and temperature regulator is at 35°C
4. Fill bottom droppers with water (or acidified brine solution)
5. The acidified brine solution is made up by adding sodium chloride to water until supersaturated and then adding a few drops of sulphuric acid
6. Fill serum bottles with the appropriate volume of sludge, substrate (e.g. stripped gas liquor and Fischer-Tropsch reaction water) and nutrients (if necessary)
7. Flush with nitrogen
8. Place serum bottles in metal holders in water bath
9. Attach pipe at the top of the gas collection dropper to serum bottle
10. Record volume of gas collected at regular intervals
11. Analyse gas composition using gas chromatograph

- **Serum bottle test procedure with alkaline solution method**

Chemicals

- Sludge
- Stripped gas liquor
- Fischer-Tropsch reaction water
- Distilled water
- Sodium hydroxide

Apparatus

- Water bath
- Temperature regulator
- Stand
- Twenty-four 250 mℓ serum bottles
- Serum bottle metal holder
- Twelve 100 mℓ plastic cylinders

Procedure

1. Ensure that water bath is filled to the correct level and temperature regulator is at 35°C
2. Make up an alkaline solution by dissolving sodium hydroxide in distilled water
3. Fill twelve serum bottles with the alkaline solution
4. Fill the other twelve serum bottles with the appropriate volume of sludge, substrate (e.g. stripped gas liquor and Fischer-Tropsch reaction water) and nutrients (if necessary)
5. Place serum bottles in metal holders in water bath
6. Place the twelve plastic cylinders at the bottom of the stand
7. Use the pipes to connect the serum bottles in the water bath in which the reaction is taking place to the serum bottles which are filled with the alkaline solution
8. Turn the bottles filled with the alkaline solution upside-down and attach to the stand above the plastic cylinders
9. Record volume of alkaline solution collected (which is a direct indication of methane collected) at regular intervals

E3. Gas chromatograph calibration and biogas testing

Chemicals

- Helium
- Nitrogen
- Carbon dioxide
- Methane

Equipment

- Shimadzu gas chromatograph
- Gas tight syringe

Switching on the gas chromatograph

1. The helium valve was opened
2. The gas chromatograph was switched on
3. The GC Real Time Analysis program and the Pollution_Research_Group method were opened
    - Oven profile: 100 °C for 8 minutes
    - Carrier gas: constant flow He, 25 mℓ/min
    - Injection: 25 °C
    - Column: Carboxen, length = 1.5 m, I.D. = 2.1 mm
4. The column temperature was checked to ensure that it was set at 100°C
5. The system was switched on and left to reach the required temperature
6. Thereafter the detector was switched on and left until the baseline stabilised

Calibrating the gas chromatograph

1. The gases that were calibrated were air, nitrogen, methane and carbon dioxide
2. The gas being calibrated was collected in a gas collection unit (bomb)
3. The calibration was started with methane gas
4. The methane line was connected to the gas collection unit, both valves on the unit were opened and the unit was flushed with methane for approximately 3 minutes
5. Both the valves were then closed
6. The button *Single Run* was clicked on and a file was opened to save the results by clicking on *Sample Login*
7. It was ensured that the program indicated that the gas chromatograph was ready and then the start button on the program was pressed

8. 0.1 mℓ of methane was then injected into the gas chromatograph and the start button on the gas chromatograph was pushed
9. When the analysis was complete the stop button on the program was pressed
10. The analyses were done for four different volumes i.e. 0.1, 0.2, 0.3 and 0.4 mℓ and each volume done in triplicates
11. The response (area) obtained was plotted against the volume in Microsoft Excel
12. The procedure was repeated for air, nitrogen and carbon dioxide

Gas testing

1. A sample of 0.4 mℓ was obtained from each bottle and injected into the gas chromatograph following the procedure above
2. Each run takes about 7-8 minutes to complete

Switching off the gas chromatograph

1. The system was switched off and left to cool for about 30 minutes
2. The detector was then switched off and the program was closed
3. The gas chromatograph was switched off and the helium valve was closed

E4. Phenol measurement

- **Colorimetric method**

Chemicals

- Ammonium chloride (NH_4Cl)
- Ammonium hydroxide (NH_4OH)
- Distilled water
- 4-aminoantipyrine
- Potassium ferricyanide ($K_3Fe(CN)_6$)
- Phenol
- Stripped gas liquor
- Fischer-Tropsch reaction water

Equipment

- 100 mℓ volumetric flasks
- Erlenmeyer flasks
- pH meter

- 1-10 mℓ pipette
- 10-100µℓ pipette
- Spectrometer

Reagent preparation

- Buffer solution
  16.9 g of ammonium chloride was dissolved in 143 mℓ of ammonium hydroxide and thereafter diluted to 250 mℓ with distilled water.

- Aminoantipyrine solution
  2 g of 4-aminoantipyrine was added to a 100 mℓ volumetric flask and diluted to 100 mℓ with distilled water.

- Potassium ferricyanide solution
  8 g of potassium ferricyanide was added to a 100 mℓ volumetric flask and diluted to 100 mℓ with distilled water.

- Stock phenol solution
  1 g of phenol was added to a 1 ℓ volumetric flask and diluted to 1 ℓ with distilled water

Standard addition method

1. Five 100 mℓ volumetric flasks were washed
2. 0, 0.2, 0.4, 0.6 and 0.8 mℓ (later modified to 0, 0.02, 0.04, 0.06 and 0.08 mℓ) of the 1000 mg/ℓ stock phenol solution was added to each of the flasks respectively
3. 10 mℓ of the sample was added to each flask and mixed
4. These were diluted to 100 mℓ with distilled water
5. 2 mℓ of the buffer solution was added to each flask and mixed to increase the pH of the solution
6. The pH of the solutions with the lowest (0 mg/ℓ) and highest concentrations (0.8 mg/ℓ) were determined with the pH meter to ensure that they were above a pH of 10
7. 2 mℓ of the aminoantipyrine solution was added to each of the flasks and mixed
8. Finally 2 mℓ of the potassium ferricyanide solution was added to each of the flasks and mixed
9. This gave a total volume of 106 mℓ, therefore giving a dilution factor of 10.6
10. The solutions were then allowed to stand for 15 minutes and thereafter the absorbance was measured with the spectrometer at 510 nm
11. Three readings were taken for each sample
12. The absorbance was plotted against the concentration
13. The graph was extrapolated until it intercepted the x-axis

14. The concentration of the phenols in the sample was calculated by multiplying the dilution factor and the x-intercept

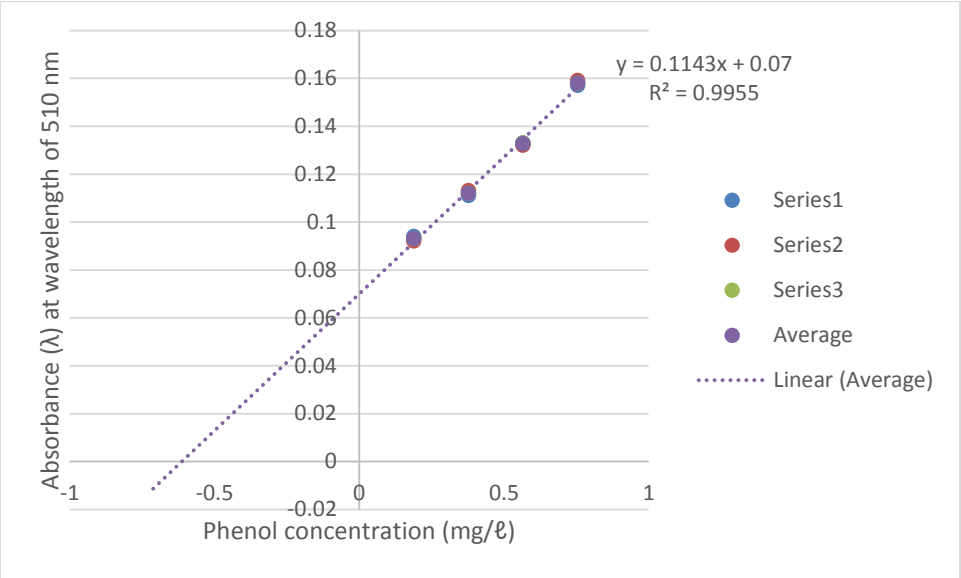

Figure E 1: Graph showing the extrapolated graph of the absorbance (λ) of light of the solution at a wavelength of 510 nm against the phenol concentration of the standard solutions

Phenol concentration of the sample = |x-intercept| × dilution factor

= (-0.07/0.1143) × (106/10)

= 0.61 × 10.6

= 6.49 mg/ℓ

- **Gas Chromatography**

Solid phase extraction

Chemicals

- Dichloromethane
- Hexane
- Phenol
- Stripped gas liquor

Equipment

- SPE vacuum
- 200 mg Strata Silica cartridges from Phenomenex
- Vacuum pump
- Two beakers for hexane and dichloromethane
- Beakers for samples
- Glass vials
- Pipette to measure out hexane, dichloromethane and samples
- 15 ml Cellstar tubes

Solid phase extraction procedure

1. Remove top of SPE vacuum and place glass vials (without the caps) in the SPE vacuum manifold
2. Replace top and ensure that the droppers fit into the glass vials and top fits securely
3. Remove yellow caps from the SPE vacuum and attach cartridges
4. Attach the vacuum pump to the SPE vacuum
5. Open SPE vacuum valves
6. Condition/equilibrate cartridges by adding 1 ml of dichloromethane to each cartridge and turn on vacuum slowly
7. Turn off vacuum after dichloromethane has passed through the cartridges
8. Condition/equilibrate cartridges by adding 1 ml of hexane to each cartridge and turn on vacuum slowly
9. Turn off vacuum after hexane has passed through the cartridges
10. Add 7 ml of sample (phenol or stripped gas liquor) to the cellstar tube, followed by 7 ml of hexane and shake thoroughly
11. Add 2 ml of this mixture to each cartridge (take sample from bottom of cellstar tube)
12. Turn on the vacuum slowly
13. Turn off vacuum after sample has passed through the cartridges
14. Wash cartridges by adding 1 ml of hexane to each cartridge and turn on vacuum slowly
15. Turn off vacuum after hexane has passed through the cartridges
16. Wash once more with 1 ml of hexane
17. Increase vacuum to just below 20 inches of mercury and leave for approximately 10 minutes to dry cartridges
18. Turn off vacuum and close SPE vacuum valves
19. Remove top of SPE vacuum remove glass vials (waste) and place new clean glass vials, without the caps, (cleaned with dichloromethane) in the SPE vacuum manifold
20. Replace top and ensure that the droppers fit into the glass vials and top fits securely
21. Open SPE vacuum valves
22. Elute by adding 1 ml of dichloromethane to each cartridge and turn on the vacuum slowly

23. Turn off vacuum after dichloromethane has passed through the cartridges
24. Elute once more with 1 ml of dichloromethane
25. Turn off vacuum and close SPE vacuum valves
26. Remove cartridges and throw away
27. Replace yellow caps
28. Remove top of SPE vacuum, remove glass vials with samples and replace caps (ensure that glass vials are labelled appropriately so as to not confuse samples)
29. Replace top and ensure that it fits securely
30. Remove vacuum pump

GC

Chemicals

- Acetone
- Sample prepared using solid phase extraction
- Helium
- Hydrogen
- Air

Equipment

- Shimadzu GC2010 Plus
- Glass vial

Switching on the GC

1. The helium, hydrogen and air valves were opened
2. The gas chromatograph was switched on
3. The computer was switched on and the GC Solution program was opened
4. The method, ReneMethod2, was opened:
    - Oven profile: 60 °C to 240 °C at 20 °C/min for 6 minutes
    - Carrier gas: constant flow He, 0.23 mℓ/min
    - Injection: 220 °C
    - Column: ZB-WAXplus, length = 30 m, I.D. = 0.25 mm
5. The system was switched on and the parameters were downloaded
6. The GC was left to reach the required temperature and stabilise
7. When the GC had reached the required parameters "Ready" was displayed on the screen
8. Before performing any runs ensure that the solvent vial is full

Calibrating the GC

1. Different concentrations of phenol were prepared using the solid phase extraction
2. The first (lowest) concentration of phenol was emptied into the 1.5mℓ vial and placed in the sample rack
3. The sample was logged in by clicking on Sample Login on the side-bar and a name for the file was entered
4. The run was then started by clicking on Start on the side-bar
5. The run takes 15 minutes to complete (phenol elutes at approximately 2.4 minutes)
6. The same sample was repeated 2 more times
7. The first concentration was removed, the next concentration was placed in the sample rack and the procedure was repeated
8. This was done for all concentrations of phenol
9. The file of the completed run was opened and the peak at 2.4 minutes was integrated
10. The retention time, area and height of the peak were recorded
11. The phenol concentration was then plotted against the area and the height on Microsoft Excel to obtain the calibration curve

Sample testing

1. The sample was emptied into the 1.5mℓ vial and placed in the sample rack
2. The sample was logged in by clicking on Sample Login on the side-bar and a name for the file was entered
3. The run was then started by clicking on Start on the side-bar
4. The run takes 15 minutes to complete (phenol elutes at approximately 2.4 minutes)
5. The same sample was repeated 2 more times
6. The file of the completed run was opened and the peak at 2.4 minutes was integrated
7. The retention time, area and height of the peak were recorded
8. Using the area of the peak of the sample and the calibration curve, the phenol concentration of the sample was determined

Switching off the GC

1. The system was switched off and left to cool down
2. The GC was then switched off
3. The helium, hydrogen and air valves were closed

F. Paired two sample t-test

The paired t-test examines if the difference between the means is significantly different from the hypothesised value of zero, $\overline{x_1} - \overline{x_2} = 0$ (Park, 2009). Microsoft Excel was used to execute the t-test to compare the two sets of data. The analysis tool *t-Test: Paired Two Sample for Means* was accessed under *Data Analysis*. The range of SMA values for the test and reference units were selected and an alpha value of 0.05 was used. A table presenting the P-value was displayed. For example, for the comparison of the 5% SGL test unit SMA and the paired reference unit SMA in set 8, the following table was obtained:

Table F 1: Table showing the results from the paired two sample t-test

	Variable 1	***Variable 2***
Mean	0.598095292	0.476312
Variance	0.210954727	0.067458
Observations	10	10
Pearson Correlation	0.832777063	
Hypothesized Mean Difference	0	
df	9	
t Stat	1.363916232	
P(T<=t) one-tail	0.102864051	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.205728102	
t Critical two-tail	2.262157163	

Therefore P = 0.103

G. Percentage inhibition

The percentage inhibition for the 15% test unit in set 8 can be calculated by averaging the percentage inhibition at each time. The percentage inhibition at each time is obtained by taking the difference between the reference SMA and the test unit SMA at each time and dividing it by the reference SMA at each time. This is then averaged.

$$Percentage\ inhibition = \left. \sum \left(\frac{SMA_{ref,t} - SMA_{test,t}}{SMA_{ref,t}} \times 100 \right) \right/ n$$

$$= \frac{1101.93}{35}$$

$$= 31\ \%$$

The confidence interval can be calculated on Excel by:

$$Confidence\ interval = tinv(probability, DOF) \times \frac{Stdev(\%\ inhibition\ at\ time\ t)}{\sqrt{n-2}}$$

$$= 20\ \%$$

Where:

$SMA_{ref,t}$ = Specific methanogenic activity of the reference unit (mℓ/gVSS.day) at time t

$SMA_{test,t}$ = Specific methanogenic activity of the test unit (mℓ/gVSS.day) at time t

n = Number of observations = 35

$probability$ = 0.05

DOF = n – 3 = 32

Therefore the percentage inhibition ranges from 11 – 51 %.

An alternative way to calculate the percentage inhibition for the 15% test unit in set 8 is by comparing the average SMA difference to the average reference SMA. The SMA difference is obtained by taking the difference between the reference SMA and the test unit SMA at each time. These values are then averaged to get the average SMA difference.

$$Percentage\ inhibition = \frac{\sum(SMA_{ref,t} - SMA_{test,t})/n}{\sum SMA_{ref,t}/n} \times 100$$

$$= \frac{2.29/35}{9.43/35} \times 100$$

$$= 24\ \%$$

This value fits into the range calculated above.

The percentage inhibition can also be calculated by comparing the cumulative methane produced in the test unit from day 2 to day 9 to the cumulative methane produced in the reference unit.

$$Percentage\ inhibition = 100 - \left(\frac{CH_{4,test} at\ day\ 9 - CH_{4,test}\ at\ day\ 2}{CH_{4,ref} at\ day\ 9 - CH_{4,ref}\ at\ day\ 2} \times 100\right)$$

$$= 1 - \left(\frac{19.66 - 4.66}{26.33 - 6.83} \times 100\right)$$

$$= 23\ \%$$

This value also falls between the range above.

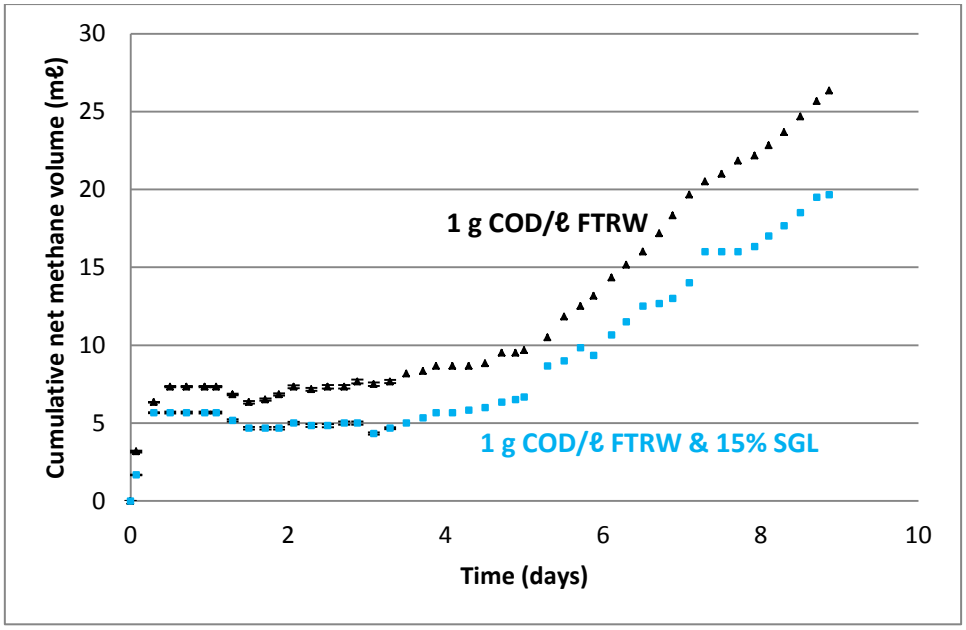

Figure G 1: Graph showing the cumulative net methane produced (mℓ) over time (days) for the 15% SGL test unit in set 8 and the matching reference test