Imperial College

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Sulfate Disinfection, Stabilisation and Heavy Metal Removal From Sludge

A Thesis Submitted For The Degree Of Doctor Of Philosophy Of The University Of London And The Diploma Of Imperial College

by

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ABSTRACT

Sulfate disinfection, stabilisation and heavy metal removal from sludge (Sulfate Digestion process) is a three-stage process which relies on the different oxidation states of the sulfur in order to remove heavy metals and destroy pathogens in sludge. The main focus of this project was on the hybrid H_2S Generator which consisted of an anaerobic continuously stirred tank reactor (CSTR) fed primary sludge as a carbon source and sulfate as an electron acceptor to stimulate H_2S production. The amount of sulfate required to re-circulate around the Sulfate Digestion process was calculated at 5 g/l for a primary sludge concentration of 2.6 g/l. This was equivalent to a Chemical Oxygen Demand:Sulfate (COD:SO_4) ratio of approximately 5:1. At this ratio, the results showed that using sulfate as a salt had no detrimental effects on total COD (TCOD) destruction of the primary sludge; however, the total solids (TS) concentration in the effluent was significantly higher compared to a completely methanogenic reactor. This was accounted for by the higher biomass yield of the Sulfate Reducing Bacteria (SRB) in the hybrid H_2S Generator.

The use of sulfuric acid instead of sodium sulfate in the range of 1.47-4.33 g/l resulted in an increase in both TS and TCOD concentration in the final effluent with decreasing COD:SO_4 ratio's in the H_2S Generators. This was shown to be as a result of a decrease in the rate of sludge particle hydrolysis, possibly due to the release of a fermentative and/or acidogenic bacteria (AcB)-specific toxin during sludge acidification with the sulfuric acid, since the final stages of digestion in terms of methanogenesis and sulfidogenesis were not adversely affected. Kinetic studies on the seed sludge from the hybrid H_2S Generators showed that a mixed microbial consortia of acetotrophic SRB (ASRB) and acetotrophic Methane Producing Bacteria (AMPB) were both responsible for acetate degradation at low sulfate concentrations (<120mg/l), while at higher concentrations, ASRB were no longer competitive. SRB were also shown to be the main oxidisers of propionate at all sulfate concentrations, whereas acetogenic bacteria (AB) rather than SRB were the main butyrate and valerate oxidisers.

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LIST OF NOTATION

NOTATION*	Kinetic values derived without values for Y and X
ΔG	Gibbs free energy of formation
μ	Specific biomass growth rate
μ_{max}	Maximum specific biomass growth rate
b	Cell decay rate
CoV	Coefficient of Variation
K_h	Hydrolysis rate constant
K_s	Half saturation constant
n	number of independent finite data points
q	flow rate
r_g	Volumetric bacterial flow rate
r_{su}	Substrate utilisation rate
S	Substrate concentration
t	time
V	Volume
W	Sum of signed ranks
X	Bacterial concentration
Y	Biomass yield constant
z	Critical value for defining the level of significance
A	Electron Acceptor Concentration
AB	Acidogenic Bacteria
ABR	Anaerobic Baffled Reactor
AcB	Acetogenic Bacteria
AD	Anaerobic Digestion
AMP	Adenosine Monophosphate
AMPB	Acetotrophic Methane Producing Bacteria
APS	Adenosine 5-Phosphosulfate
ASRB	Acetotrophic Sulfate Reducing Bacteria
ATA	Anaerobic Toxicity Assay

ATP	Adenosine Triphosphate
BMP	Biological Methane Production assay
COD	Chemical Oxygen Demand
COD:SO_4	COD to Sulfate ratio
CSTR	Completely Stirred Tank Reactor
D	Electron Donor Concentration
ds	dry solids
FID	Flame Ionisation Detector
FISH	Fluorescent In-Situ Hybridisation
GC	Gas Chromatograph
HMPB	Hydrogenotrophic Methane Producing Bacteria
HPLC	High Pressure Liquid Chromatography
HRT	Hydraulic Retention Time
HSRB	Hydrogenotrophic Sulfate Reducing Bacteria
MPB	Methane Producing Bacteria
MW	Molecular Weight
OLR	Organic Loading Rate
PS	Biodegradable Primary Sludge Concentration
RLF	Rate Limiting Factor
RMM	Relative Molecular Mass
SCOD	Soluble Chemical Oxygen Demand
SD	Standard Deviation
SMP	Soluble Microbial Products
SRB	Sulfate Reducing Bacteria
SRT	Solids Retention Time
STP	Standard Temperature and Pressure
TCD	Thermal Conductivity Detector
TCOD	Total Chemical Oxygen Demand
TOC	Total Organic Carbon
TS	Total Solids
TSS	Total Suspended Solids
VFA	Volatile Fatty Acid
VS	Volatile Solids
VSS	Volatile Suspended Solids

CHAPTER 1

INTRODUCTION

1.1 THE PROCESS OF SULFATE DISINFECTION, STABILISATION AND HEAVY METAL REMOVAL FROM SEWAGE SLUDGE

1.2 THESIS STRUCTURE

The degradation of organic matter is mediated by micro-organisms, either aerobically with the use of oxygen, or anaerobically without oxygen. Conventional sewage treatment harnesses the action of either type of organism to break down complex organic material thus significantly reducing its fermentability. The semi-solid end products of sewage treatment are termed sludge (Butler, 2000). The total amount of dry solid (ds) sludge produced annually within the EU is projected to be 8.3 million tonnes by 2005 (Langenkamp and Marmo, 1999). The main disposal options for sludge include agricultural application, incineration, land reclamation, landfill and forestry. The most economical of these is considered to be farmland application (Smith, 1996). Sewage sludge contains substantial amounts of nitrogen and phosphorus, typically 5% and 3%, respectively (Wong and Henry, 1985), both essential plant nutrients normally provided through chemical fertiliser application. As a result, sludge application onto farmland in the UK could save £15 million annually (Hall, 1992).

Additionally, the organic matter content of the sludge is reported to have soil-conditioning properties, thus improving the physical characteristics of the soil (Marinari *et al.*, 2000; Pagliai *et al.*, 1981). Whereas the K-content of sewage sludge does not contribute appreciably to the fertiliser value (Hall and Williams, 1984) the S-content may be a substitute for once aerially-deposited S, which is now in decline due to stricter regulations on SO_x emissions. However, with sludge production rates predicted to increase in the future, EU directives are coming into effect to try to deal with these vast amounts of 'waste' in economical and environmentally-friendly ways,

hence disposal options are becoming fewer and pre-treatment requirements ever stricter. A major concern discouraging the application of sludge directly onto land is the high heavy metal and pathogen content.

Council Directive 86/278/EEC of 12 June 1986 set the limit values for concentrations of heavy metals in soils. However, amendments to these limits to make them stricter have been proposed in an EU Working Document on Sludge (3rd Draft) published in 2000 (European Commission Proposal, 2000). The plans aim to improve the present situation for sludge management and include the following initiatives:

"- Reducing the quantities of potentially hazardous substances, materials, elements or compounds that end up in sewage sludge so that it complies with the concentration limits for heavy metals and organic compounds as suggested for the beneficial use of sewage sludge";

"- Maximising the share of sewage sludge suitable for the beneficial use as well as minimising the quantities of heavy metals and organic compounds released into the environment" (ibid).

It is envisaged that the new stricter limits for heavy metals will be phased in over a 25 year period. Whereas only 13% of all sludges today fail the current heavy metal limits, by 2025 this figure would rise to over 83% with the introduction of the tighter EU limits.

Concerning sludge pathogen content, the preferred method of sludge treatment in the UK is mesophilic anaerobic digestion (AD) which significantly reduces pathogen numbers (e.g bacteria Salmonella, virus Hepatitis A, protozoa Entamoeba and nematode Ascaris), but does not entirely eliminate them (Smith, 1996). An agreement, which affects all applications of sewage sludge onto agricultural land came into force in December 1998. Called the Safe Sludge Matrix, it was agreed by Water UK (representing 14 UK Water and Sewage Operators) and the British Retail Consortium. Its aim was to completely phase out the application of raw untreated sewage sludge onto agricultural land by December 2001, with the exception of land used for certain combinable crops that are further heat-processed (ADAS *et al.,* 1999).

At the time of writing, new EU legislation governing sludge pathogen levels is being anticipated. However, it is expected that the levels will not be significantly different to the current ones. Table 1.1 illustrates the Safe Sludge Matrix which shows that most sludges will require advanced treatment before their application onto most types of crop.

Table 1. 1- The Safe Sludge Matrix (ADAS *et al.*, 1999).

Crop Type	Untreated Sludges	Treated sludges	Advanced Treated Sludges
Fruit	×	×	✓
Salads	×	× 30 month harvest interval applies	✓
Vegetables	×	×12 month harvest interval applies	✓
Horticulture	×	×	✓
Combinable & Animal Feed	×	✓	✓
Grass-Grazing	×	×Deep injected or ploughed down only	✓
Grass-silage	×	✓	✓
Maize-Silage	×	✓	✓

Note: **Untreated Sludge** is any sludge which is neither treated nor enhanced treated.
Treated Sludge is sludge which has undergone biological chemical or heat treatment processes so as to significantly reduce its fermentability. It must achieve a minimum of 2 log or 99 per cent reduction of the indicator organism *Escherichia coli* (*E. coli*) across the sludge treatment process, and where the final processed sewage sludge meets the "Final Product Check".
Advanced Treated Sludge is sludge which has undergone biological, chemical or heat treatment processes so as to significantly reduce its fermentability. It must achieve a minimum of 5 log or 99.999 per cent reduction of the indicator organism *E. coli* across the sludge treatment process and where the final processed sewage sludge meets the "Final Product Check" (Lowe, 2000).

1.1 THE PROCESS OF SULFATE DISINFECTION, STABILISATION AND HEAVY METAL REMOVAL FROM SEWAGE SLUDGE

The process of ***Sulfate Disinfection, Stabilisation and Heavy Metal Removal from Sludge*** (from hereon referred to as the Sulfate Digestion process) is an alternative to conventional sewage treatment options. It was developed by Hobson *et al.*, (1994) in order to enable the application onto land of sewage sludge containing permissible levels of heavy meals and pathogens. The process consists of three main parts:

Sludge/Sulfuric Acid Reactor, H_2S Generator and H_2S Bioscrubber. Figure 1.1 illustrates this three-stage process.

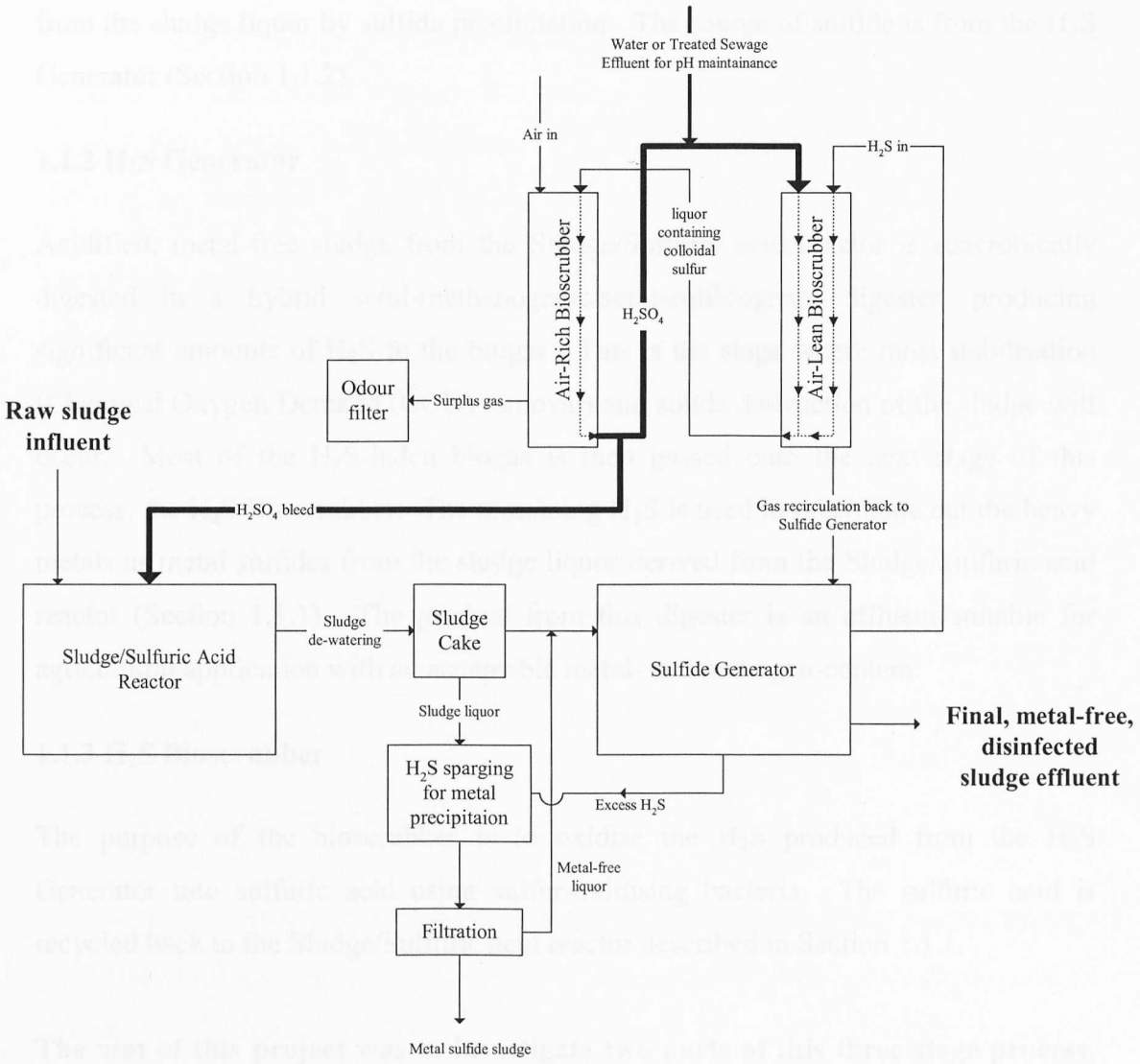


Figure 1. 1- Sulfate Disinfection, Stabilisation and Heavy Metal Removal from Sewage Sludge.

1.1.1 Sludge/Sulfuric Acid Reactor

This is the stage where influent raw and/or activated sewage sludge is received. The purpose of this stage is to solubilise heavy metals present in the sludge and to destroy pathogens. The process consists of acid leaching already used in various industries to

remove heavy metals from wastewaters or metal ores. The pH of raw sludge is between 5-6. Acid from the H_2S Bioscrubber (Section 1.1.3) reduces the pH down to 1-1.5, which is sufficient to solubilise most organically and inorganically bound heavy metals and destroy most pathogens. The solubilised heavy metals are removed from the sludge liquor by sulfide precipitation. The source of sulfide is from the H_2S Generator (Section 1.1.2).

1.1.2 H_2S Generator

Acidified, metal-free sludge from the Sludge/Sulfuric acid reactor is anaerobically digested in a hybrid semi-methanogenic/semi-sulfidogenic digester, producing significant amounts of H_2S in the biogas. This is the stage where most stabilisation (Chemical Oxygen Demand (COD) removal) and solids destruction of the sludge will occur. Most of the H_2S-laden biogas is then passed onto the next stage of this process, the H_2S Bioscrubber. The remaining H_2S is used to precipitate out the heavy metals as metal sulfides from the sludge liquor derived from the Sludge/Sulfuric acid reactor (Section 1.1.1). The product from this digester is an effluent suitable for agricultural application with an acceptable metal- and pathogen-content.

1.1.3 H_2S Bioscrubber

The purpose of the bioscrubber is to oxidise the H_2S produced from the H_2S Generator into sulfuric acid using sulfur-oxidising bacteria. The sulfuric acid is recycled back to the Sludge/Sulfuric acid reactor described in Section 1.1.1.

The aim of this project was to investigate two parts of this three-stage process, namely the H_2S Generator and H_2S Bioscrubber. The work involved looking at the effect of different sulfate levels added to the feed on sludge digestion efficiency in an H_2S Generator. In order to understand the effects of sulfate on digestion performance, it was important to also look at the microbial kinetics involved. A preliminary investigation into H_2S Bioscrubber technology was also made; for this part of the project, a new design for bioscrubber technology was proposed and built, the lay-out optimised and sulfide oxidation efficiency studied.

1.2 THESIS STRUCTURE

CHAPTER 2: LITERATURE REVIEW – The principles of conventional AD in a Completely Stirred Tank Reactor (CSTR) are covered. A comparison of hybrid AD involving sulfate reduction with conventional AD is made. The process of sulfide oxidation in the context of H_2S Bioscrubber technology is discussed. Previous investigations into technologies similar to the Sulfate Digestion process found in the literature are described.

CHAPTER 3: MATERIALS AND METHOD – A description of the apparatus, equipment and instruments used, and analytical methods are given. The statistical methods used to analyse the results are detailed.

CHAPTER 4: START UP OF A HYBRID H_2S GENERATOR AND H_2S BIOSCRUBBER – The effect of adding sulfate to a methanogenic reactor to start up an H_2S Generator is investigated. The COD:SO_4 ratio is kept constant and the source of sulfate is initially sodium sulfate. The digestion efficiency, in terms of COD and solids destruction, and biogas production is compared to a conventional methanogenic reactor. The H_2S Bioscrubber is built and set up, the lay-out is optimised, and sulfide oxidation efficiency investigated.

CHAPTER 5: EFFECT OF COD:SO_4 RATIO ON ANAEROBIC DIGESTION – Seed from the established H_2S Generator is used to establish a further three daughter hybrid reactors running at various COD:SO_4 ratios. The source of sulfate is changed from sodium sulfate to sulfuric acid. The sulfuric acid tolerance limits are explored and the sludge digestion efficiency, in terms of COD, solids destruction and biogas production from these reactors is compared to a conventional methanogenic reactor.

CHAPTER 6: BATCH-TESTS: KINETICS INVOLVED IN HYBRID ANAEROBIC DIGESTION – The aim of the kinetic tests is to compare the effect of different COD:SO_4 ratios on the hydrolysis and Volatile Fatty Acid (VFA) degradation of C4-C6 acids by mixed microbial consortia. The seed sludge used is derived from the four lab-scale reactors (3 hybrid and one methanogenic) that are started up. For the hydrolysis test, primary sludge particle solubilisation is monitored,

while for the VFA degradation test, a selective inhibitor of Sulfate Reducing Bacteria (SRB) is used to qualify and quantify at what level these micro-organisms were actually involved in the final stages of digestion. The results are used to build a profile of the microbial kinetics of the hybrid compared to the methanogenic reactors.

CHAPTER 7: CONCLUDING DISCUSSION – An overall assessment of the feasibility and limitations of running a H_2S Generator is discussed. A summary of all conclusions arrived at in this research are presented, and ideas for future work within this project are given.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION
2.2 CONVENTIONAL ANAEROBIC DIGESTION
2.3 TYPES OF ANAEROBIC REACTOR
2.4 SULFATE REDUCTION IN ANAEROBIC ENVIRONMENTS
2.5 COMPETITION BETWEEN SULFIDOGENESIS AND METHANOGENESIS
2.6 SULFIDE OXIDATION
2.7 PREVIOUS INVESTIGATIONS INTO SULFUR TECHNOLOGIES
2.8 SUMMARY
2.9 RESEARCH OBJECTIVES

2.1 INTRODUCTION

This review covers the principles of conventional AD in a CSTR and this is compared to hybrid AD involving sulfate reduction. The main factors controlling competition between methanogenesis and sulfidogenesis are highlighted and explained in order to understand the principles underlying the second stage of the Sulfate Digestion process, which is the H_2S Generator.

2.2 CONVENTIONAL ANAEROBIC DIGESTION

Conventional AD involves the complex interactions of different species of micro-organisms under oxygen-free conditions. There are a large number of benefits to using AD for treatment of liquid or sludge waste. It not only requires less land than aerobic composting or landfilling, but also reduces the waste volume and weight to be

disposed of. Whereas aerobic treatment transforms an appreciable fraction of waste to new biomass solids, AD produces an order of magnitude less solids, with the difference converted to biogas. The solids produced themselves are a form of sanitised compost and can be used as a nutrient–rich fertiliser. It is a net energy producing process, so that with proper biogas capture a high quality renewable fuel can be used, and greenhouse emissions and odours significantly reduced. In terms of economic benefits, it is one of the most cost-effective treatment options from a lifecycle perspective (Smith, 1996).

During AD, bacteria will remove hydrogen atoms from organic molecules, gaining energy in the process. The hydrogen atoms are then transferred to a hydrogen acceptor through a series of biochemical reactions. Unlike under aerobic conditions where hydrogen-oxidising bacteria oxidise H_2 with molecular oxygen as electron acceptor, under anaerobic conditions a number of different electron acceptors may be employed such as inorganic nitrate, sulfate, and carbon compounds such as CO_2 and methanol. Obligate anaerobes can only use combined forms of oxygen, while facultative anaerobes can use either free or combined forms of oxygen. The hydrogen acceptor during conventional AD is carbon dioxide, with methane and water as final reduced products (McInerney, 1988). AD can be divided into four main stages; hydrolysis, acidogensis, acetogenesis and methanogenesis.

2.2.1 Hydrolysis

The hydrolysis stage involves the break-down and solubilisation of complex organic long-chain molecules into shorter mono- or di-meric soluble ones by extra-cellular enzymes. Some 145 extra-cellular hydrolytic enzymes have been identified (*cited in* Halalsheh, (2002)) all showing a wide range of pH and temperature tolerances. Hydrolysis substrates include suspended and colloidal matter in the form of particulate carbohydrates, proteins and lipids. The group of bacteria involved are known as the fermentative bacteria.

The carbohydrate fraction of municipal sewage sludge is primarily composed of cellulose (40% w/v) derived from toilet paper and the remains of human excrement and vegetables (Pohland, 1962). Cellulose is a linear homopolymer composed of

several thousand D-glucose units linked by ß-1,4 glucosidic bonds (Chynoweth and Pullammanappallil, 1996). Its complete degradation to glucose requires four major enzymatic steps from cellulose to cello-oligosacharides to cellobiose and finally glucose (Tong and McCarty, 1991). Each step requires a different set of enzymes, all of which act in synergy (Woodward, 1991). Bacteria involved in cellulose degradation include *Bacteriodes succinogenes* and several Clostridium species. Although produced by different organisms, the cellulase properties of the enzymes are very similar. Cellulose hydrolysis occurs after the enzyme has adsorbed onto the cellulose. Aggregates called cellulosome are produced by *C. thermocellum, B. succinogenes* and *R. albus* which help to bind the cell to the substrate for subsequent hydrolysis (*cited in* Chynoweth and Pullammanappallil, 1996). By-products of cellulose beak-down include H_2, CO_2, acetic and formic acids.

Proteins are broken down by bacteria such as proteolytic clostridia into their constituent amino acids which are linked by peptide bonds. Amino acid fermentation products include VFA's, CO_2, H_2 and NH_4^+ (ammonium). Since proteins contain nitrogen and sulfur, their catabolism gives rise to N- and S-containing gases. The degradation of amino acids is a complex process involving redox reactions, and a good review can be found in McInerney (1988). As for lipids, they can form up to 30% (w/v) of sludge solids and consist of esters of tri-hydroxy alcohol glycerol. They undergo hydrolysis such that ester bonds are broken to produce glycerol, fatty acids or a combination of both known as triglycerides. Fatty acids are carboxylic acids with a hydrophobic alkyl group at one end and a hydrophilic acid group at the other. Lipases act at the water-lipid interface to solubilise triglycerides, which are themselves a major source of acetate after β-oxidation (described in section 2.2.3.1).

2.2.2 Acidogenesis

Acidogenesis involves further breakdown of end products from the hydrolysis stage and is performed by fast-growing acidogenic bacteria (AcB) such as *Pseudomonas* which have a doubling time of about 30 minutes. Carbohydrates such as hexoses and pentoses are generally converted to C2 and C3 intermediates and reduced electron carriers (e.g., NADH) via common pathways. Most anaerobic bacteria undergo

hexose metabolism via the Emden-Meyerhof-Parnas pathway which produces pyruvate as an intermediate along with NADH. The pyruvate and NADH are transformed into low molecular weight products such as lactate, propionate, acetate and ethanol (Miyamoto, 1997). Glycerol is fermented to similar products as proteins but without NH_4^+ and sulfide formation. AcB do not attack the aromatic nucleus of amino acids nor do they oxidise fatty acids. Instead, both long and short chain fatty acids are β-oxidised to acetate either by H_2-producing syntrophic bacteria, or SRB in sulfate-rich waste waters.

2.2.3 Acetogenesis

Although some acetate (20%) and H_2 (4%) are directly produced by acidogenic fermentation of sugars and amino acids, both products are primarily derived from the acetogenesis and dehydrogenation of higher volatile fatty acids by Acetogenic Bacteria (AB) (Miyamoto, 1997). Obligate H_2-producing (proton reducing) AB are capable of producing acetate and H_2 from higher fatty acids, and include *Syntrophobacter wolinii*, a propionate decomposer, and *Syntrophomonos wolfei*, a butyrate decomposer.

2.2.3.1 β-Oxidation: This pathway involves the oxidation of VFA's longer than 2C to acetate and H_2 by obligate anaerobes. Fatty acids are a major source of energy for most cells, and the first step in their utilisation is β-oxidation of fatty Acyl-Coenzyme A's (AcylCoA). In this process, the size of the fatty AcylCoA is reduced in a sequence of 4 enzymatic reactions which splits the molecule at the single C-C bond between the α and β carbons. This sequence occurs in repeating cycles where a fatty acid is progressively shortened by two carbons and is oxidised. The energy released is captured by the reduced energy carriers NADH and $FADH_2$, which are then oxidised via the electron transport chain, allowing oxidative phosphorylation to generate ATP (Green *et al.*, 1993).

2.2.4 Methane Forming Stage

Methanogens are the bacteria involved in the last stage of AD. They are members of the Archeae Kingdom and display a wide range of bacterial morphologies, including rods, cocci, sarcinae and spirilla. The methanogens can be subdivided into two

groups: the obligate chemolithotrophs (or H_2/CO_2–consumers) such as those of the order *Methanobacteriales* and *Methanococcales*. They obtain their carbon from the reduction of CO_2, with H_2 as electron and energy donor, and most of these species can also grow on formate. The methylotrophic methanogens on the other hand, not only grow on H_2 plus CO_2, but also on methanol, mono-, di- or tri-methylamine as their energy and carbon source (Anthony, 1988). Within this group also fall the acetotrophic methanogens (AMPB's) such as *Methanosaeta* and *Methanosarcina* spp. The competition between these two species is quite complex since *Methanosarcina* spp. is a methylotrophic methanogen which can grow on several substrates that may be used preferentially over acetate. In anaerobic systems, the prevalent H_2 concentrations may encourage acetoclastic methanogenesis to be the preferred pathway for *Methanosarcina* spp. so that the outcome of competition may be reliant on kinetic parameters for acetate (Raskin *et al.*, 1996). Among the known pure cultures of methanogens, the genus *Methanosarcina* is the most versatile metabolically since it can use H_2/CO_2, acetate, methanol and methylamines. All the other species are restricted to H_2/CO_2 and sometimes formate, with the exception of *Methanobaterium soehngenii* (acetate only) and *Methanococcus mazei* (methanol, methylamines, acetate) (Widdel, 1988). Since up to 70% of methane comes from the cleavage of acetate (Jeris and McCarty, 1965), and acetoclastic methanogens are very slow-growing (generation time for *Methanobacterium soehgenii* = 9 days (Mah *et al.*, 1980)), methane formation from acetate is one of the rate-limiting steps of AD. As we will see later, hydrolysis is also considered as a rate-limiting step, but the result is dependent on the type of wastewater being treated. In AD plants treating low-strength wastewaters, physical and chemical parameters are controlled in order to cater for the methanogens. The ability of methanogenic bacteria to use H_2/CO_2 and acetate is essential for sludge stabilisation since most available carbon is locked away in fermentation products before being released as biogas during methanogenesis. Typical digester gas composition is 30-35% CO_2 and 65-70% CH_4 (Cook and Boening, 1991), with traces of H_2 and H_2S.

The production and use of hydrogen in the transfer of electrons is a crucial step that allows the complex interactions between the range of bacteria involved at every stage of AD; this is known as inter-species hydrogen transfer. The formation of acetic acid from the degradation of higher chain fatty acids is coupled with simultaneous

production of large quantities of hydrogen, which is only favourable if H_2 is reduced to a low level by hydrogenotrophs (H_2-utilisers). At high H_2 levels, one response of the acid-forming bacteria is to direct their metabolism to the production of higher fatty acids (butyric, propionic) instead of acetic to reduce the production of acid and free hydrogen. Also, the oxidation of propionate and butyrate may be inhibited, leading to a decline in pH and exhaustion of buffering capacity. AcB are severely inhibited by H_2 production during the acidogenic stage since thermodynamically they require an extremely low partial pressure of 10^{-5} atm. to function. They are, therefore, only able to grow in syntrophy with hydrogen consumers. This is illustrated for propionic acid oxidation where there is a high free energy requirement (eq. 2.1) compared to oxidation with simultaneous H_2 consumption (eq. 2.2) (McInerney, 1988). Table 2.1 lists most of the known mesophilic obligate hydrogen-producing bacteria and the syntrophic organisms to which they are associated (ibid).

$$CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 \qquad \text{(eq. 2. 1)}$$

$\Delta G^{0'} = +76.1$ (kJ/reaction)

$$4CH_3CH_2COO^- + 12H_2 \leftrightarrow 4CH_3COO^- + HCO^{3-} + H^+ + 3CH_4 \qquad \text{(eq. 2. 2)}$$

$\Delta G^{0'} = -102.4$ (kJ/reaction)

The lowering of the pH inhibits Methane Producing Bacteria (MPB) and AMPB; thus during organic overload an accumulation of higher fatty acids and H_2 is expected (Benstead *et al.,* 1990). Fermentative organisms can also be induced to act as hydrogen producers due to interspecies hydrogen transfer. In the presence of hydrogenotrophs, fermentative organisms shift their metabolism to the formation of more oxidised and less reduced organic products. In one experiment, the *Ruminococcus albus* organism which ferments glucose to acetate, ethanol CO_2 and H_2 was coupled to *Vibrio succinogenes*, an organism which can only reduce fumerate to succinate. When the co-culture was grown on glucose in the presence of fumerate, electron flow was directed towards H_2 formation, and fermentation of glucose towards ethanol ceased (McInerney, 1988). Similarly, in the absence of sulfate, the SRB species of *Desulfovibrio* cannot ferment ethanol or lactate. However, in the presence of hydrogenotrophic methanogens (HMPB) they switch their metabolism to those of

acetogenic H_2-producing bacteria according to equations 2.3 and 2.4. *Desulfovibrio*, though, do not behave in the same manner in syntrophy with methanogens when VFA's are being used as substrate instead of H_2/CO_2 (Widdel, 1988).

SRB: $CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$ (eq. 2. 3)

HMPB: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ (eq. 2. 4)

Table 2. 1- Mesophilic obligate H_2-producing bacteria and associated syntrophic organisms involved in interspecies H_2 transfer (adapted from McInerney, 1988).

Organism	In culture with	Substrate
Syntrophobacter wolinii	*Desulfovibrio*, or *M. hungatei*	Propionic acid
Syntrophomonas wolfei	*Desulfovibrio*, or *M. hungatei*	Monocarboxylic saturated fatty acids (14-18 carbon)
Syntrophus buswellii	*Desulfovibrio* , or *M. hungatei*	Benzoic acid
Clostridium bryantii	*Desulfovibrio*, or *M. hungatei*	Monocarboxylic fatty acids (to 11-carbon)

Therefore, the presence of H_2-consuming bacteria such as MPB's and SRB's in co-culture will provide favourable conditions for the decomposition of fatty acids to acetate and methane (Koster *et al.*, 1986; Rebac *et al.*, 1996; Miyamoto, 1997).

2.3 TYPES OF ANAEROBIC REACTOR

Essentially, there are two types of anaerobic digesters: low rate and high rate. In both types, biomass retention within the reactor is the most important parameter to consider in order to maintain the population of the slow-growing methanogens. This is based on biomass recycling and/or retention time control within the reactor (Speece, 1996). Figure 2.1 represents some of the most common reactor configurations used to immobilise biomass. Low rate digesters are characterised by intermittent mixing, sludge feeding and sludge withdrawal, or no mixing at all (stratified). High rate reactors on the other hand are characterised by continuous mixing and shorter retention time. The oldest and most simple form of AD technology is the uncovered, unmixed lagoon with a long retention time of weeks or months. In order to improve the contact between organic substrate and bacterial biomass, mixing methods have been devised. Odour and air emissions are controlled by means of membrane covers

to collect the biogas. This type of mixed and covered lagoon is widely used outside of Europe for the treatment of industrial wastes (IEA Bioenergy, 2001). An improvement on anaerobic lagoons came in the form of a CSTR, widely applied during the 1940's. A CSTR contains a mixer to maintain good contact between biomass and the organic material. Where the biomass is separated and recycled in order to increase organic load and reduce Hydraulic Retention Time (HRT), the CSTR is called a contact reactor. CSTR's are an improvement on lagoon technologies where digestion efficiency is greater and retention times lower (measured in days).

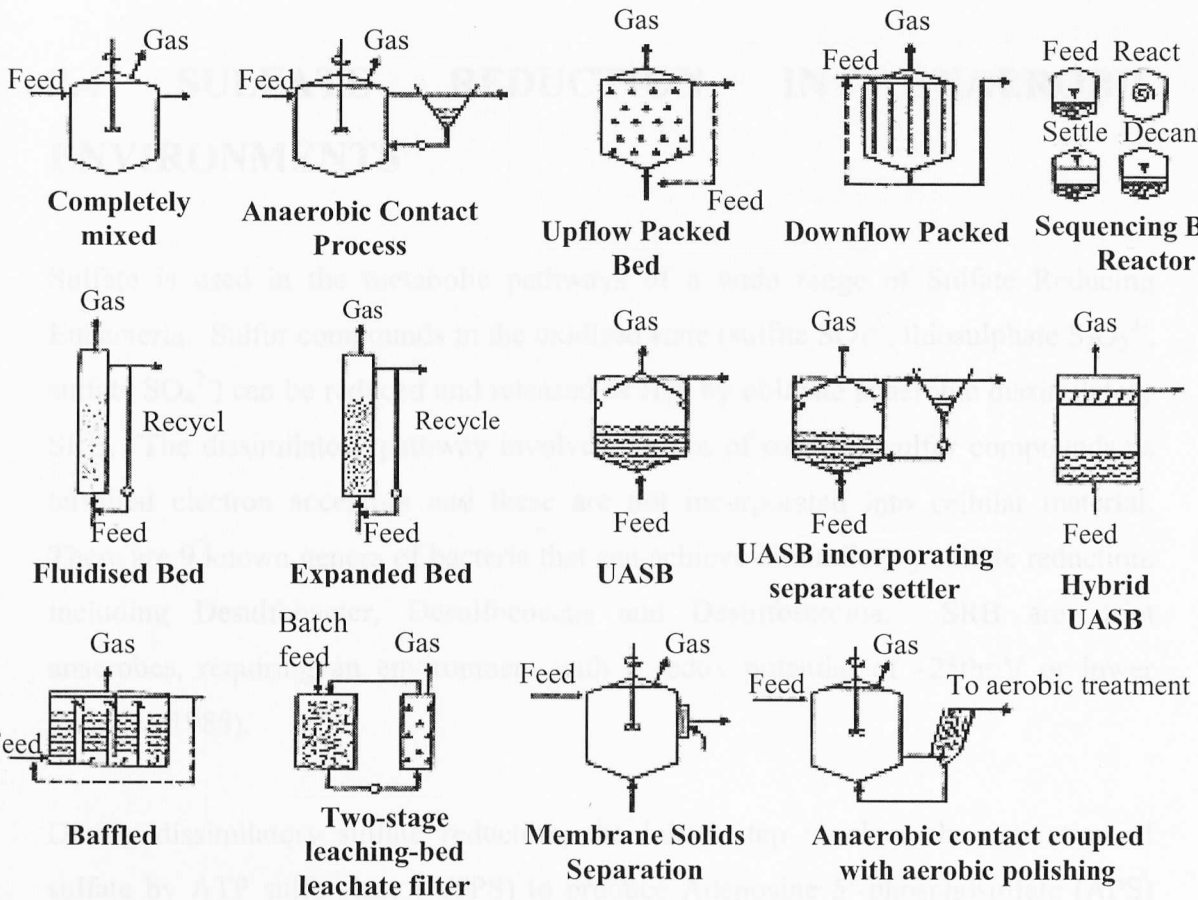


Figure 2. 1- Various reactor configurations used for biomass retention (Speece, 1996)

High rate reactors began to develop in the late 1960's and aimed at reducing retention times and therefore size of reactors to a minimum. This was possible when the HRT and Solids Retention Time (SRT) were separated, as in the Anaerobic Filter, which relies on a media, usually made of glass, wood or plastic, to retain biomass in the reactor vessel. Other high rate reactors include the Upflow Anaerobic Sludge Blanket

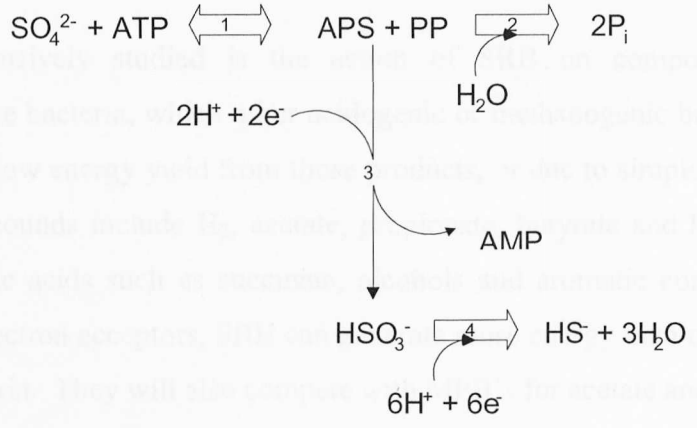
Reactor, developed by Lettinga and co-workers in the 1980's (Lettinga *et al.*, 1980), which combined the well-mixed attributes of the contact system with an internal biogas separation and clarification system and has hydraulic retention times down to less than a day. Although high rate reactors have a clear advantage over low rate reactors in terms of improved retention times, they are usually used for simple and well-defined wastewaters (McCarty and Oleszkiewicz., 1991), and cannot be used to treat sludges or wastes high in particulates. As a first step towards understanding the interaction between conventional and hybrid sulfidogenic digestion of a complex wastewater such as sewage sludge, it was decided to use a CSTR for this project.

2.4 SULFATE REDUCTION IN ANAEROBIC ENVIRONMENTS

Sulfate is used in the metabolic pathways of a wide range of Sulfate Reducing Eubacteria. Sulfur compounds in the oxidised state (sulfite SO_3^{2-}, thiosulphate $S_2O_3^{2-}$, sulfate SO_4^{2-}) can be reduced and released as H_2S by obligate anaerobic dissimilatory SRB. The dissimilatory pathway involves the use of oxidised sulfur compounds as terminal electron acceptors and these are not incorporated into cellular material. There are 9 known genera of bacteria that can achieve dissimilatory sulfate reduction, including Desulfobacter, Desulfococcus and Desulfosarcina. SRB are strict anaerobes, requiring an environment with a redox potential of –250mV or lower (Widdel, 1988).

During dissimilatory sulfate reduction, the initial step involves the activation of sulfate by ATP sulfurylase (ATPS) to produce Adenosine 5'-phosphosulfate (APS) and inorganic pyrophosphate. APS-reductase then catalyses APS to Adenosine monophosphate (AMP) and sulfite. This sulfite is finally reduced to sulfide by the enzyme bisulfite reductase (Figure 2.2 illustrates this pathway). The initial activation of sulfate by ATPS is the rate-limiting step to its subsequent participation in sulfate reduction. The physico-kinetic properties of ATPS were studied by Pletschke *et al.* (2002) in order to determine its levels in a fully methanogenic reactor ($ATPS_{MR}$), and in a sulfidogenic reactor ($ATPS_{SR}$) running at a COD:SO_4 ratio of 1:1, both running at an HRT of 2 days. The activity of the enzyme was also followed as a function of pH,

temperature, time and presence of sulfate, sulfite and sulfide. The study concluded that almost all the ATPS was located either intracellularly, or immobilised in or on the floc. The activity of $ATPS_{SR}$ was 10-20 times higher than $ATPS_{MR}$, indicating that SRB probably require high levels of ATPS to efficiently use sulfate. There was no significant pH optimum for the enzyme derived from either reactor, while the temperature optimum for $ATPS_{MR}$ and $ATPS_{SR}$ were similar at 50°C and 55°C, respectively. Sulfate and sulfide at 0-1000mg/l concentrations had no effect on ATP_{SR} activity, whereas sulfite had a slight inhibitory effect. The authors cite the lack of inhibition to ATPS by sulfate as contradictory to previous studies performed by Segel *et al.* (1987). It was speculated that the presence of exopolymeric substances in the sludge normally act as a trap for particulate matter and helps bacterial flocculation around particles thus protecting them from direct contact with hydrolytic enzymes. A change in ionic strength and composition due to the production of sulfide which is controlled by ATPS activity (e.g by increasing alkalinity, or metal precipitation) may help to destroy the floc thus releasing any particles within them and exposing them to hydrolytic enzymes.



1:ATP sulfurylase, 2: Inorganic Pyrophosphatase, 3: APS reductase, 4: Bisulfite reductase

Figure 2. 2- Dissimilatory Sulfate Reduction in SRB (adapted from Lederberg, 1992)

In agreement with this theory is a study by Dunn (1998) cited in Whiteley *et al.* (2002), which showed that SRB present in a sulfate-reducing system had a stimulating effect on the breakdown of particulate matter derived from tannery wastewater treated in a ponding system. The cyclical upwelling and resettling of residual particulate

matter into the sulfidogenic zone of the pond system achieved a high level of solids removal. The proteases and phosphatases involved in particulate matter hydrolysis showed the highest activity when either associated with, or immobilised within, the particulate matter rather than in the supernatant. Activity tests to deduce the temperature and pH activity optima of the enzymes showed them to be 60°C and pH 7 (Whiteley *et al.*, 2002). Sulfate concentrations of 200-800 mg/l inhibited protease activity by 60-80%, respectively, at pH 5. Similarly, at pH 10 there was a 25-50% drop in activity at sulfate concentrations between 400-800 mg/l, respectively, while no inhibition was apparent at pH 7. Interestingly, protease activity increased with increasing pH at sulfide concentrations of 1000 mg/l, and decreased at each measured pH with decreasing sulfide concentration. A similar pattern was observed with different sulfite concentrations at each pH (Whiteley *et al.,* 2001; Whiteley *et al.,* 2002). A drawback to these studies is that proteolytic and phosphatase activities were measured only in relation to pH, temperature, sulfate, sulfide and sulfite, whereas corresponding suspended solids measurements were not taken.

2.4.1 Acidogenesis With SRB

More extensively studied is the action of SRB on compounds derived from fermentative bacteria, which other acidogenic or methanogenic bacteria cannot utilise due to the low energy yield from those products, or due to simple metabolic inability. Such compounds include H_2, acetate, propionate, butyrate and higher chain VFA's, dicarboxylic acids such as succinate, alcohols and aromatic compounds. By using external electron acceptors, SRB can generate more energy than normally available to other bacteria. They will also compete with MPB's for acetate and hydrogen (Widdel, 1988). Table 2.2 illustrates the reduction of sulfate and related compounds, the potential energy sources and free energy production per reaction. SRB can be divided into two groups; those which can incompletely oxidise substrates to acetate, and those which are able to fully oxidise them to CO_2. The former can only use C2 compounds more reduced than acetate such as oxalate. Acetate can, however, be used as a source of organic carbon to be incorporated into cell material. Generally speaking, incomplete-oxidisers are less versatile nutritionally, but have a faster growth rate of up to 3 to 4 hours (e.g *Desulfotomaculum*). The complete substrate oxidisers will use the same substrates as the first group plus others such as the branched-chain fatty

acids, making them generally more nutritionally versatile, but slower-growing (doubling time >20hrs) (Widdel, 1988).

Table 2. 2- Reduction of sulfur and its compounds (adapted from Lederberg, 1992).

Sulfate and sulf-oxy anions	Free energy ($\Delta G^{0'}$) kJ/reaction
$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O$	-168.8
$3H_2 + HSO_3^- \rightarrow HS^- + 3H_2O$	-171.7
$4H_2 + S_2O_3^{2-} \rightarrow 2HS^- + 3H_2O$	-174.1
lactate $+ SO_4^{2-} \rightarrow$ acetate $+ H_2S + 2HCO_3^-$	-168.8
pyruvate $+ SO_4^{2-} \rightarrow$ acetate $+ 2H_2S + HCO_3^-$	-356.1
acetate $+ SO_4^{2-} \rightarrow 2HCO_3^- + H_2S$	- 47.6

2.5 COMPETITION BETWEEN SULFIDOGENESIS AND METHANOGENESIS

VFA's such as propionate and butyrate are oxidised by AB bacteria to acetate and H_2, which in turn are converted to methane by MPB. As discussed earlier, since AB require a low partial pressure of H_2 it is necessary to have H_2-consuming bacteria growing in syntrophy. However, in the presence of sulfate, AB and MPB will compete with SRB for these substrates, and the outcome of competition will determine the amount of the end-products methane or sulfide that will be produced (Lens and Hulshoff Pol, 2000). The possible competition routes between these organisms are illustrated in Figure 2.3. Thermodynamically and kinetically speaking, SRB should out-compete AB and MPB's for hydrogen, acetate, propionate and butyrate. However, in anaerobic reactors there are a host of other factors which will influence the outcome of competition between AB, MPB and SRB. These include substrate concentration and type in the feed, COD:SO_4 ratio, maximum specific utilisation rate (μ_{max}), half velocity constant (K_s), thermodynamics/free energy of the reaction, nutrient availability, adhesion properties, proximity of cells (biofilms vs. dispersed cells), sulfide inhibition, trace metals, reactor configuration (Speece, 1996), acclimatisation (Isa *et al.,* 1986), temperature and pH-optima (Omil *et al.*, 1997), many of which will be discussed below in light of previous investigations into the effect of these parameters on SRB.

Quantification of the competition between SRB and MPB can be calculated according to COD destruction via the methanogenic or sulfidogenic pathway. Ramalho (1983) reported the COD of methane, assuming all substrate removed are converted to methane (eq. 2.5). CO_2 production is not included in the calculation as it does not contribute to COD.

- $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$ (eq. 2. 5)

Or as a ***mass balance (eq. 2.6):***

$16g\ CH_4 + 64g\ O_2 \rightarrow 44g\ CO_2 + 36H_2O$ (eq. 2. 6)

Since 16g CH_4 require 64g O_2, then 1g CH_4 is equivalent to 4g COD, therefore, mass CH_4 production from 1g COD = ¼ =0.25g

This equals 0.25/16 x 22.4 = 0.35L CH_4 per gram COD at STP (eq. 2. 7)

Similarly, the COD of H_2S can also be derived:

- $H_2S + 2O_2 \rightarrow H_2SO_4$ (eq. 2. 8)

Or as a ***mass balance (eq. 2.9):***

$34g\ H_2S + 64g\ O_2 \rightarrow 98g\ H_2SO_4$ (eq. 2. 9)

Since 34g H_2S require 64g O_2, then 1g H_2S is equivalent to 1.88g COD, the mass of H_2S production from 1g COD = 0.53g

This equals 0.53/34 x 22.4 = 0.35L H_2S per gram COD at STP (eq. 2. 10)

Stoichiometrically, the number of moles of gas produced per gram COD removed is equal (at 0.016 moles) via methanogenesis or sulfidogenesis. In order to compare COD destruction via methanogenesis and sulfidogenesis, the COD_{CH_4}:COD_{H_2S} ratio can be used. Similarly, COD:SO_4 ratios (g/g) may be quoted when discussing the amount of sulfate added to a reactor compared to the COD of the influent.

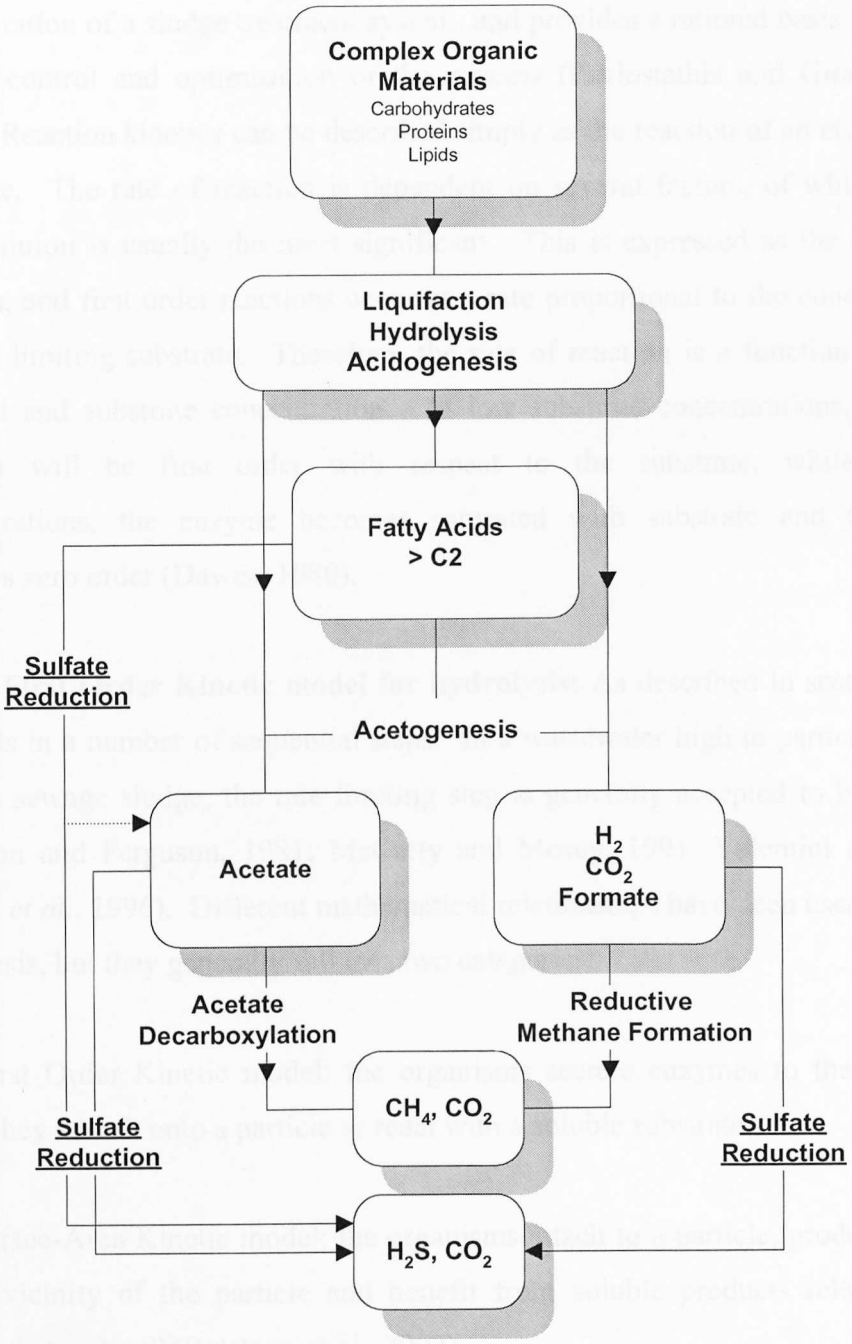


Figure 2. 3- Substrate competition between SRB, MPB and AB during anaerobic digestion (adapted from Lens and Hulshoff Pol, 2000)

2.5.1 Reaction Kinetics

Understanding the reaction kinetics involved in AD is crucial during the development and operation of a sludge treatment system, and provides a rational basis for analysis, design, control and optimisation of the process (Pavlostathis and Giraldo-Gomez, 1991). Reaction kinetics can be described simply as the reaction of an enzyme with a substrate. The rate of reaction is dependent on several factors, of which substrate concentration is usually the most significant. This is expressed as the order of the reaction, and first order reactions occur at a rate proportional to the concentration of the rate-limiting substrate. Therefore, the rate of reaction is a function of turnover constant and substrate concentration. At low substrate concentrations, an enzyme reaction will be first order with respect to the substrate, while at higher concentrations, the enzyme becomes saturated with substrate and the reaction becomes zero order (Dawes, 1980).

2.5.1.1 First Order Kinetic model for hydrolysis: As described in section 2.1, AD proceeds in a number of sequential steps. In a wastewater high in particulate matter, such as sewage sludge, the rate limiting step is generally accepted to be hydrolysis (Eastman and Ferguson, 1981; McCarty and Mosey, 1991; Valentini *et al.*, 1997; Vavilin *et al.*, 1996). Different mathematical relationships have been used to describe hydrolysis, but they generally fall into two categories:

"(1) First Order Kinetic model: the organisms secrete enzymes to the bulk liquid where they adsorb onto a particle or react with a soluble substrate;

(2) Surface-Area Kinetic model: the organisms attach to a particle, produce enzymes in the vicinity of the particle and benefit from soluble products released by the enzymatic reaction" (Batstone *et al.*, 2002).

The First Order Kinetic model is an empirical relationship which assumes that the hydrolysis rate is a linear function of the available biodegradable substrate at a certain pH and temperature. Although the simplest model, its main disadvantage is that in duplicate reactors, different hydrolysis rate constants (K_h) can be obtained due to the different particle size distributions in the raw sludge (Hills and Nakano, 1984). On the

other hand, the Surface-Area Kinetic model is a mechanistic model that assumes that enzymes are present in excess during hydrolysis of particulate matter. Research by Sanders (2001) has shown that at constant pH and temperature, the surface area available for hydrolysis is the most important parameter controlling K_h and that all other parameters are of minor importance.

The IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes (Batstone *et al.*, 2002) have adopted the First Order kinetic model based on studies by several authors such as Batstone (2000), Sanders *et al.* (2000), and Vavilin *et al.* (1996). The model has been shown to be on a par with other models such as the Surface-Area Kinetic model or Contois model (Vavilin *et al.*, 1996). Its limitation, however, is that hydrolysis proceeds according to first order kinetics if no changes in the rate limiting step or the biodegradability of the particulate matter occur during degradation (Sanders, 2001). Since first order hydrolysis constants seem to be substrate-specific, the use of literature values of K_h may be inaccurate. Moreover, it is important to describe the rate of hydrolysis as a function of the degradable particulate matter remaining and not the total particulate matter remaining. The form of the First Order Kinetic model for particulate matter hydrolysis can be described according to equation 2.11 (adapted from McCarty and Mosey, 1991):

$$\frac{d\mathrm{COD}_{pdeg}}{dt} = K_h \cdot \mathrm{COD}_{pdeg} \qquad \text{(eq. 2. 11)}$$

On integration from $\mathrm{COD}_{pdeg,0}$ to 0 yields:

$$\mathrm{COD}_{pdeg} = \mathrm{COD}_{pdeg,0}\ e^{-K_h \cdot t} \qquad \text{rearranged:} \qquad K_h = \frac{-\ln\left[\dfrac{\mathrm{COD}_{pdeg}}{\mathrm{COD}_{pdeg,0}}\right]}{t} \qquad \text{(eq. 2. 12)}$$

Where;

COD_{pdeg} = Degradable particulate COD ($kg.m^{-3}$)

$\mathrm{COD}_{pdeg,0}$ = Initial degradable particulate COD ($kg.m^{-3}$)

K_h = Hydrolysis rate constant (d^{-1})

t = Time (d)

2.5.1.2 Monod equation: The reaction rates for the soluble products of hydrolysis are described by yet another model known as the Monod equation (Barnes and Wilson, 1978; Whiteley *et al.,* 2002). The relationship is shown in equation 2.13 and illustrates that at low substrate concentrations the rate of specific biomass growth is first order with respect to substrate concentration, whereas at higher levels the rate approaches zero order.

$$\mu = \frac{\mu_{max} S}{K_s + S} \qquad \text{(eq. 2. 13)}$$

Where;

μ = Specific biomass growth rate (d^{-1})

μ_{max} = Maximum specific biomass growth rate (d^{-1})

S = Substrate concentration ($mg.l^{-1}$)

K_s = Half saturation constant equal to S when $\mu = 0.5\mu_{max}$ ($mg.l^{-1}$)

From equation 2.13, it possible to derive an expression for the volumetric rate of bacterial growth (eq. 2.14):

$$r_g = \frac{\mu_{max} XS}{K_s + S} \qquad \text{(eq. 2. 14)}$$

Where;

r_g = Volumetric bacterial growth rate ($mg.l^{-1}.d$)

X = Bacterial concentration ($mg.l^{-1}$)

In order to account for the rate of cell decay or lysis, a decay rate constant was introduced by McCarty (1966) to give equation 2.15. This decay rate is also known as the cell maintenance energy (Pirt, 1975), and although conceptually different to cell death rate, is mathematically identical (McCarty and Mosey, 1991). Values for the decay rate constant will vary according to the bacterial species, substrate- or nutrient-availability and environmental conditions. Henze and Harremoes (1983) and Siegrist *et al.* (2002) both cite a value of $\approx$ 0.050 d^{-1} at 35°C, with the latter author

experimentally verifying the constant for acetoclastic methanogens.

$$r'_g = \frac{\mu_{max} XS}{K_s + S} - bX \qquad \text{(eq. 2. 15)}$$

Where;

r'_g = Net bacterial growth rate (mg.l^{-1}.d)

b = Cell decay rate (d^{-1})

2.5.1.3 Biomass yield: The biomass yield (Y) describes the mass of bacteria produced per mass of substrate utilised. Y depends on a number of factors, including the oxidation state of the substrate and nutrients, the degree of polymerisation of the substrate, metabolic pathways used, the growth rate and reactor configuration. From an economic point of view, biomass yield is an important consideration for sewage treatment plants. Since one of the purposes of AD is to reduce the mass of sludge produced for disposal, it is important that the yield be maintained as low as possible. Since the quantity of new cells produced is reproducible for a given substrate, equation 2.16 describes the relationship between the rate of substrate utilisation and rate of growth (Metcalf & Eddy, 1991).

$$r_g = -Y r_{su} \qquad \text{(eq. 2. 16)}$$

Where;

Y = Biomass yield coefficient (mg.mg^{-1})

r_{su} = Substrate utilisation rate (mg.l^{-1}.d)

Substituting the value of r_g from equation 2.14 into equation 2.16 gives equation 2.17 for the rate of substrate utilisation.

$$r_{su} = -\frac{\mu_{max} XS}{Y(K_s + S)} \qquad \text{(eq. 2. 17)}$$

The effects of decay rate on the net microbial yield under steady state conditions are

accounted for by defining the observed biomass yield (Y_0) from equation 2.18 (Borja *et al.*, 1995).

$$Y_0 = \frac{Y}{1+(b \cdot SRT)} \qquad \text{(eq. 2. 18)}$$

Where;

Y_0 = Observed biomass yield constant

Y = Biomass yield constant

b = Cell decay rate (d^{-1})

SRT = Solids Retention Time (days)

It can be seen from equation 2.18 that Y_0 decreases with increasing SRT or, since SRT= 1/dilution rate, Y_0 decreases with decreasing dilution rate.

When sulfate reduction is to be taken into account, where both sulfate and an electron donor are used as substrates, equation 2.19 may be used specifically to describe SRB specific growth rate (Omil *et al.*, 1998):

$$\mu_{,SRB} = \mu_{\max,SRB} \frac{S}{K_{s,SRB}+S} \times \frac{SO_4^{2-}}{K_{SO_4^{2-},SRB}+SO_4^{2-}} \qquad \text{(eq. 2. 19)}$$

Where;

$\mu_{,SRB}$ = Specific biomass growth rate of SRB (d^{-1})

$\mu_{max,SRB}$ = Maximum specific biomass growth rate of SRB (d^{-1})

S = Substrate concentration ($mg.l^{-1}$)

SO_4^{2-} = Sulfate concentration ($mg.l^{-1}$)

$K_{s,SRB}$ = Half saturation constant of SRB ($mg.l^{-1}$) for substrate (S) or sulfate (SO_4^{2-})

2.5.2 Substrate Concentration And Type In The Feed

Gupta *et al.* (1994), investigated 3 CSTR's fed synthetic nutrient solution substituted with methanol, acetate and formate as substrates. Methanol and acetate were used

since they are important precursors to methanogenesis, while formate was chosen as a substitute for H_2 since both have similar $\Delta G^{0'}$/reaction. In the formate-fed reactor with excess sulfate, sulfidogenesis did not totally out-compete methanogenesis, suggesting that HMPB can compete well with hydrogenotrophic SRB (HSRB) at elevated sulfate concentrations. Overall, the authors cite reactor kinetics as the primary mechanism determining microbial speciation. In a study by Raskin *et al.* (1996) who looked at population structures in a fixed-bed biolfilm reactor, the major competitors for H_2 in the presence of sulfate have been shown to be *Methanomicrobiales* and *Methanococcales*, as opposed to *Methanobacteriales* when sulfate is limited.

From a thermodynamic and kinetic point of view, it is predicted that acetotrophic SRB (ASRB) will outcompete AMPB for acetate if excess sulfate is present (Widdel, 1988). Gupta *et al.* (1994) found this to be true where sulfate reduction totally outcompeted methanogenesis in acetate-fed chemostats, since the calculated K_s from batch tests showed that it is lower for SRB than for MPB's. In contrast, according to Ueki *et al.* (1992) who investigated the relationship between methanogenesis and sulfate reduction in AD of municipal sewage sludge, the addition of acetate markedly enhanced methanogenesis, but did not affect sulfate reduction. Also, since the degradation of propionate seemed to depend on sulfate reduction, acetate accumulated in response to the propionate degradation when methanogenesis was inhibited. This study showed that acetate is used only during methanogenesis. Thus, the role of SRB here was to degrade propionate to acetate and enhance electron flow to methanogenesis. These results also corroborate those of Polprasert and Haas (1995) who looked at the competition between SRB and MPB's for acetate in semi-continuous lab-scale reactors. The authors concluded that acetate was not a major substrate for sulfate reduction, although some SRB species were able to utilise it. This might be attributed to the fact that the MPB's specialising in acetate degradation of the genus *Methanosaeta* have a high affinity for acetate with a K_s of only 50 mg/l (Pavlostathis and Giraldo-Gomez, 1991). Fukui *et al.* (2000) looked at hydrogen, acetate and propionate uptake in the presence of sulfate, and with the addition of the specific inhibitors sodium molybdate and chloroform to inhibit SRB's and MPB's, respectively. Digested municipal anaerobic sludge was used as a seed source in

small-scale serum bottle tests. Upon MPB inhibition, propionate, butyrate, acetate and H_2 all accumulated in the bottles. On the other hand, the inhibition of SRB caused no accumulation of hydrogen, but accumulation of propionate. It was concluded that the inhibition of HMBP increased the partial pressure of H_2 in the sludge, thus inhibiting syntrophic AB that normally scavenge acetate through to butyrate. One discrepancy in this research is that it was not made clear why propionate accumulated in this case, since it is expected that SRB should be responsible for propionate uptake. The standard Gibbs free energy ($\Delta G^{0'}$) for propionate uptake by SRB is –37.7kJ (McCarty and Smith, 1986), making the overall reaction favourable thermodynamically even in the presence of high H_2 partial pressures. However, the authors did not attempt to explain this point. A similar experiment by Ueki and Ueki (1989) on municipal sewage sludge showed that in the presence of sulfate and chloroform to inhibit MPB's, acetate increased while propionate, butyrate and H_2 did not, which contradicts the previous research concerning propionate accumulation. In the study by Fukui *et al.* (2000), competition for acetate was apparent by SRB in some tests, but only when the COD:SO_4 ratio was at its lowest, and even then contributed to only 20% of the total acetate oxidation.

O'Flaherty *et al.* (1998) found that propionate degradation by a completely methanogenic suspended sludge had a lag phase of 360 days, showing the incapacity of AB's to degrade propionate. On addition of sulfate, the propionate degradation rate increased rapidly, with some acetate build-up. Contrary to this finding, Fang *et al.* (1995) found the opposite to be true. Such differences can only be attributed to differences in reactor and substrate type, nutrient availability, or even the presence of residual sulfate which may have inadvertently encouraged growth of propionate-oxidising SRB in the latter study. Although it has been reported that acetate degradation is the rate-limiting step in methanogenic reactors, where approximately 70% of methane comes from acetate cleavage, the presence of propionate can slow this process down substantially. Propionate oxidation was also shown to be the rate limiting step during sulfide inhibition of AD in a sulfate-fed reactor (Rinzema and Lettinga, 1988b). As for competition between SRB and MPB for butyrate in anaerobic digesters, limited literature had been published on this topic. SRB derived from natural habitats such as marine sediments and freshwater aquifers are known to

harbour butyrate-degrading species. A characterisation of the SRB population in a PHC-contaminated freshwater aquifer amended with specific low-molecular weight VFA's found that Desulfobacter-like species proliferated upon butyrate addition (Kleikemper *et al.*, 2002). With respect to anaerobic digesters, a study by Mizuno and Noike (1994) showed how the COD:SO_4 ratio affected the interaction between MPB's and SRB's with butyrate as the substrate. When the COD:SO_4 ratio was 6.0 or more, methane production was the predominant reaction, and over 80% of the total electron flow was used by MPB's. At a COD:SO_4 ratio of 1.5, SRB utilised over 50% of the total electron flow. A large amount of sulfate reduction (COD:SO_4 ratio <6) resulted in a decrease of methane production coupled with a rapid increase in bacterial growth. The COD:SO_4 ratio also affected the degradation pathway of butyrate and the composition of bacterial populations in the sludge. During low sulfate conditions, butyrate was degraded to acetate and hydrogen by AB, and subsequently to methane by AMPB and HMPB, whereas in sulfate-rich conditions, butyrate was first degraded into acetate by SRB, and the resulting acetate was then degraded by AMPB and SRB. A similar conclusion was arrived at by Omil *et al.*, (1996) who found that in a sulfate-rich granular sludge reactor, the use of an acetate-rich substrate favoured AMPB, while a propionate- and butyrate-rich feed favoured SRB.

2.5.3 Sulfate Concentration In The Feed

It was shown by Visser *et al.* (1993a), that in 3 UASB reactors fed synthetic nutrients and acetate, propionate and butyrate individually as substrates at COD:SO_4 ratios of 10.39:1, 3.62:1 and 0.6:1, the amount of COD removed via sulfate reduction was 5%, 17% and 21%, respectively. The corresponding sulfate removal efficiency was 81%, 90% and 18%, respectively, showing that the full potential for COD removal by sulfidogenesis was never reached at any of the sulfate concentrations used. During small-scale activity tests, it was shown that acetate was degraded at the same rate, irrespective of sludge source and sulfate presence or absence, indicating that ASRB are of less importance, and that AMPB were able to compete effectively with them. For propionate, the presence of sulfate stimulated a higher sludge activity than without sulfate, and removing sulfate from the activity tests with sludges from the low COD:SO_4 ratio reactors very much lowered the propionate utilisation rate. This shows the insignificance of propionate-degrading syntrophic acidogens in the sludge,

where propionate degradation was primarily performed by SRB. In the case of butyrate, degradation rates in the activity tests were always higher in the presence of sulfate, but when compared to activities in the reactors, butyrate degradation was still higher in the absence of sulfate. This may indicate that syntrophic, butyrate-degrading acidogens were still present in high numbers at high sulfate concentrations.

Mizuno and Noike (1998) showed that in small scale 1L CSTR's treating sucrose at various HRT's and sulfate concentrations, short HRT's (2-4 hrs) with sulfate deprivation inhibited H_2 utilisation due to the restrictively low HRT for MPB and lack of sulfate for SRB. As the HRT was increased (6-8 hrs), H_2 was subsequently converted to methane only. At a low HRT with high sulfate concentrations, H_2 was predominantly consumed by SRB and as the HRT was increased, there was competition between MPB and SRB for H_2, with the outcome depending on sulfate concentration. HSRB outcompeted HMPB provided that sulfate was present in high enough concentrations (Omil *et al.,* 1997; Rinzema and Lettinga, 1988b; Visser *et al.,* 1993a). This can be explained by the fact that HSRB obtain more energy from, and have a higher affinity for, H_2 than HMPB. This conclusion is in contrast to a study by Gupta *et al.* (1994) which showed that in a formate-fed CSTR fed excess SO_4^{2-}, HMPB competed well with HSRB. Such discrepancies may be attributed to differences in reactor and substrate type, and natural selection of micro-organisms. Battacharaya *et al.* (1996) studied the effects of feed acetate to sulfate ratio on the interaction between SRB and MPB in batch and continuous systems. The reaction for acetate utilisation by SRB was derived by combining a series of Gibbs free energy half reactions ($G^{0'}$) for acetate as the donor, SO_4 and CO_2 as electron acceptors and NH_4^+ as the nitrogen source to give equation 2.20 below:

$$CH_3COO^- + 0.9216\ SO_4^{2-} + 1.4008H^+ + 0.0312NH_4^+ \rightarrow$$

$$0.0312C_5H_7O_2N + 0.9688H_2O + 0.8744\ CO_2 + 0.9688HCO_3^- + 0.4608H_2S + 0.4608HS^- \qquad \text{(eq. 2. 20)}$$

This equation shows that SRB require an Acetate:SO_4 ratio of 0.677 mg/mg (MW acetate/MW sulfate x 0.9216) in the feed for complete utilisation of acetate. A ratio exceeding this would make the system sulfate limited. Results from serum bottle tests performed on SRB-only seed sludge confirmed this ratio quite accurately (measured

ratio was 0.664). However, during chemostat tests of 2L size CSTR's containing mixed SRB and MPB cultures fed higher acetate concentrations (>1000 mg/l compared to 332 mg/l for serum bottle tests), the maximum utilisation by SRB at the acetate:SO_4 ratio of 0.667 was only 40%, showing the inability of either organism to out-compete the other at high acetate concentrations. However, the outcome of competion may have been greatly influenced by the length that this experiment was run (< 100 days). It has been shown that it may take SRB several hundred days to predominate in a sulfate-fed reactor. Omil *et al.* (1998) demonstrated that in a UASB reactor fed acetate and excess sulfate at COD:SO_4 ratios lower than 0.67, between 250-400 days were required before ASRB increased their acetate-uptake contribution from 50-90%. Visser *et al*, (1993a) calculated that, assuming a biomass start-ratio of MB to SRB of $1x10^6$, over 500 days would pass before SRB species became dominant in a suspended growth reactor fed acetate running at an HRT of 50 days. In a full-scale upflow packed reactor treating citric acid waste at a COD:SO_4 ratio of 3:1, SRB failed to outcompete MPB, butyrate and ethanol utilising syntrophs over a 5 year period of the reactor lifetime (O'Flaherty *et al.*, 1998). The authors in the afore mentioned studies have attributed the lack of SRB dominance to their less favourable μ_{max} and K_s values than their competing counterparts. In light of this, it is possible to say that the relatively short-term length of the project may have prevented the sufficent development of a substantial SRB population able to fully contribute to COD destruction.

2.5.4 Sulfide Inhibition

H_2S produced by bacteria will either be released as gaseous H_2S, or will dissolve in water as undissociated H_2S. Depending on the pH, this dissolved sulfide can further dissociate in two steps, according to equations 2.21and 2.22 (Sarner, 1990).

$$H_2S \Leftrightarrow HS^- + H^+ \qquad \log Ka_1 = 10^{-6.88} \qquad \text{(eq. 2. 21)}$$

$$HS^- \Leftrightarrow S^{2-} + H^+ \qquad \log Ka_1 = 10^{-12.2} \text{ to } 10^{-17.1} \qquad \text{(eq. 2. 22)}$$

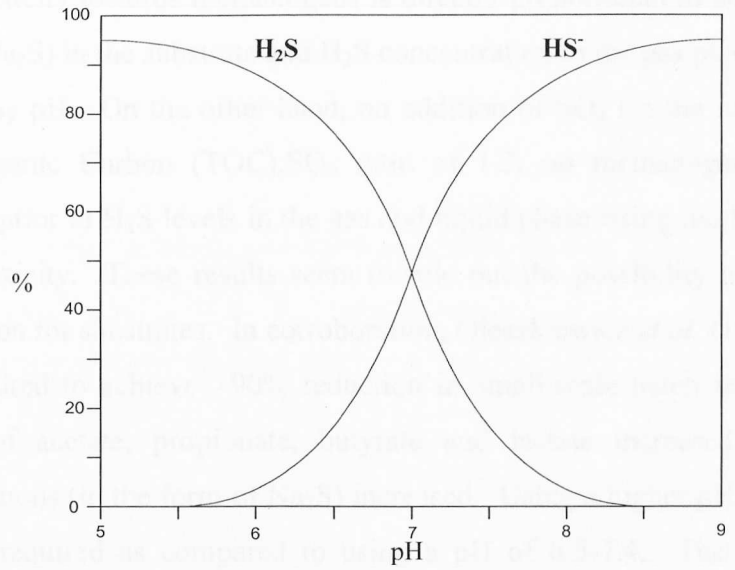


Figure 2. 4- Dependency of distribution between HS^- and H_2S on pH (Sarner, 1990).

Due to the pH dependency of the second equilibrium (eq. 2.22) it can be neglected, since no S^{2-} will appear at the pH at which AD is performed (Sarner, 1990). The distribution between HS^- and H_2S as in equation 2.21 is also pH dependent, and this relationship is illustrated in Figure 2.4. The dissociation equilibrium between H_2S and HS^- is very important in AD since unionised toxic H_2S can pass through cell membranes by diffusion much more easily than ionised HS^- (Speece, 1996). Hence, it is important to try to control the pH of the sludge to force the equilibrium towards the latter. Decreasing the partial pressure in the gas phase by increasing biogas production will force dissolved H_2S from the liquid phase to the gas phase (Sarner, 1990). Several studies have been performed which report various levels and types of sulfide which will cause inhibition of MPB's and SRB's, and Table 2.3 is a summary of some findings. Generally speaking, a free H_2S concentration higher than 150mg/l can cause process failure due to toxicity (Hulshoff Pol *et al.,* 1998). Consequently, wastewaters containing COD:SO_4 ratios lower than 10 require the incorporation of sulfide removal to prevent toxicity (Rinzema and Lettinga, 1988a).

Karhadkar *et al.* (1987) tried to determine whether competition for substrates between SRB and MPB, and/or the toxicity due to sulfide were responsible for methanogenic inhibition. They concluded from results performed in small-scale batch tests that

sulfide toxicity towards methanogens is directly proportional to added sulfide (in the form of Na_2S) in the substrate and H_2S concentration in the gas phase, which in turn is affected by pH. On the other hand, on addition of SO_4 (in the form of K_2SO_4) at a Total Organic Carbon (TOC):SO_4 ratio of 1.3, no methanogenic inhibition was observed prior to H_2S levels in the gas and liquid phase rising too high and exhibiting sulfide toxicity. These results seem to rule out the possibility of inhibition due to competition for substrates. In corroboration, Oleszkiewicz *et al.* (1989) found that the time required to achieve >90% reduction in small-scale batch tests (160 ml serum bottles) of acetate, propionate, butyrate and lactate increased as Total Sulfide concentrations (in the form of Na_2S) increased. Using a higher pH of 7.7-7.9 reduced the time required as compared to using a pH of 6.5-7.4. The authors concluded therefore, that unionised free H_2S was the main toxic agent, but without actually verifying this with HS^-/H_2S measurements (but rather calculated these from dissociation constants). Overall, the order of inhibition to Total Sulfide was propionate, acetate, butyrate and lactate, respectively. It also seemed that syntrophic bacteria were less susceptible to sulfide inhibition than MPB, and that the toxicity threshold was similar to SRB (O'Flaherty *et al.,* 1998). Any sulfide inhibition between pH 6.8 and 7.2 was shown to be related to the undissociated H_2S concentration, whereas above pH 7.2, inhibition was related to total sulfide concentration. Of the two possibilites, the threshold for 50% inhibition was much lower for undissociated H_2S than for total H_2S, indicating that in fact the latter was the more toxic form.

2.5.5 Reactor Type

The type of reactor used to treat sulfate-laden wastewaters will have a significant effect on the outcome of competition between SRB and MPB. To represent the outcome of competition between MPB and SRB in a dispersed growth reactor, an excellent study by Raskin *et al.* (1996) will be cited. In a completely mixed, fixed-bed biofilm reactor fed glucose as the sole carbon source, rRNA probing was able to identify the population structure in the biofilms. In a methanogenic reactor deprived of sulfate, SRB still comprised a significant fraction of the total population. This was attributed to the ability of SRB to grow syntrophically on lactate, ethanol, propionate, fumarate and pyruvate, and that syntrophy with HMPB eliminates the need for SRB to

reduce sulfate. Upon addition of sulfate, some or all SRB converted to using sulfate as their electron acceptor, accompanied by a rapid increase in *Desulfovibrio* and *Desulfobacterium* spp. populations (HSRB).

Table 2. 3- H_2S inhibition of MPB and SRB (Speece, 1996).

MPB Inhibition	SRB Inhibition	General Inhibition
• 50% inhibition of acetoclastic methanogens under very high COD and sulfate loading rates of 1200 mg/l H_2S (Isa *et al.*, 1986). • 50% inhibition of acetoclastic MPB by 250 mg/l H_2S (Koster *et al.*, 1986). • 50% inhibition of MPB by 100-224 mg/l total sulfide levels (Karhadkar *et al.*, 1987). • 5% H_2S in the head gas for MPB (Karhadkar *et al.*, 1987). • 100 mg/l for MPB (Oleszkiewicz *et al.*, 1989). • 240 mg/l for MPB (McCartney and Oleszkiewics, 1991).	• Acetate-fed systems SRB affected by H_2S toxicity before MPB (Maillacheruvu *et al.*, 1993; McCartney and Oleszkiewics, 1991). • No Inhibition of SRB (which were more sensitive than MPB) by 178 mg/L H_2S (Uberoi and Battacharaya, 1995). • 83 mg/l for SRB (McCartney and Oleszkiewics, 1993). • Propionate accumulation, with SRB apparently responsible for its utilisation at over 100 mg/l H_2S (McCartney and Oleszkiewics, 1991) • 2% H_2S in the headspace shut down a completely sulfidogenic batch reactor (Hobson *et al.*, 1994).	• No inhibition with acetate and propionate feed up to approximately 200 mg/l H_2S in annaerobic filter. (Maillacheruvu *et al.*, 1993). • No propionate inhibition regardless of total sulfide up to 100 mg/l and over 100 mg/l sharp drop in propionate metabolism (Rinzema and Lettinga, 1988a). • Three times as much inhibition of acetate and butyrate by 20-300 mg/l H_2S vs. propionate utilisation (McCartney and Oleszkiewics., 1991). • Process failure point in acetate-and propionate-fed chemostats 60-70 mg/l H_2S (Parkin *et al.*, 1990). • 60-75 mg/l for acetate and propionate in CSTR (Maillacheruvu *et al.*, 1993). • 100-150 mg/l for lactate and glucose in CSTR (Maillacheruvu *et al.*, 1993)

A slow but significant decrease in the population of *Methanobacteriales* and *Methanosarcinales* was also observed, but this mismatched the faster rate of decrease in methane production, showing the ability of these populations to maintain a high ribosome content for a longer period than necessary. However, the population of H_2-consuming *Methanococcales* and *Methanomicrobiales* remained constant, showing them to be the strongest competitors for H_2 against the SRB. Overall, SRB were shown to be more competitive than methanogens in sulfate-rich environments in such

a reactor. In contrast, Santegoeds *et al.* (1999) looked at the distribution of SRB and MPB in granules derived from three UASB reactors, one completely methanogenic, one methanogenic-sulfidogenic and the third completely sulfidogenic. It was shown that in the methanogenic and methanogenic-sulfidogenic aggregates, sulfide production was restricted to the outer layer with methane being produced from deeper towards the centre, and this activity matched well with the spatial distribution of the SRB and MPB populations. Between these two bands was a layer of syntrophic bacteria comprised of *Syntrophobacter* spp. offering both SRB and MPB a nutritional advantage by providing both with H_2 and acetate. In the sulfidogenic aggregates, no methane production was detected, even though the core did contain MPB surrounded by syntrophic populations, again showing the ability of MPB to retain their ribosomial activity under periods of starvation. This distribution contrasts with the thermodynamic expectations where SRB are expected to outcompete MPB and form the core of the aggregate. It was suggested that due to their better attachment characteristics (Isa *et al.* 1986), the initial aggregate may consist of MPB as the primary inoculum and these are then colonised by SRB. Interestingly, the aggregates derived from the sulfidogenic reactor lacked a population of *Desulfovibrio* spp. and *Desulfobulbus* spp., which were otherwise present in the methanogenic reactors. This is in contrast to the research by Raskin *et al.* (1996) which showed a rapid increase in the population of *Desulfovibrio* spp. upon sulfate addition. The more nutritionally-versatile *Desulfosarcina* and *Desulfococcus* species were predominant instead. This may be due to the difference in the type of HSRB predominating in this instance, which was not detectable by the rRNA probes used. In summary, with attached-growth or granule reactors, mass transfer limitations, microbial colonisation and adhesion properties seem to be more important in determining the outcome of competition than in dispersed growth systems where kinetics and thermodynamics are the dominant mechanisms controlling competition (Raskin *et al.*, 1996).

2.5.6 pH, Temperature And SRT

The various micro-organisms in an anaerobic sludge all have different pH and temperature optima. Since the growth rates of SRB and MPB are similar over the pH range of 7.0 to 7.5, other factors such as sulfate concentration, substrate affinity, sulfide inhibition and microbial content of the seed sludge will determine the outcome

of competition. From a purely physiological point of view, it is expected that below a pH of 7.0 MPB's would outcompete SRB's due to the more favourable conditions, whereas at elevated pH's (greater than 7.5), SRB's would overtake (Widdel, 1988). In fact, the growth kinetics of pure and mixed MPB and SRB cultures over the pH range of 6.8-9 on various substrates (acetate, propionate, butyrate, ethanol and lactate) show little variation in substrate affinities of the micro-organisms (O'Flaherty *et al.,* 1998).

The AD process is also sensitive to temperature fluctuations, so that a decrease in temperature may accompany an increase in VFA concentration as the metabolism rate of fermentative bacteria is reduced. Another factor increasing digester sensitivity to temperature is the Organic Loading Rate (OLR), since a combination of high substrate concentration and low rate of metabolism can lead to digester failure. On the other hand, thermophilic conditions (55-65°C) in anaerobic digesters will usually increase metabolic rates by 50-100% above the mesophilic range, but lead to a much lower net yield of biomass. The effects of non-mesophilic conditions on the competition between SRB and MPB for substrates have been examined, and various studies have been performed at psychrophilic and thermophilic conditions. Rebac *et al.* (1996) looked at the effect of sulfate on propionate and butyrate degradation in a psychrophilic anaerobic expanded granular sludge bed (EGSB) reactor. Under these conditions, sulfate seemed to enhance the degradation of propionate by about 35% compared to control bottles. In contrast, the degradation of butyrate remained unaffected, indicating that acidogenic butyrate oxidisers can compete well with SRB. It was also calculated that the resulting acetate from propionate and butyrate degradation was not used by SRB but by MPB. A summary of the various advantages and disadvantages of psychrophilic and thermophilic digestion can be found in Speece (1996).

Short–term low temperature shocks (15 and 13°C) have been used to try to alter the competition between SRB and MPB in a UASB reactor. Results indicated that there was no significant change in competition, although a slight decrease in COD (acetate, propionate, sulfate) removal efficiency was observed (Omil *et al.,* 1997). Rintala and Lettinga (1992) showed the effect of high temperatures on the competition between SRB and MPB for hydrogen, acetate and propionate in small-scale bioassays and 1.2L UASB reactors. The research concluded that there was a significant change from

methane production to sulfate reduction of acetate as the temperature of the reactor was changed from mesophilic (37°C) to thermophilic (55°C), and seemed to be dependent on seed sludge acclimatisation and kinetic factors. In terms of propionate degradation, conclusions could not be drawn due to differences in results between the small–scale serum bottle tests and the UASB reactors. Visser *et al.* (1993b) used high temperature short-term shocks to mesophilic systems, and observed a decrease in methanogenic sludge activity at temperatures between 55-65°C.

With respect to the effect of SRT on the competition between SRB and MPB for substrate, 2L CSTR's fed acetate and propionate, each run at HRT's of 15, 25 and 40 days showed no significant difference in effluent H_2S, dissolved sulfide and sulfate reduction rates between the different SRT's (Parkin *et al.,* 1990).

2.5.7 Summary Of Competition Between SRB And MPB

The factors which have been mentioned such as temperature, pH, sulfate concentration etc. will act together to give a unique set of conditions for each experiment. This inevitably leads to variable outcomes for the competition between different sets of organisms, in particular when concerned with kinetic values such as μ_{max}, K_s and Y on certain substrates. However, certain trends can be identified despite differences between the studies.

Direct competition between SRB and MPB seems to focus on the final product of digestion; H_2/CO_2. Even at high COD:SO_4 ratios, complete suppression of SRB activity is difficult to achieve since hydrogen oxidation by HMPB is easily out-competed by HSRB. On the other hand, achieving completely sulfidogenic conditions at low COD:SO_4 ratios is also difficult since HMPB can compete very well with HSRB (Oude Elferink *et al.*, 1994). With short chain VFA's, ASRB should be able to outcompete AMPB for acetate based on thermodynamic considerations. The evidence from the various studies reviewed in Section 2.5 has shown that this is rarely the case, and that ASRB cannot compete effectively against AMPB. Nevertheless, ASRB have been found to be able to compete to a limited degree, especially if acetate levels are very high and sulfate is not limited. SRB in sulfate-rich anaerobic environments, therefore, seem not to be in direct competition with MPB. It is known that MPB's

cannot oxidise propionate directly (Widdel, 1988). Instead, AB assume this role, and in fact up to 15% of methane produced in mesophilic anaerobic reactors may be derived from propionate degradation (Gujer and Zehnder, 1983). Although AB are primarily responsible for propionate degradation, and completely methanogenic reactors are able to treat propionate as a sole carbon source successfully (Fang and Chui, 1995), SRB are predicted to predominate due to their higher maximum specific growth rates in sulfate-rich environments (μ_{max} 0.89 d^{-1} for *Desulfolobus propionicus* and 0.1-0.2 d^{-1} for *Syntrophobacter wolinii* – Rinzema and Lettinga., 1988b). Since propionate degradation in methanogenic sludges is reliant on low H_2 partial pressures, sulfidogenic propionate degradation, which is much less dependent on low H_2, seems more reliable and less susceptible to organic shock-loads. Longer chain VFA's such as butyrate have not been identified as significant substrates for SRB in anaerobic reactors, although the literature available in this area is limited. Of the physical factors discussed such as sulfide toxicity, temperature, pH and SRT, all appear to be minor contributors to the end result of competition compared to reactor configuration.

The superior attachment ability of MPB's compared to SRB's to form granules or biofilms in certain reactor configurations may protect them against toxicity due to mass transfer limitations, and also help prevent their washout. Overall, in dispersed-growth reactors, substrate affinity and COD:SO_4 ratio's seem to be the main parameters controling competition. One of the principal roles of SRB during sulfidogenic anaerobic sludge digestion has been consistently identified as H_2/CO_2 oxidation in competition with HMPB as well as propionate degradation in competition with AB, with a minor role for ASRB for acetate in competition with AMPB.

2.6 SULFIDE OXIDATION

On the oxidative side of the sulfur cycle, reduced inorganic sulfur compounds serve as electron donors for anaerobic phototrophic bacteria or provide energy for the growth of colourless sulfur bacteria. The latter are subdivided into five families: Thiobacteriaceae, Beggiatoaceae, Achromaticeae, Sulfolobus and Thiodendron (Keunen, 1989). 'Colourless sulfur bacteria have been found growing at pH 9.0 and

pH 1.0, at 4°C and at 95°C, and at dissolved oxygen concentrations ranging from air-saturation to anaerobic levels' (Robertson and Kuenen, 1992). This statement emphasises the diversity and tolerance levels of these bacteria.

Of interest in this project are the genus *Thiobacilli* within the Thiobacteriaceae family. They can thrive under highly acidic environments at pH 1-2. At such low pH's, most heavy metals in a sludge will solubilise, and can thus be removed by sulfide precipitation. The mesophilic Thiobacilli species which can tolerate such pH's are *T. thiooxidans*, *T. albertis, T. ferrooxidans* and *T. acidophilus.* The former three species consist of obligate chemolithoautotrophs (require an inorganic sulfur source for energy, and obtain their cell carbon from the fixation of CO_2), while *T. acidophilus* is a facultative autotroph (can grow chemolithoautotrophically or heterotrophically with complex organic compounds providing both carbon and energy) (Robertson and Kuenen, 1992). Table 2.4 illustrates the oxidation reactions of inorganic sulfur compounds by Thiobacilli and other colourless sulfur bacteria.

Table 2. 4- Oxidation of sulfur and its compounds (Kuenen, 1989).

Sulfide
$H_2S + 2O_2 \rightarrow H_2SO_4$
$2H_2S + O_2 \rightarrow 2S + 2H_2O$
$5H_2S + 8KNO_3 \rightarrow 4K_2SO_4 + H_2SO_4 + 4N_2 + 4H_2O$
Elemental Sulfur
$2S° + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$
$5S° + 6KNO_3 + 2H_2O \rightarrow 3K_2SO_4 + 2H_2SO_4 + 3N_2$
Sulf-oxy anions
$Na_2S_2O_3 + 2O_2 + H_2O \rightarrow Na_2SO_4 + H_2SO_4$
$4Na_2S_2O_3 + O_2 + 2H_2O \rightarrow 2Na_2S_4O_6 + 4NaOH$
$2Na_2S_4O_6 + 7O_2 + 6H_2O \rightarrow 2Na_2SO_4 + 6H_2SO_4$
$5\ Na_2S_2O_3 + 8NaNO_3 + H_2O \rightarrow 9Na_2SO_4 + H_2SO_4 + 4N_2$

The biochemical pathways by which these species oxidise sulfur compounds are not yet fully understood (Kelly *et al.*, 1989), and are beyond the scope of this project. Most species of *Thiobacillus* precipitate elemental sulfur extracellularly during

growth on sulfur compounds. This is most likely the consequence of conversion of these compounds at the periplasmic space on the surface of a cell, to elemental sulfur. Catalysis occurs by enzyme systems located in or external to the cell membrane (Kelly *et al.,* 1989).

2.7 PREVIOUS INVESTIGATIONS INTO SULFUR TECHNOLOGIES

Traditionally, anaerobic treatment of sulfate-rich wastewaters has been avoided due to the production of H_2S gas. H_2S is not only malodorous in low concentrations, but concentrations of 600 ppm can be fatal in 30 minutes, while 1,000 ppm (0.1%) can be fatal in only 2 to 3 minutes. H_2S also contributes to effluent COD when in the aqueous state. On oxidation to sulfuric acid (either microbiologically- or chemically-mediated), it can cause corrosion-damage to pipes.

There are physical and chemical means of removing H_2S from effluents (ion-exchange resins, absorption into aqueous/organic solvents, chemical oxidation), but these methods usually transfer the pollution from one waste stream to another, are expensive and may not be 100% efficient. Recently, a number of biotechnologies have been developed in order to take advantage of the diversity of states in which sulfur compounds can occur, and hence their oxidising or reducing capabilities. Table 2.5 is a summary of these technologies, which include the removal of organic COD, sulfur, nitrogen and heavy metals. Treatment of sulfate-containing wastewaters has so far mainly focused on the treatment of landfill leachate (Henry and Prasad, 2000), mine water drainage (Maree and Strydom, 1985; Maree and Strydom, 1987) spent sulfuric acid (Stucki *et al.,* 1993) and industrial waste waters (Sarner, 1990) from the pulp and paper industries. Commercially, a process similar to the Sulfate Digestion process does currently exist known as the THIOPAQ® process, although its purpose is to treat sulfate-rich leachate or industrial waters. In a first reactor, sulfate is reduced biologically to sulfide, with ethanol or hydrogen acting as the electron donor at an HRT of 4 hrs. Depending on the waste stream, dissolved sulfide is then used to precipitate out heavy metals as insoluble metal sulfides. In a second fixed-film submerged reactor with an HRT of 0.5 hrs, sulfide is oxidised back to S^0. Removal

efficiencies of 99% for heavy metals and 85% for sulfate were attained (Buisemann, 1996; Sarner, 1990).

Table 2. 5- Applications of the sulfur cycle in environmental biotechnology (Lens and Hulshoff Pol, 1999).

Application	Sulfur Conversion Utilised	Typical Waste Stream
Wastewater Treatment		
➢ Removal of oxidised sulfurous compounds (sulfate, sulfite and thiosulfate)	➢ SO_4^{2-} and/or SO_3^{2-} reduction + partial S^{2-} oxidation to S^0	➢ Industrial wastewaters, acid mine drainage and spent sulfuric acid
➢ Sulfide removal	➢ Partial S^{2-} oxidation to S^0	➢ Industrial wastewaters
➢ Heavy metal removal	➢ SO_4^{2-} reduction	➢ Extensive treatment in wetlands or anaerobic ponds; high rate reactors for process water, acid mine drainage and ground water
➢ Nitrogen removal (denitrification)	➢ S^{2-}, S^0 and $S_2O_3^{2-}$ oxidation	➢ Domestic wastewater, agricultural run-off
➢ Removal of xenobiotics	➢ SO_4^{2-} reduction	➢ Wastewaters from textile, paper and explosives manufacturing
➢ Microaerobic treatment	➢ Internal sulfur cycle in a biofilm	➢ Domestic sewage
Off-gas Treatment		
➢ Biofiltration of gases	➢ Oxidation of S^{2-} and organo-sulfur compounds	➢ Biogas, malodourous gases from composting and farming
➢ Treatment of scrubbing waters	➢ SO_4^{2-} and/or $S_2O_3^{2-}$ reduction + partial S^{2-} oxidation to S^0	➢ Scrubbing waters of SO^{2-} rich gases
Solid waste treatment		
➢ Bioleaching of metals	➢ S^{2-} oxidation	➢ Sewage sludge, compost
➢ Desulfurisation	➢ Organo-sulfur oxidation	➢ Waste rubber
➢ Gypsum processing	➢ SO_4^{2-} reduction	➢ Waste gypsum depots
Treatment of soils and sediments		
➢ Bioleaching of metals	➢ S^{2-} oxidation	➢ Dredged sediments and spoils
➢ Phytoextraction	➢ SO_4^{2-} uptake by plants	➢ Dredged sediments and spoils
➢ Degradation of xenobiotics	➢ SO_4^{2-} reduction	➢ PCB contaminated soil slurries

2.7.1 Research Into The H_2S Generator

The H_2S Generator described in Section 1.1.2 on which this project focuses has the possibility of using readily available sulfuric acid to disinfect and remove heavy metals from sewage sludge (rather than dilute wastewaters) which in itself acts as a

cheap and abundant carbon source. Research into this area was originally encouraged in 1950's during a serious shortage of sulfur in the UK. The idea was to microbiologically reduce oxidised sources of sulfur such as sulfate (which was available in large quantities and at low cost) into sulfide and subsequently oxidise the H_2S into sulfur. The most suitable organic material available in terms of quantity and cost was sewage sludge (Butlin *et al.*, 1956). Studies were in fact performed by Butlin *et al.* (1956; 1960) to investigate and optimise sulfur production from municipal sewage sludge using calcium sulfate as a source of sulfur.

Later, similar research was carried out by Ueki *et al.* (1988) who looked more closely at the substrate distribution between MPB and SRB fed on municipal sewage sludge supplemented with sodium sulfate. By a series of inhibition tests, the research concluded that MPB mainly utilised acetate, whereas SRB degraded longer-chain fatty acids and possibly propionate so that during municipal sludge digestion, the two processes of methanogenesis and sulfidogenesis did not compete but shared the role of hydrogen-scavenger. More recently, pilot studies were carried out to investigate complete and hybrid sulfidogenesis of sewage sludge (Hobson *et al.*, 1994). The production of H_2S was initiated by seeding the reactors with a source of SRB. Domestic sewage sludge was anaerobically digested and run in a purely sulfidogenic reactor.

Metcalf and Eddy (1979) showed sludge to have the approximate formula: $CH_{1.9}O_{0.3}N_{0.24}$. From this equation, the oxygen needed to break down the sludge to CO_2 and H_2O was calculated and provided by sulfate in the form of sulfuric acid or calcium sulfate (eq. 2.23) (Kolmert *et al.*, 1997).

$$H_2SO_4 + 1.75\ CH_{1.9}O_{0.3}N_{0.24} \rightarrow H_2S + 1.75CO_2 + 1.03H_2O + 0.42\ NH_3 \qquad \text{(eq. 2. 23)}$$

Four 20L digesters were set up; the first two digesters (A, B) were run together as purely sulfidogenic, while the others (C, D) were set up as a hybrid reactor (semi-methanogenic semi-sulfidogenic), and purely methanogenic, respectively. The source of sulfate for the first digester (Digester A) was calcium sulfate only. The second reactor (Digester B) was fed a mixture of calcium sulfate and sulfuric acid, with the

latter predominating at a maximum of 84% of the total. Both digesters were started as methanogenic digesters, and when stable, were converted to purely sulfate digesters. In Digester A, the hydraulic retention time ranged from 20-70 days, with the estimated amount of calcium sulfate to digest the sludge being dosed. A gas re-circulation system was installed, whereby the gas produced was stripped of H_2S and then re-circulated back to the bottom of the digester to strip out any remaining CO_2 and H_2S. It was observed that decreasing the hydraulic retention time from 70 to 28 days, and increasing the gas re-circulation rate to 250 ml/minute caused peak values of H_2S to be produced. The theoretical destruction of volatile solids was calculated to be 40%. Assuming this, H_2S yields were estimated to be 0.35 g/g Volatile Suspended Solids (VSS) from the equation 2.23 above, and actual yields matched quite closely.

As for Digester B, the rate of acid dosing was gradually increased over a 30-day period until the input reached the estimated amount required to digest the sludge. This, however, caused the pH of the sludge to fall below 7 if no action was taken, and digestion would cease. Hence, the dose was reduced to less than the theoretical requirement, which decreased volatile solids (VS) destruction to between 15% and 40%, while the calcium sulfate input was kept constant. H_2S production rose to 0.35-0.45 g/g as the sulfuric acid dose was increased, equivalent to a maximum of 8 g/d. This was achieved when the HRT was reduced to 22 days. The carbon balance performed on the reactor showed that inputs and outputs were equal. Digester C, which was run as a hybrid sulfidogenic/methanogenic reactor was compared in performance to the other 3 reactors. The hybrid reactor was regularly dosed with sulfuric acid as a sulfate source, and had an HRT of 12 days as a minimum. The hybrid and purely methanogenic digesters achieved similar VS destruction percentages. However, when compared to the purely sulfidogenic reactors, it was observed that the hybrid reactor achieved a greater level of destruction than in the purely sulfidogenic reactors, and achieved this in a shorter HRT. Since the work of Hobson *et al.* (1994), an attempt was made to start off a reactor as purely sulfidogenic by Forrest (1999). The reactor was not seeded with a SRB source, but instead regularly fed with calcium sulfate. Even after several months, no SRB were established, hence no significant H_2S was produced.

2.7.2 Investigations Into Sludge/Sulfuric Acid Reactors

2.7.2.1 Metal solubilisation: Acidification experiments conducted on waste activated sludge showed that solubilisation rates and ultimate removal efficiencies for all metals increase with time and decreasing pH and solids concentrations (Wozniak and Huang, 1984). Limitations did exist regardless of the length of acidification, with maximum solubilisation achieved after 12 hours. The following order of removal efficiency compares results obtained for different pH's in a sludge with 2.5% solids (ibid):

pH 1.5 Zn > Cd = Cu = Ni >Pd = Cr
pH 2.0 Zn > Cd > Ni > Cu > Pb > Cr
pH 3.0 Zn = Ni > Cd > Cr = Pb > Cu

An investigation into the effect of sludge solids concentration (range 8-30 g/L) on metal solubilisation efficiency found that average solubilisation rates were inversely proportional to sludge solids concentration. In addition, the fertiliser value of the sludge remained intact, with no significant reduction in nitrogen or phosphorus. There was, however, a significant decrease in Total Suspended Solids (TSS) and VSS for all sludge solid concentrations (Tyagi *et al.,* 1997). Jenkins *et al.* (1981) compared sulfuric acid to a strong chelating agent, ethylenediaminetetracetic acid (EDTA) as a means of effecting metal solubilisation in various types of sludges (primary, waste activated). Results for acid dosing showed that Cu was the most difficult metal to solubilise, with only 20% of the theoretical total possible solubilised at pH 2. The authors suggested that the reason for this was the high affinity of copper to form tightly bound organic complexes. Increasing dosing time from 1 hour to 24 hours did not have a significant effect on copper, but did increase the solubility of Cd by 6 fold, and Pb by 3 fold. Removal efficiencies for Fe, Ni, Zn and Cr were over 75% after 24 hours of treatment at pH 2. As for solubilisation due to EDTA addition, the authors found that metal removals did not correspond to theoretical solubilisation constants, or show any similarity to solubilities effected by acid dosing. However, they did not speculate on the reasons for this. A drawback to acid dosing of raw sludge is that the authors estimated that 0.5 metric tons of acid would be required per dry metric ton of sludge for any significant metal solubilisation extent, increasing costs, reactor size due to dilution, and sludge-dewatering requirements.

In studies performed by Blais *et al.* (1992a), sludge of varying solids concentration was subjected to acid treatment and two microbial leaching processes, then compared. Acid treatment consisted of dosing the sludge with sulfuric acid, while microbial leaching consisted of using *T. thiooxidans* fed elemental sulfur, and iron oxidising *T. ferrooxidans* fed on ferrous sulfate. It was found that for an undigested sludge with a concentration of approximately 1 0g/l, sulfuric acid dosing lowered the pH of the sludge the most to 1.47, compared to 1.77 and 2.74 for microbial leaching with *T. thiooxidans* and *T. ferrooxidans*, respectively. On the other hand, comparing mean metal solubilisation yields for all sludges tested (variable TS), the use of sulfur-oxidising bacteria allowed for a better solubilisation for all the metals tested (Al, Cd, Cr, Cu, Mn, Ni, Pb, Zn) than acid dosing. However, the use of iron-oxidising bacteria achieved better solubilisation than acid dosing for only half of the metals tested (Cd, Cu, Mn, Zn). The authors speculated that the longer leaching time needed for microbial leaching (5 days for *T.thiooxidans* and 10 days for *T.ferrooxidans*) than acid leaching (1 day) could explain the higher efficiencies of metal solubility achieved by the former. Later research showed that acclimatising *T.ferrooxidans* already in the sludge achieved satisfactory metal leaching levels (Tyagi *et al.,* 1992).

2.7.2.2 Pathogen removal: Investigations into pathogen removal by acid treatment were also performed by Blais *et al.* (1992b; 1997). Pathogen destruction was achieved by in-situ acid production by a mixture of indigenous *Thiobacillus* strains fed powdered sulfur or sulfur tablets (the former was found to be more efficiently utilised), and different sludge concentrations were used. It was concluded that sludge concentration was not a significant factor for growth of the Thiobacilli, since for all sludge concentrations the same pH was reached after 3 days. Overall, a pH of < 2.7 was required to destroy coliforms, and faecal streptococci destruction occurred after 2 days (pH > 2.7). In comparison, Wong and Henry, (1985) who used *T. ferrooxidans* and ferrous sulfate as a substrate to treat anaerobically digested sludges found that high concentrations of suspended solids inhibited pathogen destruction.

2.7.3 Investigations Into H_2S Bioscrubber Technology

In this project, the purpose of a bioscrubber was to capture H_2S gas produced by the H_2S Generator, and then oxidise it to sulfuric acid by sulfur oxidising bacteria of the genus *Thiobacillus*, as illustrated in Table 2.4. This technology has already been investigated by various authors, culminating in the THIOPAQ®Scrubber technology from PAQUES Bio Systems BV of the Netherlands.

2.7.3.1 The THIOPAQ®Scrubber: The process consists of several steps; first, H_2S is passed into an alkaline liquid (pH 8-9) in a counter-current mode, where the gas is absorbed according to equation 2.24 (Lens and Hulshoff Pol, 2000):

$$H_2S_{(gas)} + OH^- \rightarrow HS^-_{(liquid)} + H_2O \qquad \text{(eq. 2. 24)}$$

The aqueous solution is then passed into an air lean bioscrubber containing *Thiobacilli* bacteria which are attached to a matrix and fed carbon dioxide as their carbon source to oxidise the sulfide into elemental sulfur. Since the sulfur is hydrophilic in nature, clogging problems are reduced (eq. 2.25):

$$HS^- + 0.5O_2 \rightarrow S^\circ + OH^- \qquad \text{(eq. 2. 25)}$$

An advantage of this process is that the OH^- ions are recycled. Full oxidation of the sulfide can also be performed in air-rich conditions, where sulfuric acid is the end result (eq. 2.26):

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \qquad \text{(eq. 2. 26)}$$

Currently, there are 20 units of the Paques Bio System in operation around the world. In Eerbeek, the Netherlands, where the technology was tested on a paper industry wastewater generating H_2S, the bioscrubber reduced output H_2S gas concentration from up to 35,000ppm to 10ppm (Janssen, 1999). Figure 2.5 shows an example of such an installation.

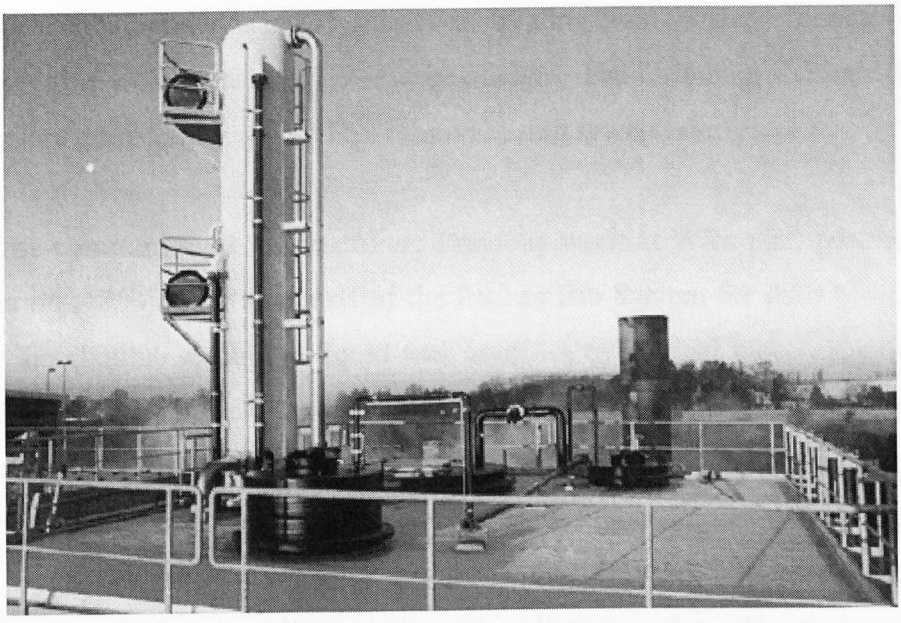


Figure 2. 5- An example of a THIOPAQ®Scrubber installation (Paques, 2003)

2.7.3.2 Biogas desulfurisation using iron ferrate: An alternative approach was carried out by Guoqiang *et al.* (1994), where a pure culture of *Thiobacillus ferrooxidans* was used. Ferric (Fe^{3+}) sulfate was used to absorb biogas, subsequently oxidising the sulfide to elemental sulfur, and reducing the ferrate to ferrous (Fe^{2+}) sulfate (eq. 2.27):

$$H_2S + Fe_2(SO_4)_3 + H_2S \rightarrow 2FeSO_4 + H_2SO_4 + S° \qquad \text{(eq. 2. 27)}$$

The ferrous sulfate was then passed onto the packed column bioreactor, where it is re-oxidised to its ferric state by *T. ferrooxidans* (eq. 2.28):

$$2FeSO_4 + 0.5O_2 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O \qquad \text{(eq. 2. 28)}$$

Thus the iron sulfate is recycled, and H_2S removed. The disadvantage of this technology is the need to use an external oxidising agent (ferric sulfate) for H_2S removal (although continuously recycled).

The above technologies are mainly aimed at treating waste-water effluents such as leachate or mine waters, rather than sewage sludge. The following sections describe previous work done to investigate H_2S removal using sewage sludge.

2.7.3.3 One-compartment bioscrubber: Previous work at WRc plc., performed by Hobson *et al.* (1994), used the basis of the Paques Bio System for their bioscrubber. However, no alkaline scrubbing liquid was used. A cylindrical bioscrubber with an approximate 2L capacity containing packing material similar to Raschig rings was employed. H_2S entering the bioscrubber was absorbed by effluent from a secondary sewage treatment plant. This was used as the bioscrubber liquor to keep the media wet and to provide nutrients for the bacteria. The sulfuric acid produced by the bacteria was washed out with the bioscrubber liquor, and a pH of about 1 was maintained by replacing this liquor with fresh secondary effluent. Figure 2.6 illustrates this apparatus.

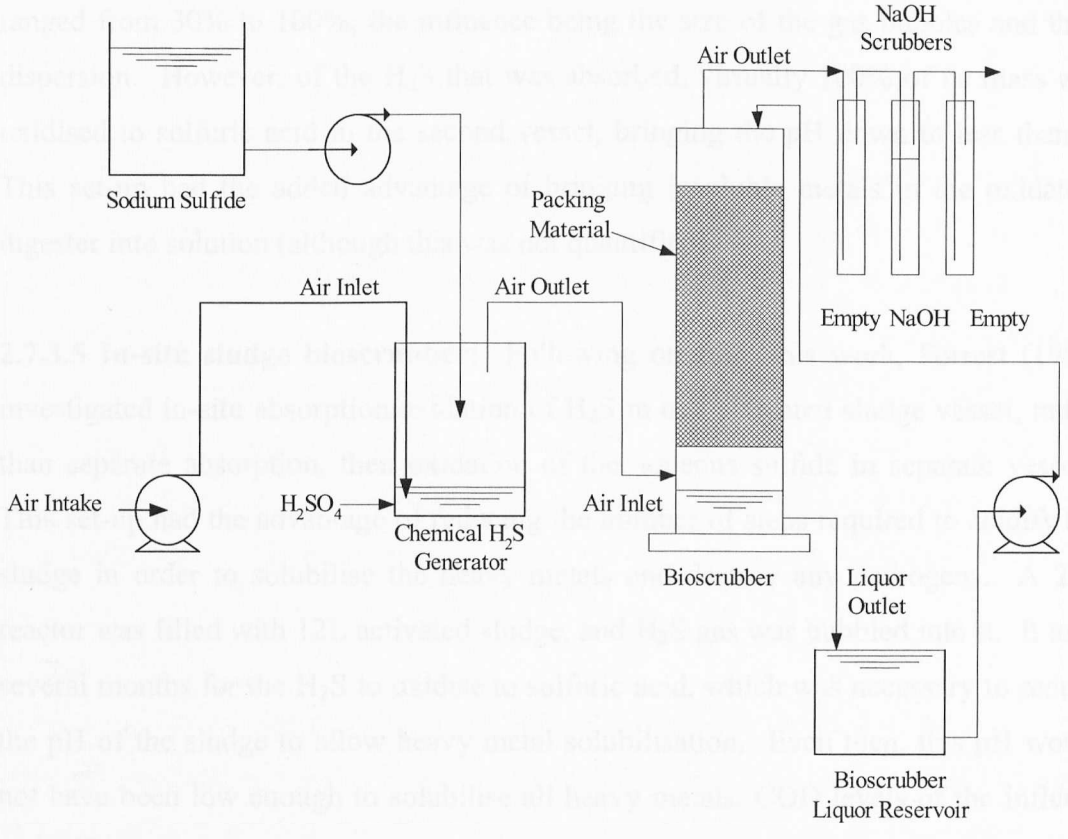


Figure 2. 6- One-compartment Bioscrubber.

Results for this set-up were encouraging, and H_2S removal ranged from 63% to 100%. The disadvantages were that the equipment was prone to breakdown due to blockage of the holes in the packing support plate, rendering the bioscrubber unworkable for more than 2 weeks at a time. Since acid produced at this stage would be added to the Sludge/Sulfuric Acid Reactor to solubilise heavy metals, this would increasing the potential plant size due to dilution effects of the acid on sludge. In the overall system, the scrubbed, H_2S-free gas would eventually be recirculated back to the anaerobic H_2S Generator in order to increase gas-stripping efficiency. This recirculated gas in this arrangement would contain oxygen due to the aeration of the bioscrubber, and will disrupt AD.

2.7.3.4 Sludge absorption/oxidation bioscrubber: An alternative study was carried out at a later date by Hobson *et al.* (1994) to investigate other methods of sulfide oxidation. This consisted of two separate digesters, where one 5L activated sludge vessel absorbed the H_2S, while another 10L oxidation digester was used to oxidise the absorbed H_2S to sulfuric acid. The amount of H_2S actually absorbed in the first vessel ranged from 30% to 100%, the influence being the size of the gas bubbles and their dispersion. However, of the H_2S that was absorbed, virtually 100% of its mass was oxidised to sulfuric acid in the second vessel, bringing the pH down to less than 2. This set-up had the added advantage of bringing insoluble metals in the oxidation digester into solution (although this was not quantified).

2.7.3.5 In-situ sludge bioscrubber: Following on from this work, Forrest (1999) investigated in-situ absorption/oxidation of H_2S in one activated sludge vessel, rather than separate absorption, then oxidation of the aqueous sulfide in separate vessels. This set-up had the advantage of reducing the number of steps required to acidify the sludge in order to solubilise the heavy metals and destroy any pathogens. A 20L reactor was filled with 12L activated sludge, and H_2S gas was bubbled into it. It took several months for the H_2S to oxidise to sulfuric acid, which was necessary to reduce the pH of the sludge to allow heavy metal solubilisation. Even then, this pH would not have been low enough to solubilise all heavy metals. COD levels of the influent and effluent remained constant, suggesting no reduction in the carbon content of the sludge. However, once the reactor stabilised, the H_2S was oxidised to elemental

sulfur (either microbially- or chemically-mediated) but no further, resulting in an accumulation of total solids in the effluent, with no acid generation.

2.8 SUMMARY

This chapter covered the principles of conventional AD, including the different stages involved and types of AD reactors that exist commercially to take advantage of this microbial function. A description of sulfate reduction in anaerobic environments by SRB and their role in enhancing electron flow during digestion was also carried out. Although many physical factors interact to influence the outcome of competition between SRB and MPB, a comparison of the various studies that have looked at sulfate reduction in anaerobic digesters allowed several conclusions to be drawn. In sulfate-rich anaerobic environments, the evidence points to the fact that SRB and MPB may only be in direct competition for H_2/CO_2. For acetate, it has been shown that ASRB rarely outcompete AMPB due to the lower K_s of the former, and the main competition for higher VFA's such as propionate and butyrate lies between AB and SRB.

Although there have been many studies in the field of sulfate reduction, most organic substrates used consisted of low molecular weight VFA's such as acetate or single organic compounds such as ethanol. The studies which had used more complex wastewaters, such as sewage sludge, were interested in looking solely at parameters which affected the competition between SRB and MPB. The effects of sulfidogenic conditions (with sulfuric acid as a sulfate source, instead of sulfate salts as in previous studies) on sludge digestion and stabilisation had not been considered except as part of a preliminary study by Hobson *et al.* (1994) who developed the Sulfate Digestion idea.

Since a CSTR configuration is to be used in this project, the lack of support media prevents one type of microorganism to be favoured over the other due to attachment. In mesophilic anaerobic digesters, physical characteristics such as temperature, pH, SRT and sulfide toxicity seem to have similar effects on SRB and MPB, which means it is conceivable to expect the competition to be controlled mainly by kinetic

phenomena (μ_{max}, K_s), which in turn are strongly influenced by COD:SO_4 levels. From these conclusions, several objectives were defined for this project. Since completely sulfidogenic conditions were shown to have a detrimental effect on sludge digestion efficiency, the first objective was to look at the viability of running a semi-methanogenic semi- sulfidogenic reactor, which produced enough H_2S to recycle through the 3-stage Sulfate Digestion process. The use of sulfuric acid with the sludge feed as a sulfate source has implications regarding the pH drop in the anaerobic reactor, so that the limitations must also be defined. The effect on sludge digestion efficiency is of primary importance in order to give an accurate assessment of the feasibility of the Sulfate Digestion process. Hand in hand is the understanding of the kinetics involved which, as mentioned earlier, is controlled by the sulfate concentration in the feed, hence varying the COD:SO_4 ratio is another objective. The kinetic data collected would also help in future modelling of sulfidogenic AD.

The literature review also covered the principles of sulfide oxidation and the technology that has evolved around this concept. It was seen that *Thiobacilli* involved in sulfide oxidation are versatile in nature when considering environmental conditions such as pH and temperature. From experiences of Paques Bio Systems B.V, it is also expected that the substrate demands should be limited to only CO_2 as carbon source, and H_2S as energy source, due to the obligate chemolithoautotrophic nature of the most likely *Thiobacilli* species to develop in the bioscrubber environment. However, all the technologies described above require air circulation to maintain aerobic conditions and allow the full oxidation of H_2S to S^0 and then to H_2SO_4. In this project, since it would be necessary to re-circulate the biogas back to the H_2S Generator once it has been scrubbed of sulfide (to act as a purge gas), connecting the biogas line to an aerobic bioscrubber would cause air to enter the H_2S Generator and inhibit it. As a result, a new design to cater for the anaerobic needs of the H_2S Generator is necessary.

2.9 RESEARCH OBJECTIVES

1) To determine the extent of sulfate reduction required in an H_2S Generator in order for a sufficient amount of sulfur to be recycled through the three stages of the Sulfate

Digestion process. Initially, sodium sulfate will be used rather than sulfuric acid in order to start-up the H_2S Generator without compromising pH integrity as a result of acid addition. The effect of hybrid sulfate digestion on the effluent sludge quality compared to conventional anaerobic treatment will also be investigated;

2) To investigate the effect of using sulfuric acid as a source of sulfate in an H_2S Generator. This is the ultimate aim of the project since sulfuric acid must be used to solubilise heavy metals from the raw primary sludge. Lab-scale reactors will be run at different COD:SO_4 ratios, and the sludge treatment efficiency observed to see whether there is a difference in digestion efficiency using sulfate as an acid rather than a salt;

3) To build a profile of the microbial kinetics involved during sulfate digestion compared to conventional anaerobic methanogenic digestion, including areas such as hydrolysis, sulfidogenesis and methanogenesis using small-scale batch tests with seed sludge derived from large-scale reactors running at various COD:SO_4 ratios;

4) To design, build and set-up an H_2S Bioscrubber that can be connected to a H_2S Generator without compromising the anaerobic conditions in the latter reactor. The sulfide oxidation efficiency of the bioscrubber will also be investigated.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION
3.2 REACTOR DESIGN
3.3 EXPERIMENTAL SET-UP
3.4 ANALYTICAL METHODS
3.5 STATISTICAL ANALYISIS OF DATA
3.6 SOFTWARE FOR KINETIC-DATA ANALYISIS

3.1 INTRODUCTION

The reactors used in this project, feed constituents and start-up procedures are described, and analytical methods explained. The data handling methods are also demonstrated. A large part of this project involved setting up, preparing and maintaining previously idle analytical instruments for gas chromatography. The methods used, mechanical adaptations made and analytical protocols devised for each instrument are explained in the relevant sections.

3.2 REACTOR DESIGN

Six CSTR's labelled M, S, A, B, C and D were used in this project. The main body of each reactor consisted of a glass jar of 8L capacity (2L headspace volume) for Reactors M, S and A, and 5L capacity (1L headspace volume) for reactors B, C and D. The reactors all had four ground glass inlets circled around one central inlet for the stirrer, all measuring 19/26 mm (internal/external diameter). In order to prevent air from entering the reactors via the stirrer inlet and during feeding and extraction of sludge, a draft tube was fitted to these inlets. This consisted of inflexible rubber tubing pushed through the inlet. The outer surface of the tube and the inner surface of the inlet were sealed together with silicon gel so that the tube extended from the top of

the inlet down below the sludge level. A natural seal was therefore created between the surface of the sludge in the tube and external air. A vacuum pump was used for extraction and feeding, and the effluent tested immediately for various parameters before further biochemical reactions could take place. Flexible narrow tubing ran from a third inlet from the CSTR to the bottom of an acidified, water-filled gas collection column placed in an open 40L container. Acidified water was displaced from the column into the container as gas was collected. For Reactor S, A, B, C and D, a chemical H_2S scrubber was fitted to the fourth inlet, and this is described in section 3.4.2. A fifth inlet for all reactors was stoppered with a rubber septum from which biogas samples could be extracted via a syringe and analysed. All reactors were placed in a water bath and kept at a constant temperature of 34°C +/- 1°C.

3.3 EXPERIMENTAL SET-UP

At the start of this project, two reactors were in operation: Reactors M and S. These were started up from another 20L 'seed bank' CSTR running at a >100 day HRT and fed a mixture of primary and secondary sludge at ~2.5% TS. Reactors M and S were initially run as completely methanogenic reactors with HRT's of 40 days, and then gradually reduced to 20 days over a 4-month period. On reaching steady state (minimum three times the HRT), Reactor S was converted into a hybrid H_2S Generator, while Reactor M was maintained as a methanogenic reactor throughout the project to act as a control. In order to promote sulfidogenic conditions in Reactor S, 1L of seed sludge from an Anaerobic Baffled Reactor (ABR) treating tannery wastes high in sulfates was added (Freese, 2001). The mass of sodium sulfate required in the reactor was weighed out and supplemented with the feed, and the results for this part of the project are discussed in Chapter 4. Once hybrid conditions had been established and digestion efficiency investigated, the source of sulfate was changed to sulfuric acid and the reactor name changed to Reactor A to avoid confusion.

The purpose of using sulfuric acid was to mimic the original intentions of this project which were to acidify raw sludge in order to eliminate pathogens and reduce its metal content before being digested. The seed from Reactor A was used to start up reactors

B, C and D. All reactors were maintained at 20 day HRT throughout the project and their results are discussed in Chapter 5.

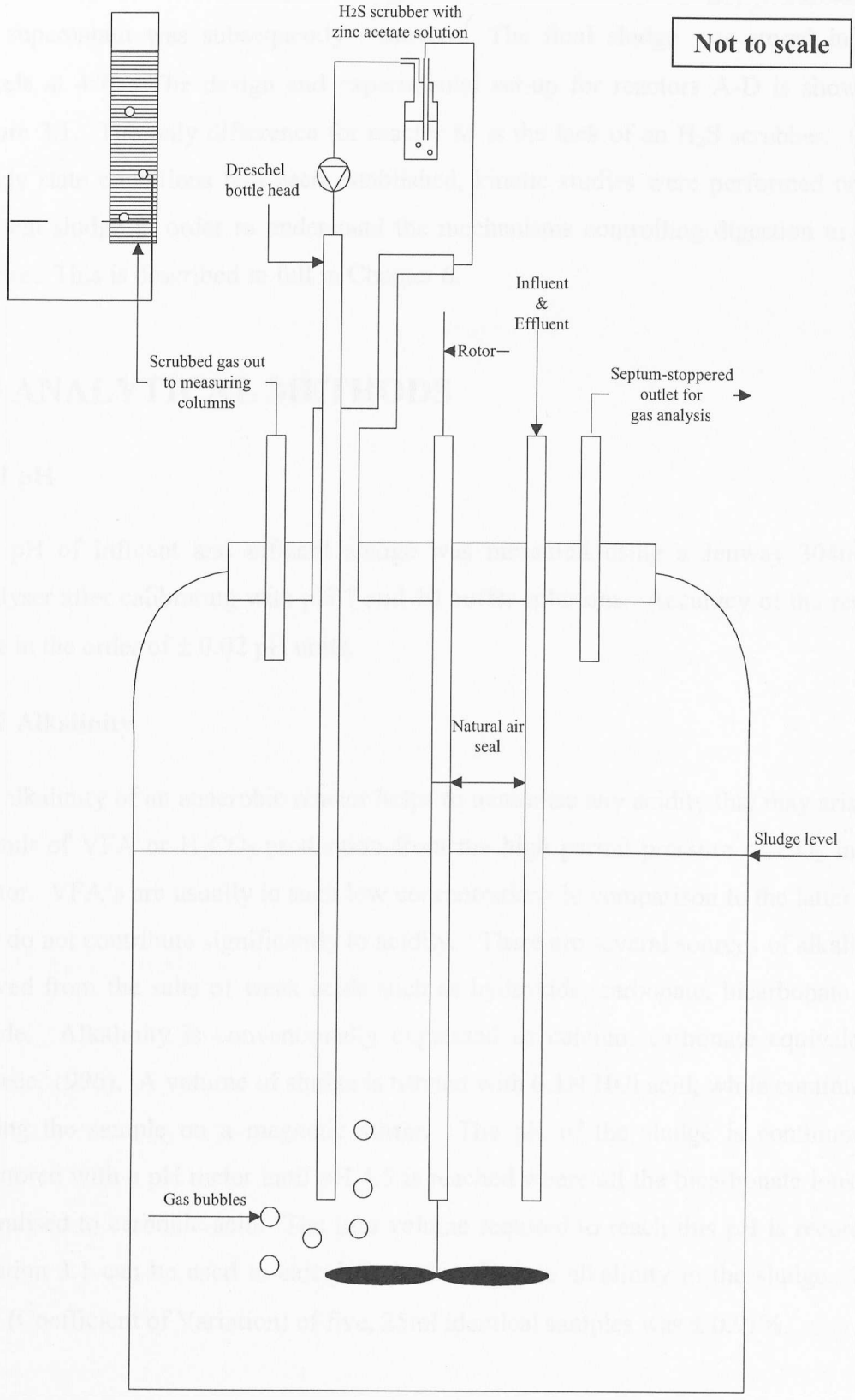


Figure 3. 0- Experimental layout for Reactors A-D.

Throughout the project, all six reactors were fed screened and settled primary sewage sludge originating from a local housing estate. It was thickened to the desired concentration (1.0-3% TS) by allowing the solids to settle over-night in barrels and the supernatant was subsequently removed. The final sludge was stored in 50L barrels at 4°C. The design and experimental set-up for reactors A-D is shown in Figure 3.1. The only difference for reactor M is the lack of an H_2S scrubber. Once steady state conditions had been established, kinetic studies were performed on the effluent sludge in order to understand the mechanisms controlling digestion in each reactor. This is described in full in Chapter 6.

3.4 ANALYTICAL METHODS

3.4.1 pH

The pH of influent and effluent sludge was measured using a Jenway 3040 Ion Analyser after calibrating with pH 7 and 10 buffer solutions. Accuracy of the results were in the order of ± 0.02 pH units.

3.4.2 Alkalinity

The alkalinity of an anaerobic reactor helps to neutralise any acidity that may arise as a result of VFA or H_2CO_3 production from the high partial pressure of CO_2 in the reactor. VFA's are usually in such low concentrations in comparison to the latter that they do not contribute significantly to acidity. There are several sources of alkalinity derived from the salts of weak acids such as hydroxide, carbonate, bicarbonate and sulfide. Alkalinity is conventionally expressed as calcium carbonate equivalence (Speece, 1996). A volume of sludge is titrated with 0.1N HCl acid, while continually stirring the sample on a magnetic stirrer. The pH of the sludge is continuously monitored with a pH meter until pH 4.5 is reached where all the bicarbonate ions are neutralised to carbonic acid. The titre volume required to reach this pH is recorded. Equation 3.1 can be used to calculate the bicarbonate alkalinity in the sludge. The CoV (Coefficient of Variation) of five, 25ml identical samples was ± 0.91%.

$$CaCO_3 \text{ equivalence (mg/L)} = \frac{AT \cdot N \cdot 50000}{S} \qquad \text{(eq. 3.1)}$$

Where;

AT = volume of acid titrated (ml)

N = Acid Normality

S = sludge sample size (ml)

3.4.3 Gas Volume

Biogas volume was measured by collecting the biogas produced from a reactor under acidified water containing methyl orange solution in order to indicate acidity. To calculate the equivalent biogas volume collected by water displaced in the water columns, equation 3.2 was used.

$$V = \frac{M \cdot a \cdot ((SAP - L) + M)}{SAP} \qquad \text{(eq. 3.2)}$$

Where;

V = Gas volume at atmospheric pressure and temperature (ml)

M = Water volume displaced (cm)

a = ml gas·cm column height^{-1} (manufacturers specifications) = 19.984

SAP = Standard Atmospheric Pressure in cm water gauge = 1029.38

L = Length of column (cm) = 84.62

3.4.4 Gravimetric Gaseous Sulfide Detection

A gravimetric method was employed to determine the mass of sulfide produced from the sulfidogenic reactors. A Dreschel bottle head was fitted to an inlet of reactors A-D so that the shank extended below the level of the sludge. The biogas from the reactor was passed through two consecutive 250ml Dreschel bottles containing saturated zinc acetate solution in deionised water to precipitate out the H_2S as zinc sulfide. The second Dreschel bottle down the line acted as a saturation indicator (turning cloudy when the first Dreschel bottle was fully saturated). The scrubbed biogas was then recirculated back below the sludge level to strip out more H_2S from the liquid. This system is an improvement on previous NaOH-containing gas

scrubbers used in a similar project (Hobson *et al.*, 1994) where absorption of CO_2 would have occurred. The zinc sulfide was collected and filtered through a dried and weighed 70mm diameter Whatman #1 filter paper under vacuum pressure. The filter paper and filtrate were subsequently dried in a microwave for 12 minutes under full power, left to cool and re-weighed. The mass of precipitated H_2S was calculated from equation 3.3. Five, 20ml identical samples derived from the same solution containing precipitated zinc sulfide had a COV of ±1.4%

$$(CH_3COO)_2\,Zn.2H_2O + H_2S \rightarrow ZnS + 2(CH_3COOH) + 2H_2O \qquad \text{(eq. 3.3)}$$

3.4.5 Colormetric Gaseous Sulfide Detection

Dräger Tubes were used to determine any residual concentration of H_2S in the reactor headspace. The tubes consist of sealed glass tubes between 10 and 20 cm long and 0.5 cm diameter. The tubes contains either Cu_2SO_4 for the detection of high H_2S concentrations (0.2-7%, manufacturers repeatability ± 5%), or lead acetate crystals for lower concentrations (0.2-2000ppm manufacturers repeatability ± 1%). The ends of the glass tube are broken off and inserted into a pump. The correct number of strokes are made to pass the correct volume of gas through the tube (typically 100 ml). The H_2S concentration in the sample is determined by the length of the discolouration through the tube after passing a known volume of biogas through it. The disadvantage of this method is that a relatively large volume of gas must be drawn through the tube, hence this method is not suitable for H_2S detection in gas samples below 100ml volume. Equation 3.4 was used to convert ppm values to mg/m^3:

$$mg/m^3 = \frac{P \cdot MW \cdot ppm \cdot 1000}{82.05 \cdot T} \qquad \text{(eq. 3.4)}$$

Where;

P = Pressure (atm)

MW = Molecular Weight of gas ($g.mol^{-1}$)

T = Absolute Temperature (°K)

82.05 = Molar Gas Constant ($J.(K.mol)^{-1}$)

Henry's Law can be used to predict the concentration of unionised hydrogen sulfide $[H_2S]_{(aq)}$ in solution depending on the gaseous sulfide concentration $[H_2S]_{(g)}$ since $H_2S_{(aq)}$ should be in equilibrium with the gas-phase H_2S (eq. 3.5). Henry's constant is highly dependent on temperature so that the lower the temperature the higher the constant (lower solubility) (Weber *et al.*, 1996);

$$[H_2S]_{(aq)} = \frac{P_{\{H_2S\}}}{\mathcal{K}_H} \qquad \text{(eq. 3.5)}$$

Where;

$[H_2S]_{(aq)}$ = concentration of H_2S in the aqueous phase in mol/m^3

$P_{\{H2S\}}$ = partial pressure of H_2S in atm

$\mathcal{K}_H$ = Henry's constant for H_2S (11.9×10^3 atm-m^3/mol at 35°C)

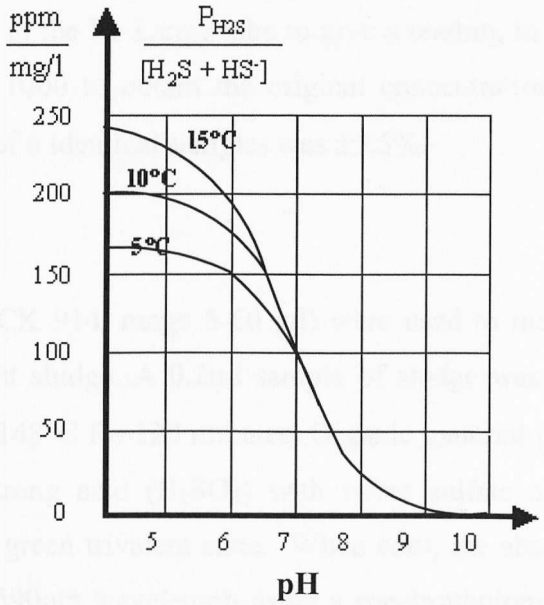


Figure 3. 1- Equilibrium H_2S concentration in the atmosphere per unit concentration in the water phase (Lens *et al.*, 2000).

Overall, the pH of solution will determine the fraction of unionised $H_2S_{(aq)}$. As the pH decreases, a higher proportion of the total sulfide will be in the $H_2S_{(aq)}$ form, which in turn will increase the concentration of $H_2S_{(g)}$. The solubility of $H_2S_{(g)}$ is also

determined by the temperature of the solution so that the lower the temperature, the higher the solubility; this relationship is clearly illustrated in Figure 3.2.

3.4.6 Total Dissolved Sulfide

Dr Lange tubes (LCW 053, range 0.1- 2.0 mg/l) were used to measure total dissolved sulfide in liquid samples. Hydrogen sulfide and acid-soluble metallic sulfides convert N,N-dimethyl-p-phenylenediamine to methylene blue in the presence of potassium dichromate as an oxidising agent. The intensity of colour as measured spectrophotometrically is directly proportional to sulfide concentration. The method advised by Dr Lange is for the detection of sulfide in clear liquid samples, hence it was necessary to adapt it for sulfide detection from silty sludge samples. A sample of sludge was collected via syringe (1ml) and injected into a stoppered glass 120ml McCarty bottle containing 19ml of 0.1M zinc acetate solution. This was to 'fix' the sulfide in the samples as zinc sulfide. 1ml of this solution was then diluted to 50ml with deionised water to reduce interference from solid particles, and 2ml of this final solution was tested in the Dr Lange tube to give a reading in mg/l. The final reading was multiplied by 1000 to obtain the original concentration of total sulfide in the sample. The CoV of 6 identical samples was ±5.5%.

3.4.7 COD

Dr Lange tubes (LCK 914, range 5-60 g/l) were used to measure total COD of the influent and effluent sludge. A 0.2ml sample of sludge was added to the Dr Lange tube and heated to 148°C for 120 minutes. Organic material is oxidised by potassium dichromate in a strong acid (H_2SO_4) with silver sulfate as catalyst; this reduces dichromate into its green trivalent state. When cool, the absorbency of the resulting solution is read at 590nm wavelength using a spectrophotometer. For soluble COD, LCK 514 Dr Lange tubes (range 100-2000mg/l) were used with 2ml samples. Prior to analysis, the sludge was centrifuged at 10,000rpm for 10 minutes, and filtered through a 0.45 µm disposable Nalgene^{TM} filter to remove any suspended matter. For effluent samples from the sulfide reactor, any dissolved sulfide can interfere with the COD measurement. Therefore, interference was removed by acidifying the bulk sludge with concentrated HCl and then purging with Nitrogen gas for 10 minutes to strip out

the sulfide gas before removing samples for analysis. The CoV of 8 identical samples was ±4.1%.

3.4.8. Sulfate

Dr Lange tubes (LCK153, range 40-150mg/l SO_4, LCK 353, range 150-900mg/l SO_4) were used to determine aqueous sulfate levels; the method is based on the precipitation of barium chloride as barium sulfate. The intensity of the resulting opaque white solution is measured spectrophotometrically at 420nm because the intensity is proportional to sulfate concentration. The sludge sample to be tested was centrifuged at 10,000 rpm for 10 minutes prior to sampling. The volume required was 5ml for the lower concentration, and 2ml for the higher concentration. The CoV's for the two ranges of 6 identical samples were ±4.0% and 3.4%, respectively. These tubes were used for routine analysis of SO_4 concentrations in the reactors. For the analysis of a larger numbers of samples during batch tests, or to detect for concentrations lower than 40mg/l, the method described in Standard Methods (APHA, 1990) was used and adapted to facilitate the analysis of a 3ml sample volume (rather than 100ml) so that the volume of buffer and sample was 0.6ml and 3ml, respectively. This method is based on the same principle used for the Dr Lange.

3.4.9 TS, VS, TSS and VSS

The TS, VS, TSS and VSS of influent and effluent sludge samples were determined according to that described in Standard Methods (APHA, 1990). Duplicate measurements were taken to minimise error. The CoV 6 identical samples was ±1% for all types of solids.

3.4.10 Particle Size Distribution (PSD)

A 500ml sample of sludge was passed through sieves of different sized apertures sitting one on top of the other in sequence (1.0ml, 0.850, 0.710, 0.600, 500, 0.425, 0.300, 0.180, 0.106, 0.075mm) with the largest on the top. A gentle jet of tap-water was used to wash down the sludge from one sieve to the next. The particles held in each sieve were collected in crucibles and TSS and VSS measured according to section 3.4.9.

3.4.11 Gas Analysis

A Perkin-Elmer 8700C Gas Chromatograph-Thermal Conductivity Detector (GC-TCD) was used to detect for CO_2, N_2 and CH_4 in the biogas. Two glass columns (1.5m long x 6mm o.d x 4mm i.d) were purchased (Analytical Columns, Croydon). One was packed with Molecular Sieve 5A (80-100 mesh size) and the other with Porapak S (80-100 mesh size). The column ends were attached to the injection ports by adapting the fittings, which were originally intended for capillary columns, via a series of reducers. High purity helium at a pressure of 50 PSI was used as carrier gas eliminating the need for any gas purifiers along the line. The velocity of the carrier gas through the columns was measured with a manometer at the waste end of the column and adjusted to 1 ml/second using the make-up and capillary modules at the front of the instrument. The injection port was fitted with a Valco 10-port multifunctional Air Actuated valve. It was believed that the valve was configured to be able to split the gas sample between the two columns hence able to detect not only for N_2, CO_2, CH_4 through the Molecular Sieve column, but also H_2S through the Porapak column. However, the configuration of the valve, as seen in Figure 3.3 shows that the gas sample is only passed through one column, while carrier is passed through the other. It was decided to use the Molecular Sieve column for N_2, CO_2, CH_4 analysis, while H_2S was analysed separately as described in section 3.4.4-3.4.6.

The oven, injector and detector temperatures were maintained at 45°C, 60°C and 125°C, respectively, throughout the run. The protocol for analysis can be found in Appendix A1. In order to calibrate the GC with the different gases, the calibration gas mixtures (Air Products plc) in Table 3.1 were used. The outlet valve of the gas cylinders were fitted with a rubber septum from which a 20 ml gas sample was collected in a glass syringe. The syringe needle was extracted and quickly stoppered with a rubber bung until ready for analysis. In order to dilute the calibration gas mixtures by 50%, the syringe was first filled with air to the 10ml mark and then injected into the septum of the gas cylinder and filled with the gas mixture to the 20 ml mark. N_2 concentration in the air was assumed to be 79.8%. The CoV of 10 identical samples was ±1% for all gases.

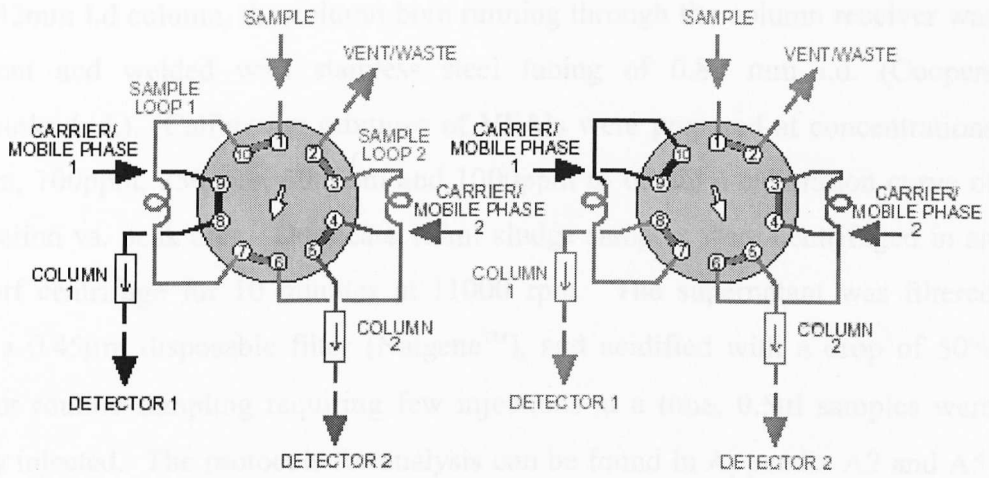


Figure 3. 2- Valco 10-port Air Actuation Valve (Vici, 2000)

Table 3. 1- Concentration of calibration gases used to calibrate the GC-HWD.

N_2 Conc. %	CO_2 Conc. %	CH_4 Conc. %
79.80	0.00	0.00
0.00	40.01	59.99
0.00	34.58	65.42
39.87	20.05	29.99
39.90	17.29	32.71

3.4.12 GC-FID For VFA Analysis

VFA's were measured using a Perkin-Elmer 8500C Gas Chromatograph-Flame Ionisation Detector (GC-FID) fitted with a DB-FFAP liquid phase 30m x 0.542 mm capillary column (J&W Capillary Columns) with hydrogen as carrier gas, and an air/hydrogen mix as combustion gas. Samples were introduced onto the capillary column by way of a split/splitless injector known as a Programmable Temperature Vaporiser (PTV). The PTV temperature was maintained at 340°C during sample analysis by passing filtered compressed air over the module at 60 PSI. The detector end of the column was originally designed to accept a 0.25mm i.d column. In order to

fit a 0.542mm i.d column, the column bore running through the column receiver was drilled out and welded with stainless steel tubing of 0.80 mm i.d. (Coopers Needleworks Ltd.). Calibration mixtures of VFA's were prepared at concentrations of 50ppm, 100ppm, 250ppm, 500ppm and 1000ppm to obtain a calibration curve of concentration vs. peak area. Duplicate 1.5ml sludge samples were centrifuged in an Eppendorf centrifuge for 10 minutes at 11000 rpm. The supernatant was filtered through a 0.45µm disposable filter (Nalgene™), and acidified with a drop of 50% HCl. For routine sampling requiring few injections at a time, 0.5µl samples were manually injected. The protocols for analysis can be found in Appendix A2 and A5. The CoV of 15 identical samples was ± 2.1%.

For the analysis of a large number of samples, an Air Actuated AS 8300 Autosampler with a 100-sample capacity was attached to the FID. Compressed air for the PTV and hydrogen gas for the column were divided and diverted to the autosampler to drive the air actuation, and carry the sample to the syringe needle, respectively. The air pressure was maintained at 50 PSI and H_2 pressure at 10 PSI. The original manual injection analysis protocol was adapted for the autosampler, and can be found in Appendix A3-A5. Any blockages that occurred down the line between the syringe needle and the sampling probe were removed by 'flushing' the system with a sample of concentrated methanol at a higher H_2 pressure (20 PSI). Failing that, a 1ml syringe with a wide bore needle was filled with methanol and gently forced down the end of the still-attached syringe needle so as to wash any particles backwards through the system and out through the sampling probe.

3.4.13 HPLC For VFA Analysis

Towards the end of the project, some samples were analysed with a Perkin Elmer series 4 High Pressure Liquid Chromatography (HPLC) fitted with an ISS-100 Autosampler. This was due to the failure of the GC-FID. The column consisted of an ion exchange resin (Biorad-Aminex) with 0.1M H_2SO_4 as carrier solvent; the flowrate was 0.5ml/min at 60°C. The components were detected using a Perkin Elmer LC 290 UV/VIS Spectrophotometer at 210nm, and peak areas calculated and printed out using a Philips Data acquisition system. The CoV of 10 identical samples was ±1.2%.

3.4.14 Anions And Cations

The concentrations of anions and cations were measured by ion chromatography using a Dionex DX-120 Ion Chromatograph. Anions were detected with an AS 144-mm Dionex ION PAC column (4x250mm) using 3.5mM Na_2CO_3 and 1.0mM $NaHCO_3$ as the mobile phase. Cations were detected with a CS12A Dionex ION PAC column (4x250mm) with 2mM methanesulphonic acid as the mobile phase. Flow rates of 1ml/min at ambient temperature using autosuppressed conductivity detection were used. A Dionex AS40 Autosampler was used. Calibration curves were constructed to determine concentrations. The CoV of 14 identical samples was ±0.8%.

3.4.15 Gram Staining Of Bacterial Samples

Bacteria fall into two groups according to their cell structure, which can be distinguished using Gram stain. The cell wall of both groups of bacteria contain a framework of meurin molecules consisting of parallel polysacharide chains, regularly cross-linked by short peptide chains. In Gram positive bacteria, the meurin is infilled with polysaccharide and proteins to form a thick rigid box, and will stain violet with Crystal Violet stain. Gram negative bacteria have thinner walls, where the meurin is coated with a smooth lipid-rich layer, which does not stain violet with Crystal Violet, but does stain red with Safronin O (Green *et al.*, 1993).

To prepare a bacterial slide, a drop of deionised water was placed in the centre of a glass slide and a heat-sterilised wire loop was used to transfer the desired microbial sample into the drop of water. The resulting sample was smeared over a 3x1 cm section of slide and allowed to air-dry for a few minutes. To fix the smear, the underside of the slide was quickly passed over a flame three times. Fixing causes the bacterial cytoplasm to coagulate and stick to the slide. To stain the bacteria, the slide was flooded with 0.5% w/v Crystal Violet solution and left for 30 seconds. The stain was washed off with Lugol's iodine, left for another 30 seconds, and then washed off with distilled water. A 50/50 acetone/ethanol solution was used to wash off excess colour (3 seconds) and immediately washed with water to prevent excessive decolourisation. Next, Safronin O was used to flood the slide, the stain left for 1 minute and then washed off with water. The stain was blotted with tissue paper and

covered with a cover-slip. A drop of immerison oil was placed on the cover slip and the slide examined under a light microscope (Zenith XSZ-G).

3.5 STATISTICAL ANALYISIS OF DATA

3.5.1 Standard Deviation

The Standard Deviation is a measure of the variance of a set of results from the mean. The formula for calculating Standard Deviation is equation 3.6.

$$SD = \sqrt{\frac{\sum_{i}^{n}(x_i - \bar{x})^2}{n-1}} \qquad \text{(eq. 3.6)}$$

Where;

SD = Standard Deviation

n = Sum of independent finite data points

xi = Data point

$\bar{x}$ = Mean of all data points

3.5.2 Student's *t*

Student's t is a statistical tool that can be used to compare results from different experiments. The t-test for the comparison of means is shown in equation 3.7.

$$t = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{s_{pooled}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \qquad \text{(eq. 3.7)}$$

Where;

t = Student's t

$$s_{pooled} = \sqrt{\frac{SD^2{}_1(n_1 - 1) + SD^2{}_2(n_2 - 1)}{n_1 + n_2 - 1}}$$

Here, s_{pooled} is a pooled standard deviation making use of both sets of data. The value for t from equation 3.16 is compared to t in a Student's t-table, which can be found in Appendix C. If the calculated t is greater than the tabulated t at the 95% confidence level, the two results are considered to be different.

3.5.3 Wilcoxon Signed-Rank Test

The Wilcoxon Signed-Rank test is a non-parametric alternative to the Students t-test for independent samples that are not normally distributed. The mechanism for calculating the significance of a difference between two sets of samples can be found under reference Vassar (2003).

The equations used to perform the Wilcoxon Signed-Rank test include equations 3.8 and 3.9:

$$SD_W = \sqrt{\frac{n(n+1)(2n+1)}{6}} \qquad \text{(eq. 3.8)}$$

and,

$$z = \frac{W - 0.5}{SD_w} \qquad \text{(eq. 3.9)}$$

Where;

W = Sum of signed ranks
n = Sum of independent finite data points
z = Critical value for defining the level of significance

The table of critical values for 'z' can be found in Appendix C. The significance test used in this project is directional rather than two-tailed non-directional.

3.5.4 Coefficient Of Variation

The coefficient of variation is a measure of relative dispersion of a set of results. It can be used to ascertain the accuracy of measurement of a group of identical samples (e.q 3.10).

$$CoV = 100\left(\frac{SD}{\bar{x}}\right) \qquad \text{(eq. 3.10)}$$

3.5.5 Propagation Of Uncertainty

For the addition and subtraction of uncertainty such as standard deviations, the absolute uncertainty is used as shown in equation 3.11.

$$x_4 = \sqrt{x^2_1 + x^2_2 + x^2_3} \qquad \text{(eq. 3.11)}$$

For the multiplication and division of uncertainty, the percent relative uncertainty is used (eq. 3.12):

$$x_4 = \sqrt{\%x^2_1 + \%x^2_2 + \%x^2_3} \qquad \text{(eq. 3.12)}$$

3.6 SOFTWARE FOR KINETIC-DATA ANALYISIS

NLREG is a statistical analysis program that performs linear and non-linear regression analysis, surface and curve fitting. NLREG determines the values of parameters for an equation whose form has been specified, and fits a set of data values to a set equation. It does not transform the function into a linear form, leading to more accurate results. The software is able to handle linear, polynomial, exponential, logistic, periodic, and general nonlinear functions. This programme was used to determine K_s and μ_{max} values from the experimental kinetic data obtained. See the web-page reference under Sherrod (1996) for more details of the software function. An explanation of how the data was entered into the programme, and how the results were interpreted can be found in Appendix D.

CHAPTER 4

START-UP OF A HYBRID H_2S GENERATOR[1] AND H_2S BIOSCRUBBER

4.1 INTRODUCTION

4.2 PREPARATORY STUDIES & RESULTS FOR H_2S GENERATION

4.3 H_2S BIOSCRUBBER DESIGN, OPERATION & RESULTS

4.4 DISCUSSION

4.5 SUMMARY

4.1 INTRODUCTION

As discussed in Section 2.7 of Chapter 2, previous investigations into sulfide generation in a completely sulfidogenic reactor yielded lower TS destruction levels than in a hybrid or methanogenic reactor. Moreover, a greater amount of hydrogen sulfide was produced than was required for the next stage of the process, the Sludge/Sulfuric Acid reactor (Hobson *et al.*, 1994). The amount of sulfate to be passed around the Sulfate Digestion system hinges on the amount of sulfuric acid required to bring the pH of raw primary sludge down to pH 1.5-2 in the Sludge/Sulfuric Acid Reactor. This low pH is necessary to solubilise heavy metals and destroy pathogens in the raw primary sludge. Therefore, a preliminary experiment was performed to establish the concentration of H_2SO_4 required, and this figure converted to sodium sulfate equivalent. Following on from this, a hybrid H_2S Generator was started-up (Reactor S) to investigate its sludge digestion efficiency compared to a conventional methanogenic reactor (Reactor M). Both reactors were

[1]Published as:

Lowrie, D., Hobson, J. and Stuckey, D.C. (2002): Sulfate disinfection, stabilisation and heavy metal removal from sewage sludge - process description and preliminary results. Wat. Sci. Tech., 45 (10), pp. 287-292. Proceedings of the 9th World Congress on Anaerobic Digestion, Antwerp-Belgium, 2-6 September, 2001.

started off as completely methanogenic by batch-feeding them raw primary sludge once a day.

The feed for Reactor S was then supplemented with sulfate (at the established amount in the preliminary experiment) in the form of sodium sulfate to initiate sulfidogenic conditions. At this stage in the project, it was important to start-up a hybrid sulfidogenic reactor without compromising its stability due to pH decrease from adding concentrated sulfuric acid; hence the use of sodium sulfate. Previously, in the study by Hobson *et al*, (1994), intermittent dosing instead of single batch-feeding of sulfuric acid was necessary to prevent the pH of the reactor plummeting. The reactor design and experimental set-up for Reactors M and S were described in Chapter 2. The analytical techniques described in Chapter 3 were used to measure pH, Total COD, VFA's, TS and VS in order to compare digestion efficiency between Reactors M and S.

The second part of this Chapter in Section 4.3 deals with the design and set-up of an H_2S Bioscrubber. Once the bioscrubber had been built, it was necessary to evaluate the basic hydrodynamics operating within the bioscrubber in order to optimise the liquid hold-up within the bisocrubber. Subsequently, it was necessary to pack the bioscrubber with suitable media on which sulfur oxidising bacteria could grow. To seed the packing material, a broth containing all the elements necessary to encourage *Thiobacillus* growth was prepared and seeded with a potential source of this bacteria, and this was added to the bioscrubber. The source of H_2S for the bioscrubber was from a separate hybrid H_2S Generator that was set-up especially for this bioscrubber as an economic source of sulfide. It was also intended to prevent the risk of aerating the experimental H_2S Generator (Reactor S) that was set-up and discussed in this Chapter. Due to pressure-related problems of fluid and gases passing from one bioscrubber compartment to the next, the anaerobic digester was inadvertently being sporadically aerated so that the reactor shut-down and never fully recovered. As a result, the source of sulfide was changed to a chemical H_2S Generator.

The bioscrubbers were continuously analysed for sulfide levels in the biogas and sulfide and sulfate in the effluent liquor stream. At the end of the experimental stage,

swab samples from the packing media were analysed under a light microscope for microbiological growth.

4.2 PREPARATORY STUDIES & RESULTS FOR H_2S GENERATION

4.2.1 Sulfate Addition

The first step to establishing how 'hybrid' Reactor S should be was to calculate the mass of SO_4 as sodium sulfate to add to the feed of the hybrid reactor. A volume of fresh raw sludge was divided into five, 200 ml volumes in beakers. The sludge was allowed to settle for a few hours, and different volumes of supernatant liquor were carefully removed from each beaker to obtain different solids concentrations. One hundred ml from each beaker was used to test for TS concentration, while the remaining 100 ml was titrated with 10% sulfuric acid with constant stirring using a magnetic stirrer. Figure 4.1 shows the concentration of H_2SO_4 as sulfate required to decrease the pH of raw sludge at various solids concentrations.

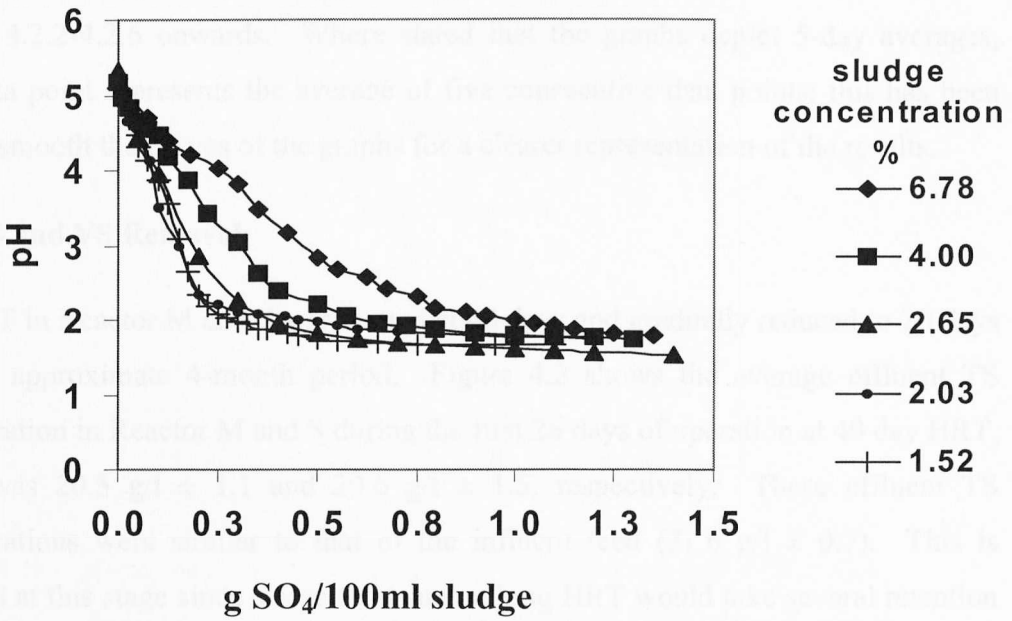


Figure 4. 1- Titration curve of different concentrations of raw primary sludge with H_2SO_4.

As expected, the higher the TS concentration, the higher the buffering capacity (Sreekrishnan *et al.*, 1993; Tyagi *et al.*, 1997), and the greater amount of acid require to reduce the pH to the same level as a sludge with a lower TS concentration. For a TS concentration of sludge between 2-2.65%, approximately 5 g/l (± 1g) of sulfate as H_2SO_4 was required to bring the pH down to 1.85. This concentration will vary with the type and freshness of sludge, but was used as a guide in this project. The feed sludge for Reactor S was therefore supplemented with 5g/l sulfate as sodium sulfate once stable methanogenic conditions had been established. The feed concentrations was approximately 2% TS (± 0.7%) for both reactors during the sulfate addition period in Reactor S. According to Metcalf and Eddy (1979) sludge has the approximate formula presented in equation 2.23, for which the Relative Molecular Mass (RMM) was calculated as 83.46. Since 64g of oxygen are required to completely oxidise 83.46 g of sludge, the TS:COD ratio is equal to 0.77 g/g. Therefore, the concentration of sulfate added is equivalent to a COD:SO_4 ratio of 5.2:1. The amount of sodium sulfate added was varied according to the feed COD strength to maintain this ratio.

Once the amount of sulfate required in a hybrid H_2S generator was established, lab-scale reactors were set up and the results of these trials are depicted below between Section 4.2.2-4.2.6 onwards. Where stated that the graphs depict 5-day averages, each data point represents the average of five consecutive data points; this has been done to smooth the curves of the graphs for a clearer representation of the results.

4.2.2 TS and VS Removal

The HRT in Reactor M and S was started at 40 days and gradually reduced to 20 days over an approximate 4-month period. Figure 4.2 shows the average effluent TS concentration in Reactor M and S during the first 26 days of operation at 40 day HRT, which was 20.5 g/l ± 1.1 and 20.6 g/l ± 1.5, respectively. These effluent TS concentrations were similar to that of the influent feed (21.6 g/l ± 0.7). This is expected at this stage since a reactor at such a long HRT would take several retention times for the effect of solids digestion to become apparent. The effluent concentration in both reactors gradually decreased at similar rates compared to the feed, and by day 69, a further reduction in HRT to 26.6 days was made. Despite fluctuations in the

influent TS concentration between day 69 and 109 (26.6 day HRT), effluent TS concentrations in both reactors remained fairly stable and did not fluctuate to the same extent as the feed, indicating that the reactors were reaching stable operating conditions. The HRT of both reactors were subsequently reduced to 20 days on day 110 and retained at this HRT throughout the rest of the run (224 days total run-length). As seen from Table 4.1, between days 110 and 153 before sulfate addition commenced, the effluent TS concentration in both reactors was similar (~12 g/l). The stability of the reactor is apparent by the relatively low SD for both reactors of 1.3 g/l, despite a fluctuation in the influent TS concentration. On day 154, the feed of Reactor S was supplemented with sodium sulfate.

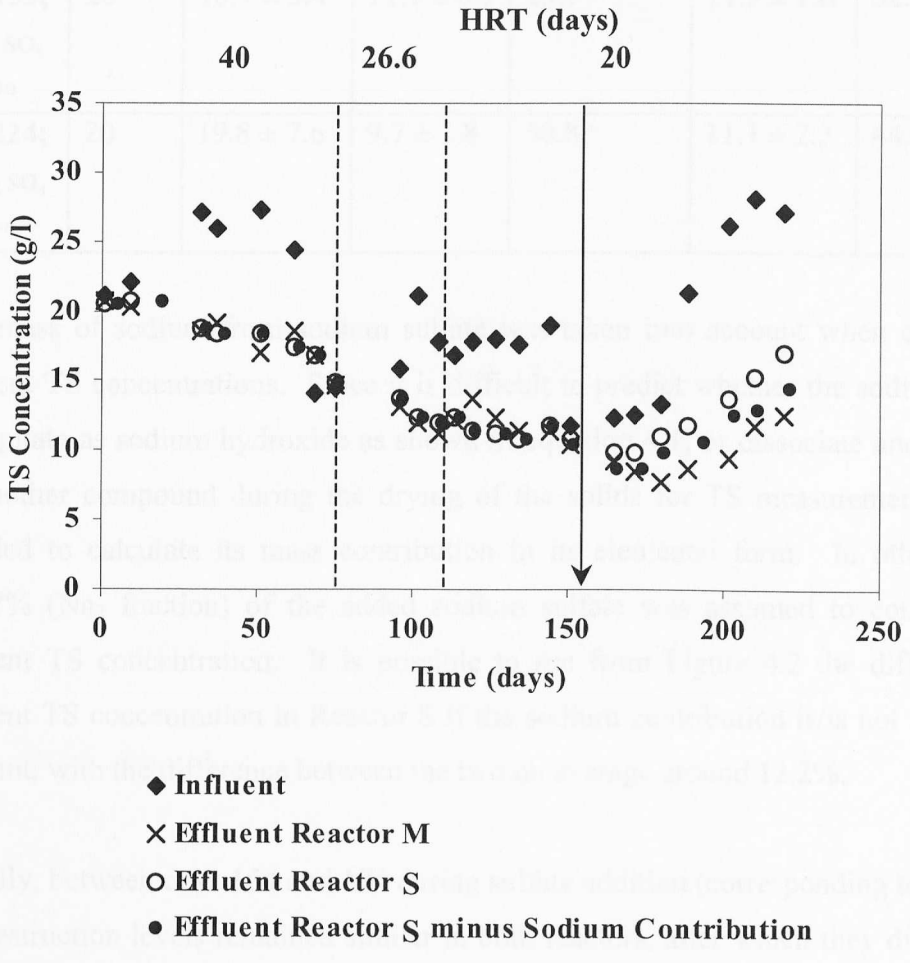


Figure 4. 2- 5-day average TS concentration in the feed and effluent of Reactors M and S. Dotted lines indicate HRT (above graph) during the period between the lines; arrow represents initial SO_4 feeding in Reactor S.

Table 4. 1- Summary of average TS concentrations and TS destruction levels in Reactors M and S at various points during reactor lifetimes. Mass of sodium from Na_2SO_4 taken into account.

Day	HRT	Influent	Effluent Reactor M		Effluent Reactor S	
	Days	Average TS Conc. (g/l)	Average TS Conc. (g/l)	TS Destruction (%)	Average TS Conc. (g/l)	TS Destruction (%)
0-26	40	21.6 ± 0.7	20.5 ± 1.1	5.3	20.6 ± 1.5	4.6
27-68	40	25.4 ± 4.6	18.2 ± 1.2	28.0	18.2 ± 1.1	28.4
69-109	26.6	16.8 ± 4.1	13.6 ± 2.1	19.2	13.9 ± 1.9	17.5
110-153; before SO_4 addition	20	16.9 ± 3.4	11.9 ± 0.9	29.7	11.5 ± 1.0	32.2
154-224; during SO_4 addition	20	19.8 ± 7.6	9.7 ± 1.8	50.8	11.1 ± 2.3	44.0

The mass of sodium from sodium sulfate was taken into account when calculating effluent TS concentrations. Since it is difficult to predict whether the sodium would precipitate as sodium hydroxide as shown in equation 4.1, or dissociate and combine as another compound during the drying of the solids for TS measurements, it was decided to calculate its mass contribution in its elemental form. In other words, 32.37% (Na_2 fraction) of the added sodium sulfate was assumed to contribute to effluent TS concentration. It is possible to see from Figure 4.2 the difference in effluent TS concentration in Reactor S if the sodium contribution is/is not taken into account, with the difference between the two on average around 12.2%.

Initially, between days 154 and 174 during sulfate addition (corresponding to 1 HRT), TS destruction levels remained similar in both reactors, after which they diverged so that the TS concentration in the effluent of Reactor S began to rise in comparison with that of Reactor M for the period between days 175-224. Despite a large SD in the influent TS concentration of 7.6 g/l, the SD of the effluent concentrations of Reactors M and S remained very stable. Overall, the average TS destruction during the whole period of sulfate addition was 50.8% in Reactor M, but lower for Reactor S at only

44.0%. Taking into account the sodium mass contribution from sodium sulfate, Figure 4.3 represents average VS destruction levels during the lifetime of the two reactors.

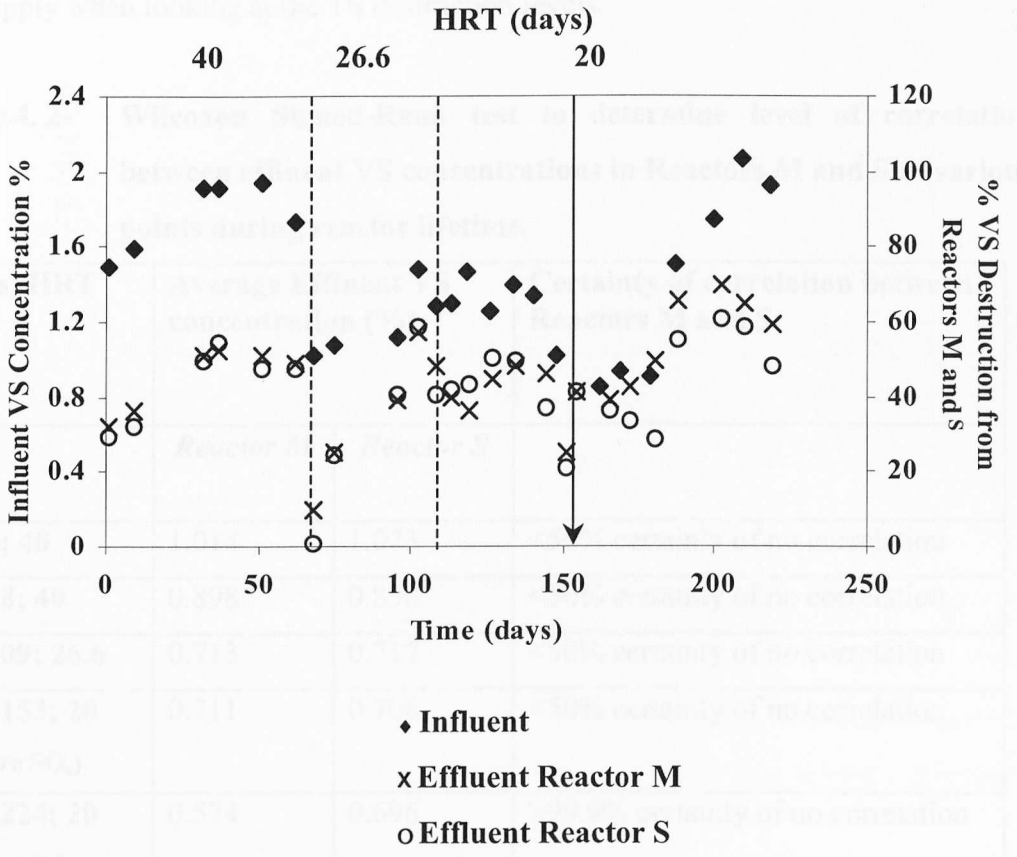


Figure 4. 3- 5-day average VS destruction in Reactors M and S. Dotted lines indicate HRT (above graph) during the period between the lines; arrow represents initial SO_4 feeding in Reactor S.

As expected, similarities can be drawn between TS and VS destruction levels in both reactors. During the first 26 days, VS destruction levels reached 34.2% for Reactor M and 30.3% for Reactor S. This increased to an equal average maximum of 50.7% in both Reactors M and S between days 27-68. During sulfate addition between days 154 and 224, the VS destruction in both reactors rose further to 59.4% in Reactor M, but not as high for Reactor S at only 50.8%. In order to establish whether there was a significant difference between VS destruction levels in the two reactors, a Wilcoxon Signed-Rank test (see Section 3.5.3) was used to analyse the data at different time-periods during the lifetime of the reactors. Table 4.2 shows the results of the statistical analysis where it can be seen that there is over 99.9% confidence that the

VS concentration in Reactors M and S are significantly different during the period of sulfate addition. This is opposed to days 27-153 prior to sulfate addition when there was <50% certainty that the data were not different. These same levels of uncertainty also apply when looking at the TS destruction levels.

Table 4. 2- Wilcoxon Signed-Rank test to determine level of correlation between effluent VS concentrations in Reactors M and S at various points during reactor lifetime.

Days; HRT	Average Effluent VS concentration (%)		Certainty of correlation between Reactors M and S
	Reactor M	*Reactor S*	
0-26; 40	1.014	1.073	<50% certainty of no correlation
27-68; 40	0.898	0.896	<50% certainty of no correlation
69-109; 26.6	0.713	0.717	<50% certainty of no correlation
110-153; 20 (before SO_4)	0.711	0.706	<50% certainty of no correlation
154-224; 20 (after SO_4)	0.574	0.696	>99.9% certainty of no correlation

4.2.3 Total COD and VFA Destruction

Total COD (TCOD) concentrations in the influent and effluent were measured from day 110 onwards in reactors M and S, which is the period when the HRT was reduced to 20 days. As seen from Figure 4.4, while the TCOD concentration in the influent increases towards the end of the run, the TCOD concentration in the effluent from both reactors remains relatively stable. Destruction levels are an average of 64% in Reactor M (19.1±5.8 g/l) and 63% in Reactor S (18.7±6.0g/l) during the sulfidogenic period of Reactor S.

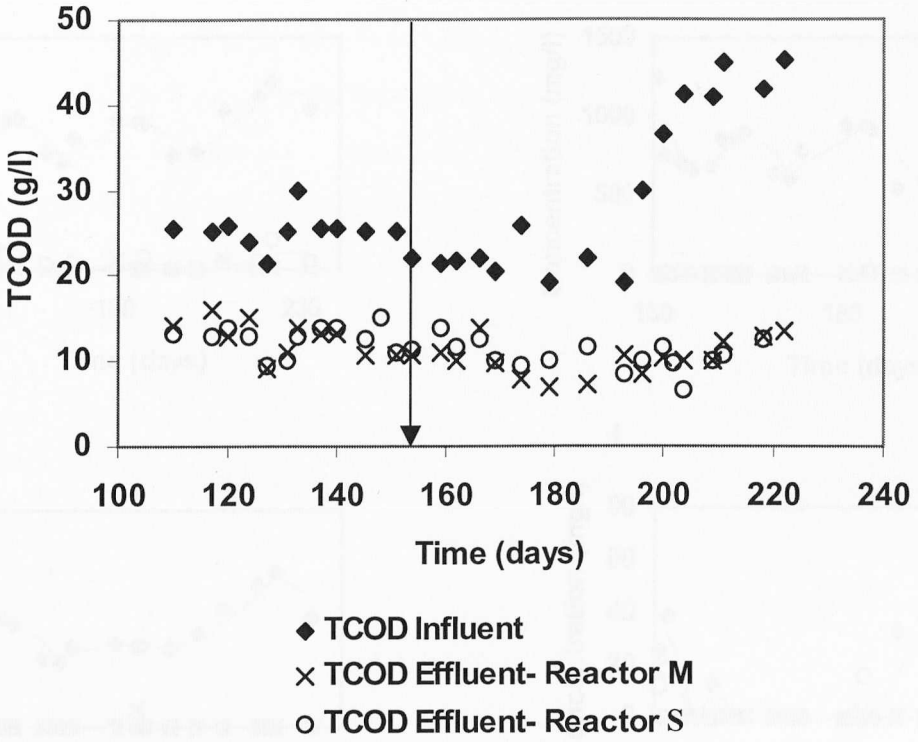


Figure 4. 4- Influent and Effluent TCOD concentrations in Reactors M and S at 20 day HRT. Points represent 5-day averages; arrow represents initial SO_4 feeding in Reactor S.

Contrary to the results of the solids destruction levels, a Wilcoxon Signed-Rank test to compare effluent TCOD concentrations in both reactors during this period showed <50% confidence that the results were different. In addition, although the soluble COD (SCOD) concentration in the effluent was not measured directly, VFA concentrations were, which represent part of the SCOD excluding Soluble Microbial Products (SMP's). Depending on factors such as substrate feed type and HRT, SMP's may account for between 0-100% of SCOD. An account of SMP characterisation and formation can be found in Kuo (1993), and Barker and Stuckey (2001).

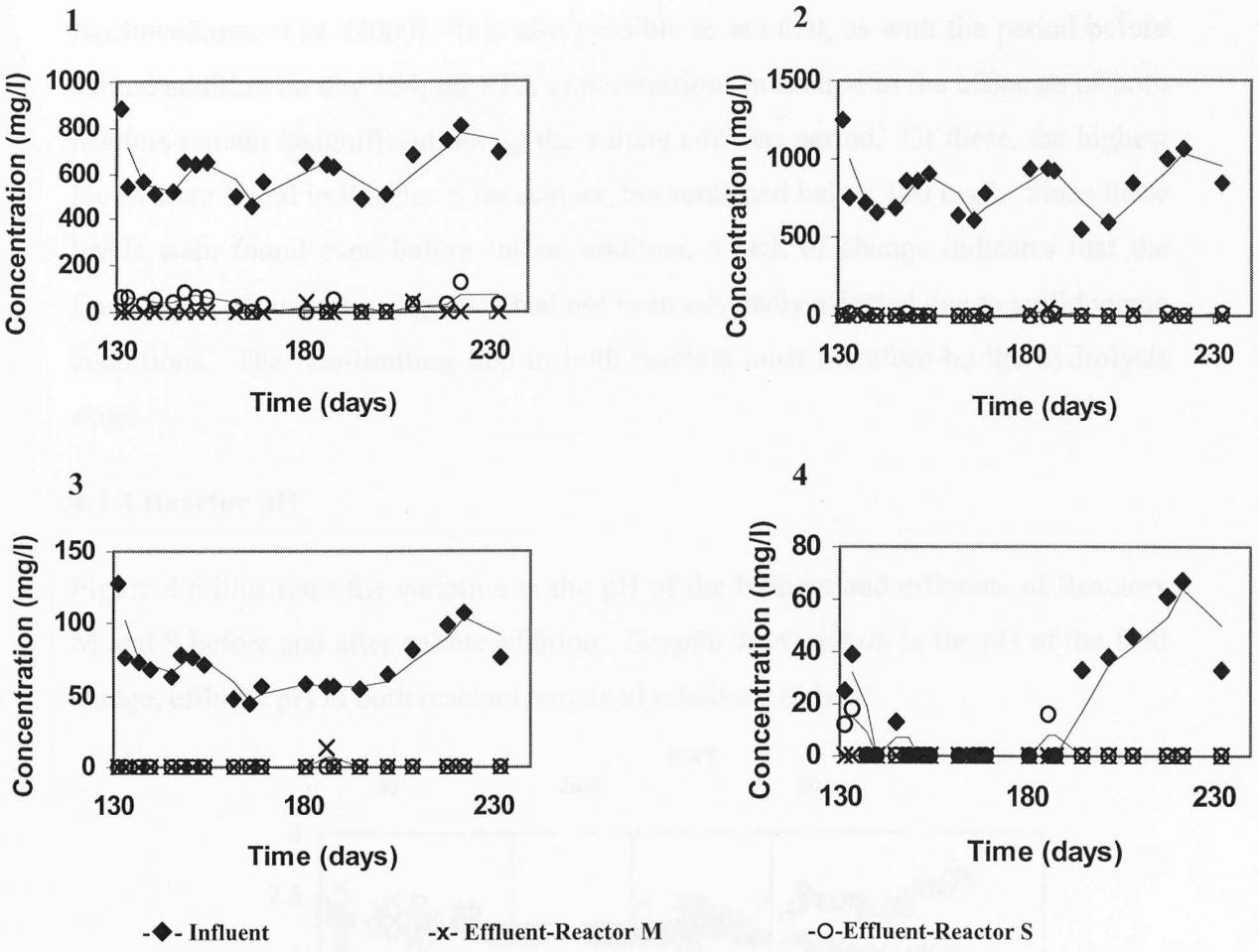


Figure 4. 5- VFA concentrations in the Influent and Effluent of Reactors M and S. Graph 1: Acetate, Graph 2: Propionate, Graph 3: N-butyrate, Graph 4: N-Valerate. Sulfate addition commenced on day 154 in Reactor S.

In this project, it was of interest to measure VFA levels, since their concentration in the effluent can indicate the level of stability in a reactor. Figure 4.5 represents the different VFA levels in the influent and effluent of Reactors M and S. Although the graphs illustrate influent VFA concentrations, these levels only represent initial levels in the feed sludge, and do not reflect the total VFA's that would be produced as a result of hydrolysis and acidogenesis. Therefore, it is inaccurate to represent VFA destruction percentages in this case. As seen in Graphs 1-4 in Figure 4.5, the levels of all VFA's in the influent fluctuate steadily, reflecting the periods when the sludge is degrading into VFA's during storage, and then diluted from the addition of fresh batches of raw sludge. A study of anaerobic production and degradation of VFA's at

psychrophilic temperatures by sludges from various cold habitats can be found in Nozhevnikova *et al.* (2000). It is also possible to see that, as with the period before sulfate addition on day 154, all VFA concentrations measured in the effluents of both reactors remain insignificant during the sulfate addition period. Of these, the highest levels were found in Reactor S for acetate, but remained below 100 mg/l. Since these levels were found even before sulfate addition, a lack of change indicates that the final stages of anaerobic digestion had not been adversely affected due to sulfidogenic conditions. The rate-limiting step in both reactors must therefore be the hydrolysis stage.

4.2.4 Reactor pH

Figure 4.6 illustrates the variation in the pH of the influent and effluents of Reactors M and S before and after sulfate addition. Despite the variation in the pH of the feed sludge, effluent pH in both reactors remained relatively stable.

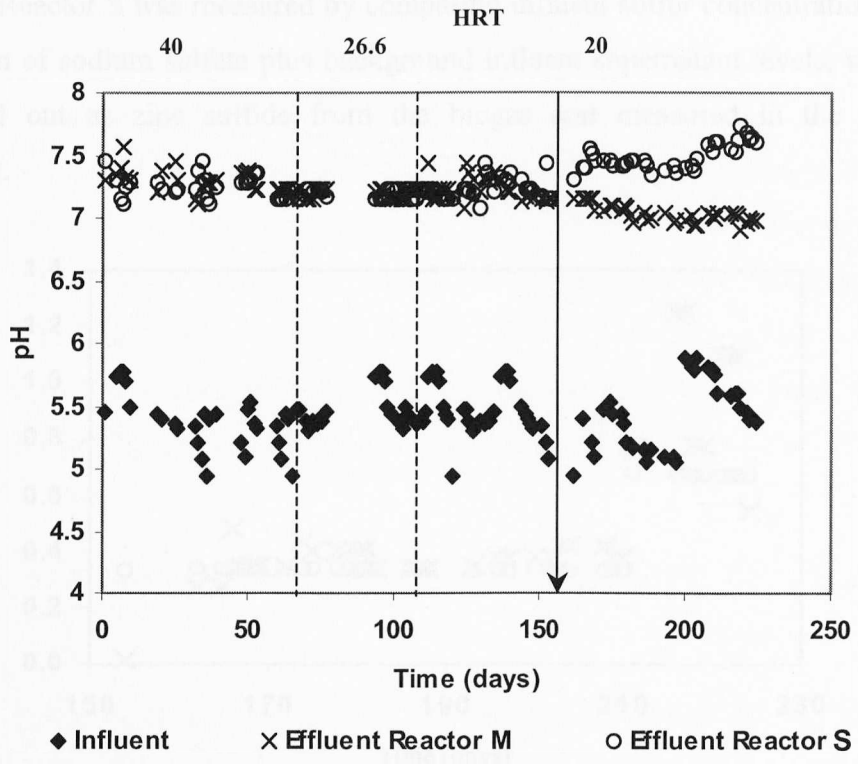


Figure 4. 6- Variation in pH of the influent and effluent of Reactors M and S. Dotted lines indicate HRT (above graph) during the period between the lines; arrow represents initial SO_4 feeding in Reactor S.

However, it can be seen that there is a noticeable and immediate increase in the pH of the effluent from Reactor S after sulfate addition is commenced up until the end of the run. This increase from an average of 7.2 (± 0.05) to 7.5 (± 0.15) may be due to the production of sodium hydroxide according to equation 4.1.

$$4H_2 + Na_2SO_4^{2-} \rightarrow 2NaOH + H_2S + 2H_2O \quad \text{(eq. 4.1)}$$

The pH of a reactor can have a significant effect on bacterial growth rates (O'Flahery *et al.*, 1998; Visser *et al.*, 1996). The pH of 7.5 in Reactor S after sulfate addition suggests that that pH may favour SRB over MPB's for certain substrates.

4.2.5 Sulfate Reduction and Biogas Production

Reactor S was first seeded with 100 ml effluent from an ABR treating tannery waste high in sulfate on day 154 along with sodium sulfate addition. The sulfur mass balance in Reactor S was measured by comparing influent sulfur concentrations from the addition of sodium sulfate plus background influent supernatant levels, with that precipitated out as zinc sulfide from the biogas and measured in the effluent supernatant.

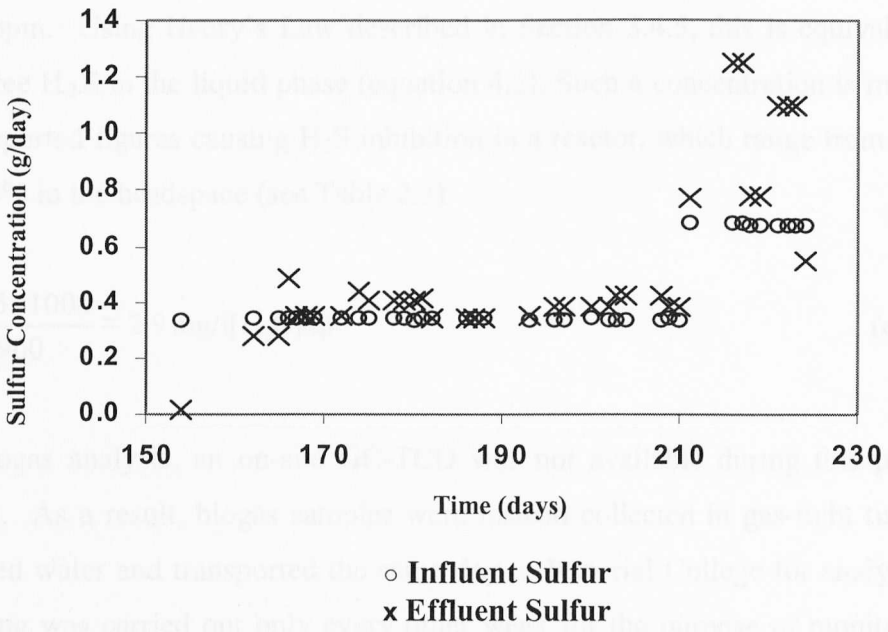


Figure 4. 7- Sulfur mass balance in Reactor S.

The zinc sulfide precipitate was collected once to twice a week and the mass averaged out for that period and converted to sulfur equivalent. The H_2S production from Reactor M was so low in comparison to the sulfidogenic reactor that its concentration was not measured and assumed negligible.

It is clear from Figure 4.7 that sulfide production did not commence immediately, but required over a week to reach maximum levels. The amount of sulfur entering and leaving the reactor matched closely up to day 210, indicating 100% sulfate reduction. After this period, when influent solids concentrations increased (as well as sodium sulfate to maintain the correct COD:SO_4 ratio) the amount of sulfur leaving was 19% higher than that entering. The 'extra' sulfur collected may also be partly attributed to sulfur already present within cell proteins and cell walls. This does not fully explain such a large discrepancy, but does suggest that sulfidogenesis levels had not equilibrated to the new levels of sulfate.

Overall, with effluent sulfate levels of ≤20 mg/l (compared to an influent of 5 g/l), these results suggest 100% reduction of the added sulfate. Due to the constant stripping of the biogas through the zinc acetate solution, the headspace concentration of H_2S, which was measured approximately once a week always remained below 1000 ppm. Using Henry's Law described in Section 3.4.5, this is equivalent to 2.9 mg/l free H_2S in the liquid phase (equation 4.2). Such a concentration is much lower than reported figures causing H_2S inhibition in a reactor, which range from 20mg/l to up to 5% in the headspace (see Table 2.3).

$$\frac{34.076 \times 1000}{11900} = 2.9\,\text{mg/l}[H_2S]_{(aq)} \qquad \text{(eq. 4.2)}$$

For biogas analysis, an on-site GC-TCD was not available during this part of the project. As a result, biogas samples were instead collected in gas-tight tubes under acidified water and transported the same day to Imperial College for analysis. Such sampling was carried out only every other week for the purpose of monitoring CH_4 concentrations in both reactors. For the 6 samples collected over the period 110-224 days (20 day HRT), CH_4 concentration in Reactor M and S were 70.2% ± 4.6 and 52.5% ± 6.8 respectively. The SD for CH_4 in Reactor S was higher due to the

variable COD:SO_4 during the fluctuating influent concentration towards the end of the run. Total biogas volumes were measured almost daily. Data prior to day 110 are unreliable and have been discounted due to technical problems encountered with the biogas volume counter system used at the time. After day 110, upside-down water columns containing acidified water were used for biogas volume measurements as described in section 3.4.3. The data for CH_4, H_2S and biogas volume measurements were used to calculate the equivalent TCOD destruction levels from equations 2.7 and 2.10. It was then possible to compare the results of this method with those measured chemically by Dr Lange tubes; the difference is illustrated in Figure 4.8.

Between days 110-153, the COD destruction levels from the two methods match quite closely, and indeed the results from both reactors were very similar, as reflected in Table 4.3. From day 154 onwards during sulfate addition, the results from the two methods differed to a greater extent. There was an immediate increase in TCOD destruction levels as measured by the Dr Lange method, whereas this was not the case for COD measured as biogas. The difference in the two results may be attributed to a combination of factors including:

a) Due to the slow growth of MPB (in combination with a long HRT), a change in influent feed concentrations may take several retention times before becoming apparent as COD released as methane. TCOD measurements on the other hand are a reflection of the increased gap between influent and effluent TCOD concentration, rather than an increase in actual microbial activity levels;

b) An underestimation of biogas volume due to the solubility of CO_2 and CH_4, and subsequent loss through the effluent;

c) A loss of biogas sample during transportation for biogas analysis, therefore underestimating methane composition;

d) Residual dissolved CH_4 and H_2S which may over-estimate TCOD levels by the Dr Lange method;

e) Low number of biogas samples collected to make a viable comparison.

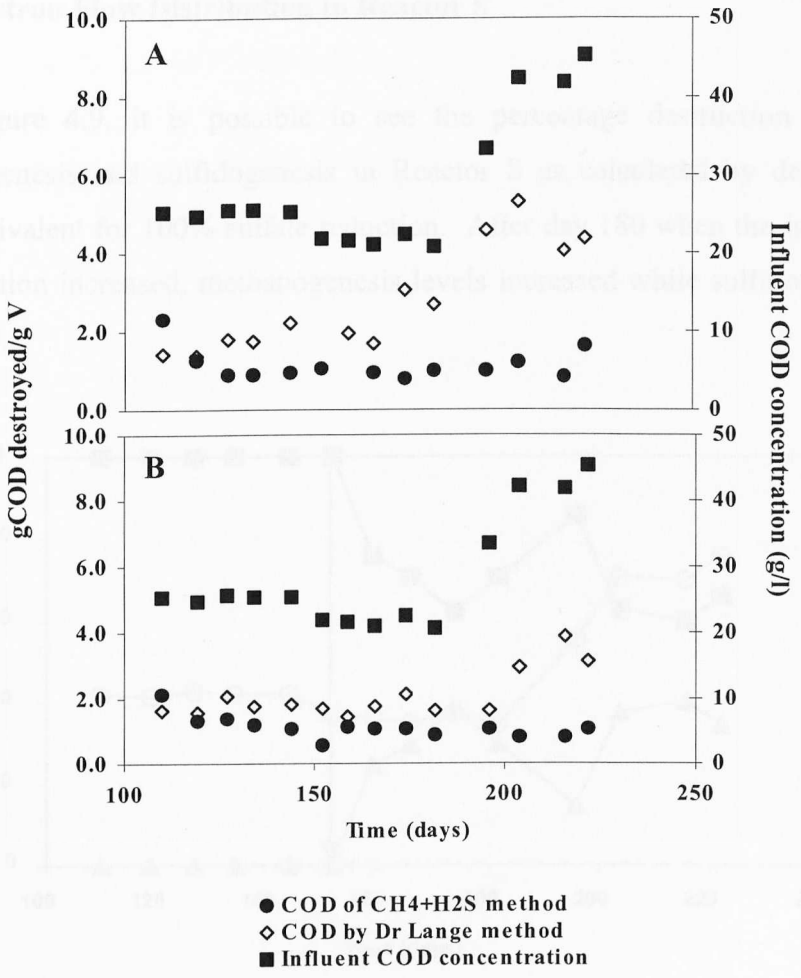


Figure 4. 8- **TCOD destruction in Reactor M (Graph A) and Reactor S (Graph B) calculated using two methods. Points represent 5-day averages.**

Table 4. 3- **Comparison of results for two methods used to calculate specific COD destruction in Reactors M and S.**

Days	Reactor	Specific COD Destroyed (gCOD/gVS)		Difference between methods
		COD of CH_4, or COD of CH_4+H_2S	COD by Dr Lange	
110-153	M	1.23 ± 0.54	1.70 ± 0.33	28%
110-153	S	1.27 ± 0.52	1.74 ± 0.18	27%
154-224	M	1.06 ± 0.29	3.46 ± 1.31	69%
154-224	S	0.98 ± 0.14	2.32 ± 0.88	58%

4.2.6 Electron Flow Distribution in Reactor S

From Figure 4.9, it is possible to see the percentage destruction of COD via methanogenesis and sulfidogenesis in Reactor S as calculated by determining the COD equivalent for 100% sulfate reduction. After day 180 when the influent TCOD concentration increased, methanogenesis levels increased while sulfidogenesis levels decreased.

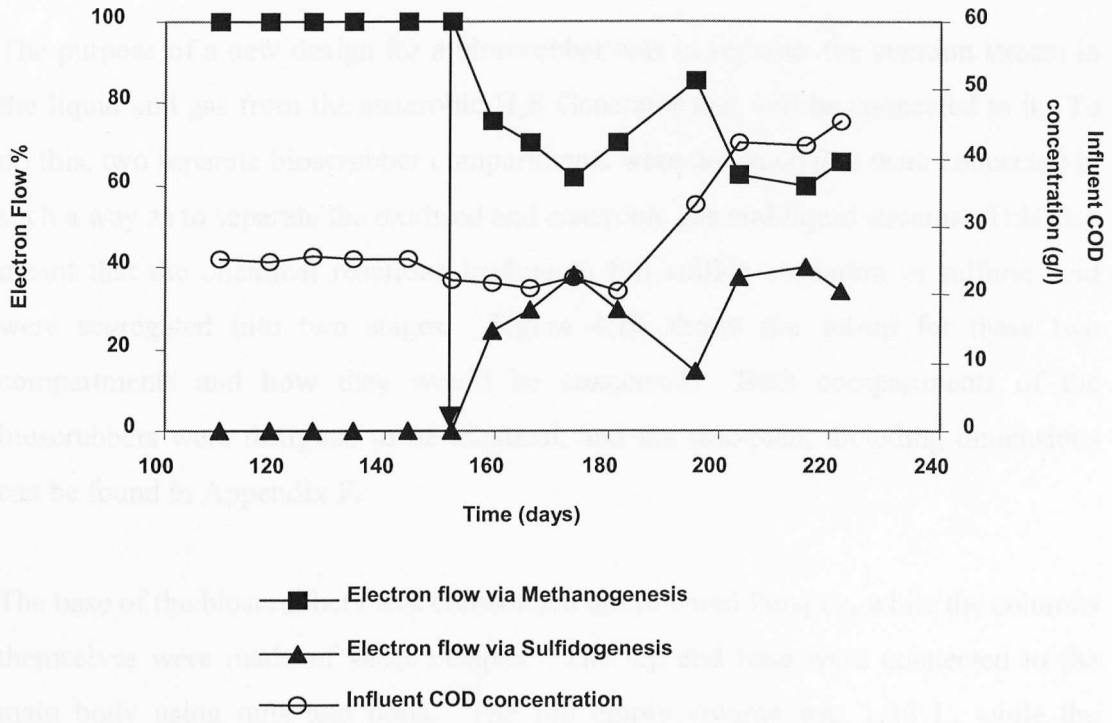


Figure 4. 9- Electron flow between methanogenesis and sulfidogenesis in Reactor S at a COD:SO_4 ratio of 5.2:1. Points represent 5-day averages. Arrow represents day of SO_4 addition to feed of Reactor S.

This trend was reversed when the COD:SO_4 was adjusted to the initial ratio of 5.2:1 by adding more sodium sulfate. Although the target for the theoretical COD:SO_4 ratio was 5.2:1, the actual ratio as calculated from the measured influent TCOD and SO_4 concentrations was 5.45:1 due to the fluctuation and variability in the influent

TCOD concentration. The average flow of electrons via methanogenesis and sulfidogenesis during sulfate feeding was 71%: 29% (± 11) respectively.

4.3 H_2S BIOSCRUBBER DESIGN, OPERATION & RESULTS

4.3.1 Design

The purpose of a new design for a bioscrubber was to separate the aeration stream in the liquid and gas from the anaerobic H_2S Generator that will be connected to it. To do this, two separate bioscrubber compartments were designed that were connected in such a way as to separate the oxidised and anaerobic gas and liquid streams. This also meant that the chemical reactions leading to full sulfide oxidation to sulfuric acid were segregated into two stages. Figure 4.10 shows the set-up for these two compartments and how they would be connected. Both compartments of the bioscrubbers were designed to be identical, and the side-plan, including dimensions can be found in Appendix F.

The base of the bioscrubbers was constructed out of cored Perspex, while the columns themselves were made of sheet Perspex. The top and base were connected to the main body using nuts and bolts. The full empty volume was 1.36 L, while the effective empty volume was 1.17 L for each bioscrubber. The bioscrubber therefore consisted of two compartments; one air-lean and one air-rich. H_2S entering the air-lean compartment was chemically oxidised down to elemental sulfur and no further. The sulfur-containing liquor was then passed onto the air-rich bioscrubber where it was further biologically oxidised to sulfuric acid by the *Thiobacilli*. The sulfuric acid was then re-circulated back to the air-lean bioscrubber to keep the bacteria moist. The pH of the sulfuric acid was maintained at the desired level by occasional pumping of fresh secondary activated sludge liquor into the acid stream. The temperature inside the bioscrubbers could be maintained at the desired level using a water jacket. In this project, room temperature was used (20°C ±4).

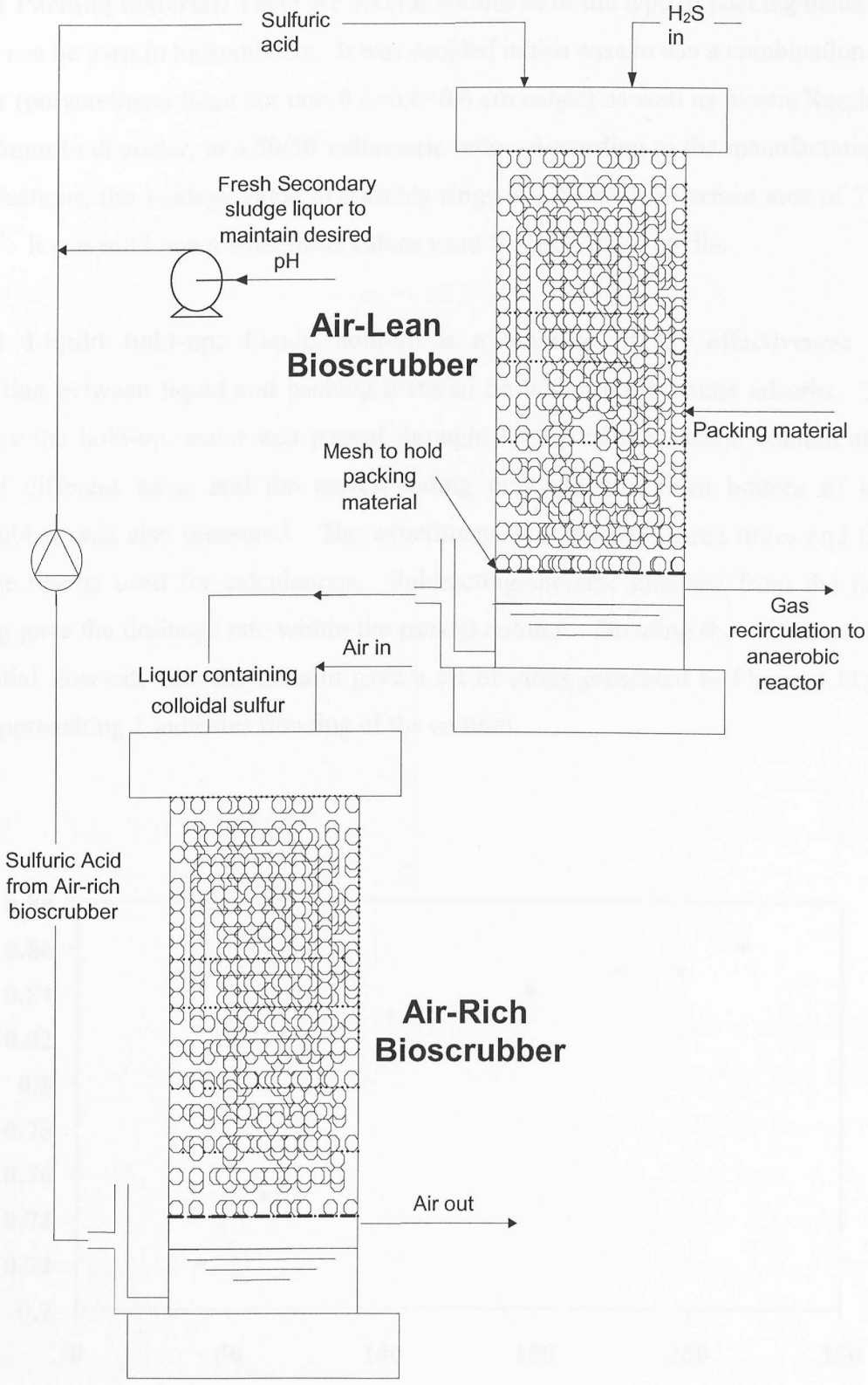


Figure 4. 10- Connection of the two H_2S Bioscrubber compartments.

4.3.1.1 Packing material: There are several options as to the type of packing material which can be used in bioscrubbers. It was decided in this case to use a combination of Linpor (polyurethane foam cut into 0.6×0.6×0.6 cm cubes) as well as plastic Raschig rings 6mm in diameter, in a 50/50 volumetric ratio. According to the manufacturer's specifications, the voidage value of Raschig rings is 72% with a surface area of 774 $m^2 \cdot m^{-3}$. It was not known what these values were for the Linpor media.

4.3.1.2 Liquid hold-up: Liquid hold-up is a measure of the effectiveness of contacting between liquid and packing material on which the biomass adsorbs. To measure the hold-up, water was passed through the top of the packed column at a several different rates, and the corresponding drainage rate from bottom of the bioscrubber was also measured. The experiment was repeated three times and the average results used for calculations. Subtracting the rate entering from the rate leaving gave the drainage rate within the packed column. Dividing the difference by the initial flow-rate into the column gave a set of ratios presented in Figure 4.11; a ratio approaching 1 indicates flooding of the column.

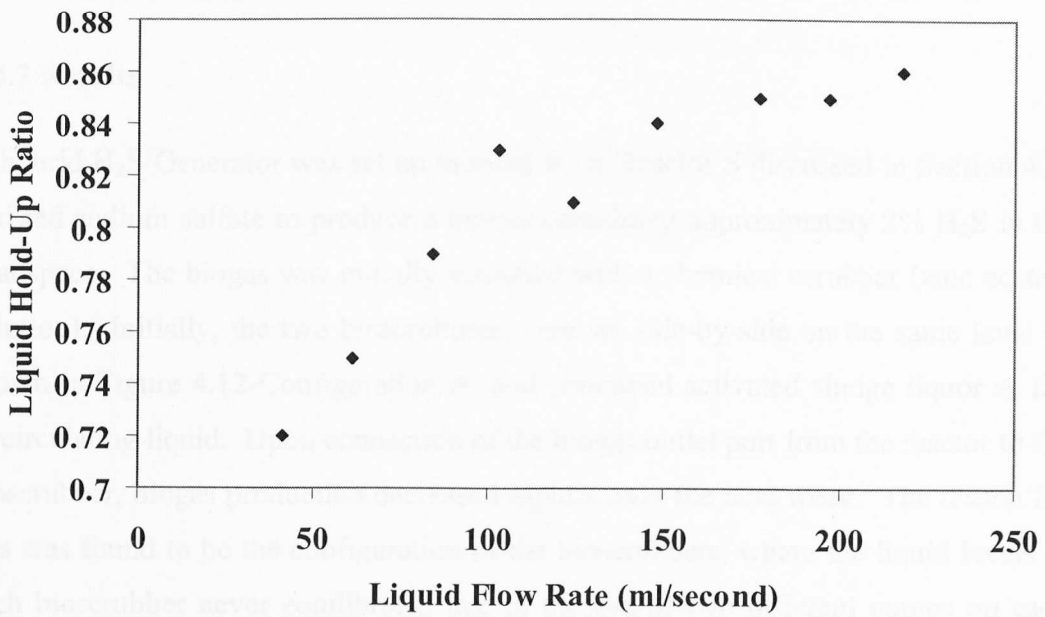


Figure 4. 11- The liquid hold-up in the packed bioscrubber.

The results show that at initial flow rates up to approximately 100 ml/sec, the ratio is linear, after which the curve changes to an asymptotic form. It was therefore decided to choose a median flow-rate of 100 ml·sec^{-1} as a starting point. To prevent flooding of the air outlet ports in each bioscrubber, the volume of liquid re-circulating around the two bioscrubbers was measured as 200 ml.

4.3.1.3 Seeding of the bioscrubbers with *Thiobacilli*: In order to seed the packing material with a source of sulfur oxidising bacteria, a 2L broth containing essential elements and nutrients for their growth was prepared (see Table G1-Appendix G) in a conical flask and seeded with 100 ml activated sludge. The temperature of the flask was maintained at 30°C in a water bath to encourage faster growth. The flask was covered with aluminium foil to reduce evaporation losses and aerated via a ceramic diffusing stone to maintain aerobic conditions at a rate of 10ml/second. The volume of liquid was topped up to the original level with tap-water once a week. The pH of the liquid was also measured once a week until a pH of 3 was achieved (after approximately 4 weeks), indicating *Thiobacillus* activity from oxidation of the available sulfur to sulfuric acid. The packing material was then soaked in the broth overnight to allow the adsorption of the bacteria before placing the packing material in the two bioscrubbers.

4.3.2 Results

A hybrid H_2S Generator was set up (seeded from Reactor S discussed in Section 4.3) and fed sodium sulfate to produce a biogas containing approximately 2% H_2S in the headspace. The biogas was initially scrubbed with a chemical scrubber (zinc acetate solution). Initially, the two bioscrubbers were set side-by-side on the same level as shown in Figure 4.12-Configuration A, and contained activated sludge liquor as the re-circulating liquid. Upon connection of the biogas outlet port from the reactor to the bioscrubber, biogas production decreased rapidly over the next week. The reason for this was found to be the configuration of the bioscrubbers, where the liquid levels in each bioscrubber never equilibrated due to the use of two different pumps on each liquid line. Since the pumps could never match the same flow rates, flooding was caused in the air-rich bioscrubber, while the liquid level could not be maintained high

enough in the air-lean bioscrubber to prevent escape of the biogas from the H_2S Generator.

In the next step, the pump on the liquid line from the bottom of the air-rich bisocrubber to the top of the air-lean bioscrubber was discarded. It was hoped that the combined suction of the other pump would force liquid from the bottom of the air-lean to the top of the air-rich bioscrubber as shown in Figure 4.12-Configuration B. However, it was seen that the rate of flow in this tube depended greatly on the position of the tubing, so that again, flow rates could not be completely controlled. Finally, the two bioscrubbers were placed on two different levels, and the direction of the air flow into the air-rich bioscrubber was changed to increase the pressure above the liquid surface to prevent liquid from escaping through the air outlet tube (Figure 4.13). An air-lock was created in the liquid line between the bottom of the air-rich bioscrubber and the top of the air-lean bioscrubber to allow free-drainage of the liquid, all of which resulted in eliminating the pressure problems.

By the time these pressure problems were resolved, the hybrid H_2S Generator had shut down due to excessive aeration, and never fully recovered its original performance. As a result, a chemical H_2S Generator was set-up instead, where sulfuric acid was dosed continuously in a solution of sodium sulfide in a sealed conical flask. The resultant gas was purged with N_2 and re-circulated around the air-lean bioscrubber. The following sections present the data for various aspects of the bioscrubber performance.

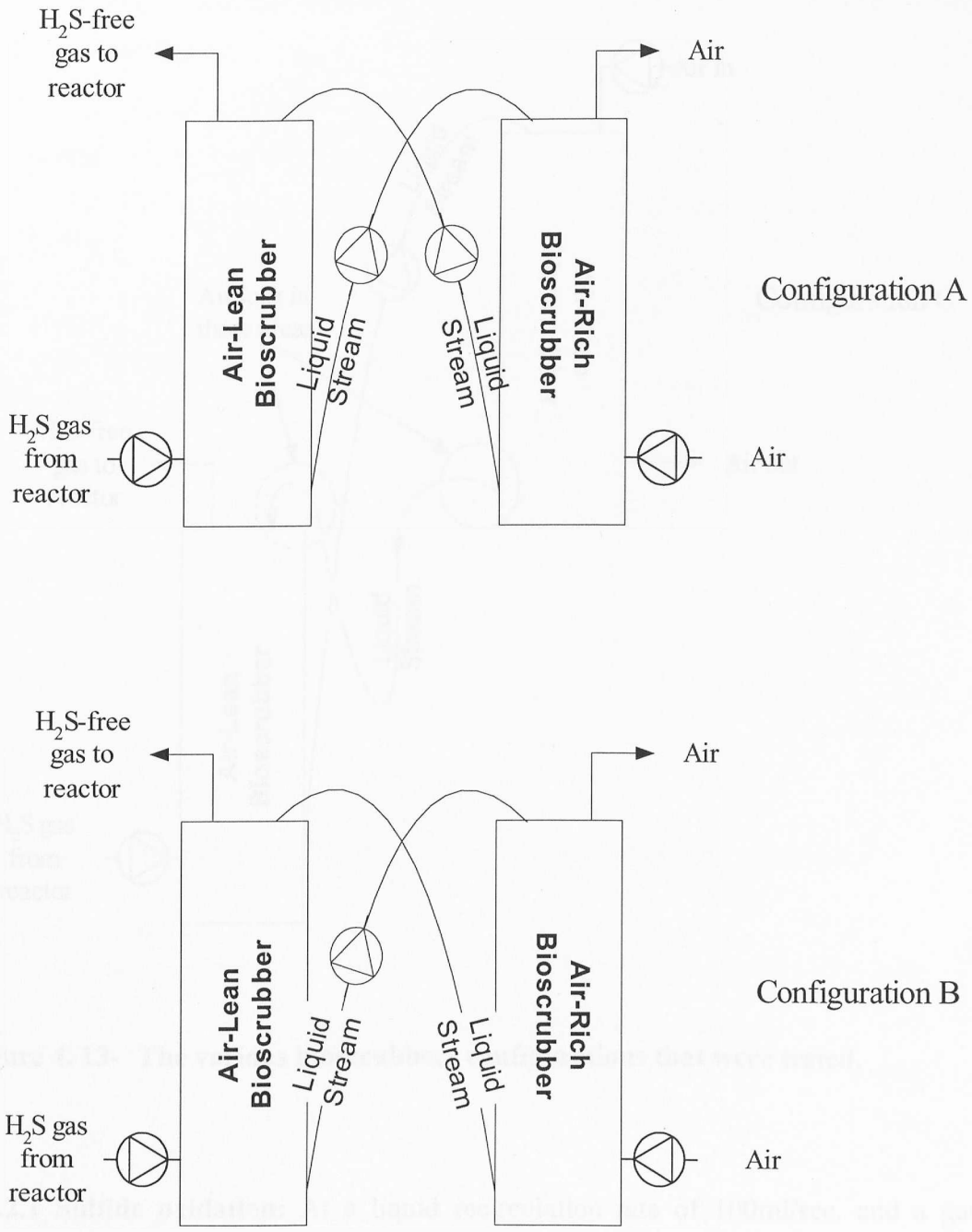


Figure 4. 12- The various bioscrubbers configurations that were tested.

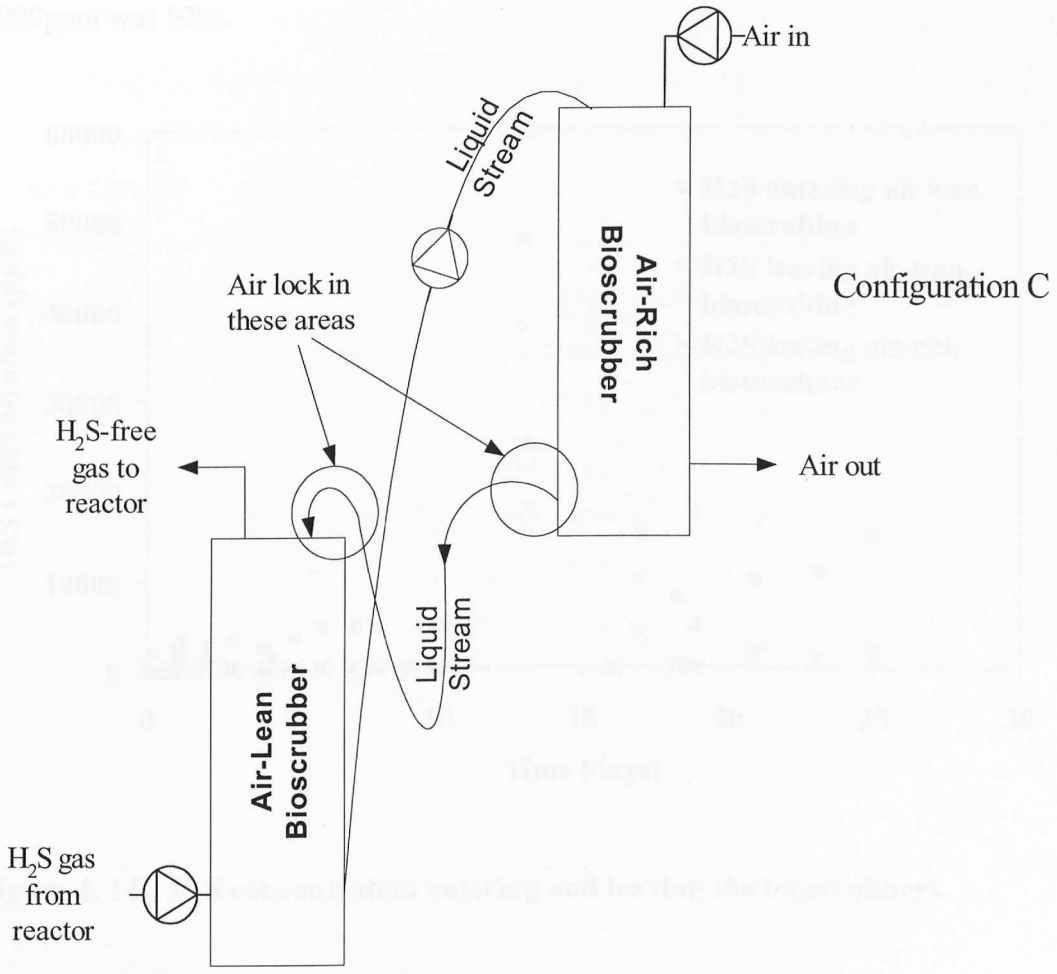


Figure 4. 13- The various bioscrubbers configurations that were tested.

4.3.2.1 Sulfide oxidation: At a liquid recirculation rate of 100ml/sec. and a gas residence time of 39 minutes (50 $L \cdot day^{-1}$ in a 1.36 L bioscrubber), the graph in Figure 4.14 represents the H_2S concentration entering and leaving the air-lean bioscrubber, as well as the H_2S concentration leaving the air-rich bioscrubber. The results show that up to day 11, when H_2S concentrations were kept below 5000 ppm, complete oxidation of the sulfide occurred in the air-lean bioscrubber. On day 12, the H_2S levels inadvertently rose to 4.8%; a concentration which was not substantially oxidised in the air-lean bioscrubber, but was slightly oxidised in the air-rich bioscrubber. When the H_2S concentration was lowered down to 1.5%, overall sulfide oxidation efficiency from both bioscrubbers rose once more, as illustrated in Figure

4.15. Overall, the average efficiency of sulfide removal at an average concentration 8000ppm was 92%.

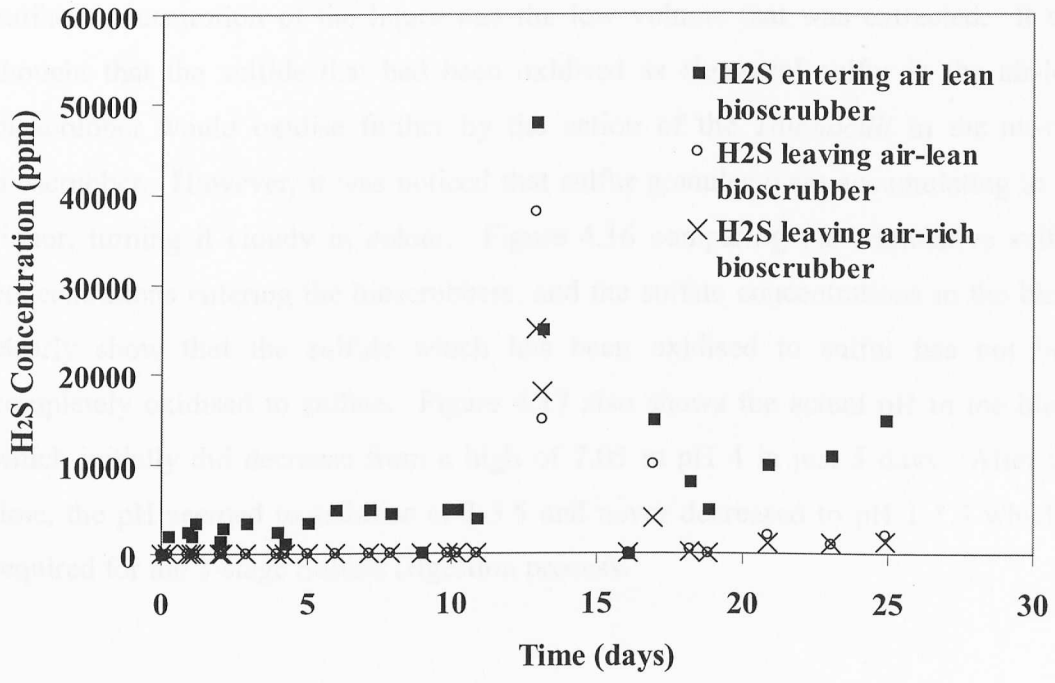


Figure 4. 14- H_2S concentration entering and leaving the bioscrubbers.

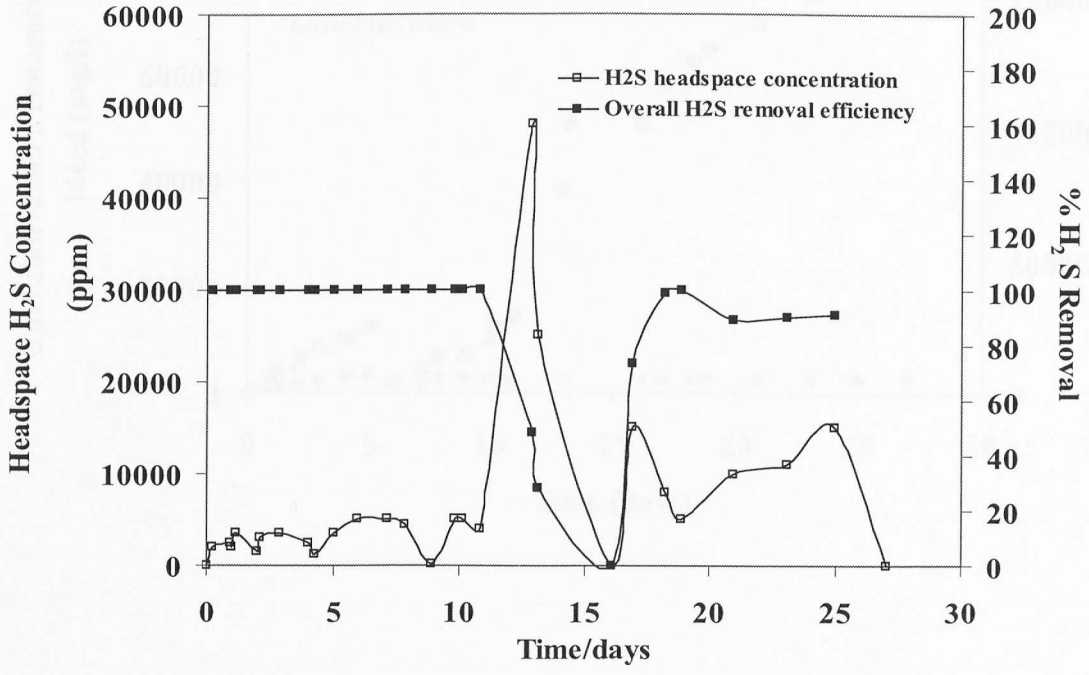


Figure 4. 15- Overall H_2S removal efficiency from the bioscrubbers.

4.3.2.2 Sulfate production: Throughout the 30-day period of the experiment, liquor was bled from the system once a week at a rate of 30 ml·week^{-1} and replaced with fresh activated sludge liquor. This was not expected to significantly affect the pH or sulfate concentration of the liquor due the low volume that was extracted. It was thought that the sulfide that had been oxidised as elemental sulfur in the air-lean bioscrubber would oxidise further by the action of the *Thiobacilli* in the air-rich bioscrubber. However, it was noticed that sulfur granules were accumulating in the liquor, turning it cloudy in colour. Figure 4.16 comparing the cumulative sulfide concentrations entering the bioscrubbers, and the sulfate concentrations in the bleed, clearly show that the sulfide which has been oxidised to sulfur has not been completely oxidised to sulfate. Figure 4.17 also shows the actual pH in the bleed, which initially did decrease from a high of 7.05 to pH 4 in just 5 days. After this time, the pH seemed to stabilise at 3-3.5 and never decreased to pH 1-1.5 which is required for the 3-stage Sulfate Digestion process.

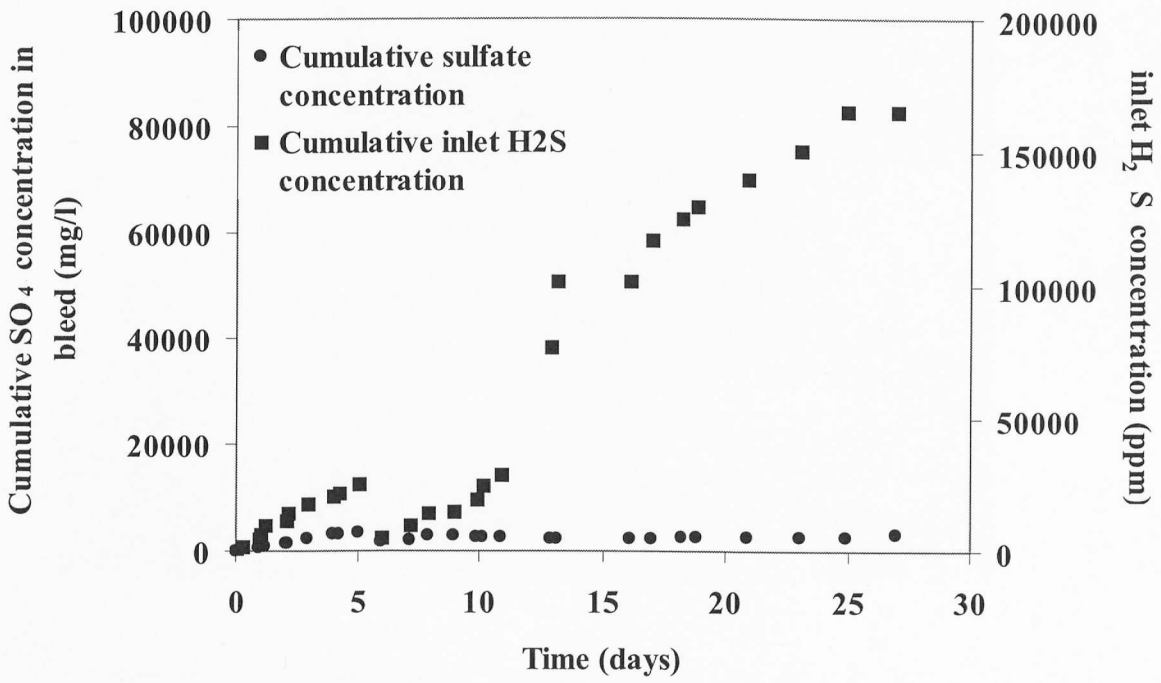


Figure 4. 16- Cumulative sulfide and sulfate concentrations entering and leaving the bioscrubber, respectively.

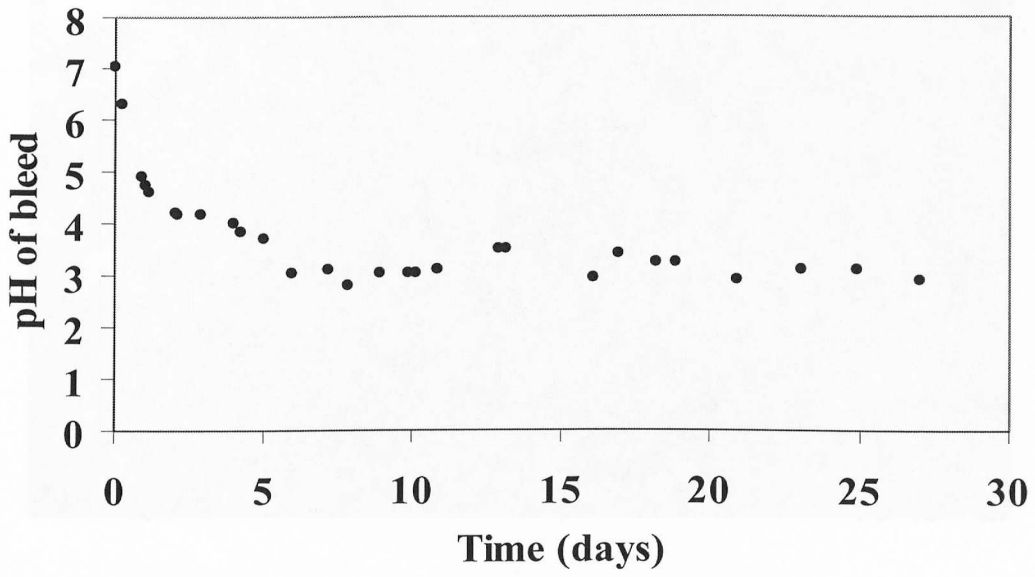


Figure 4. 17- pH of the bleed liquor leaving the air-rich bioscrubber.

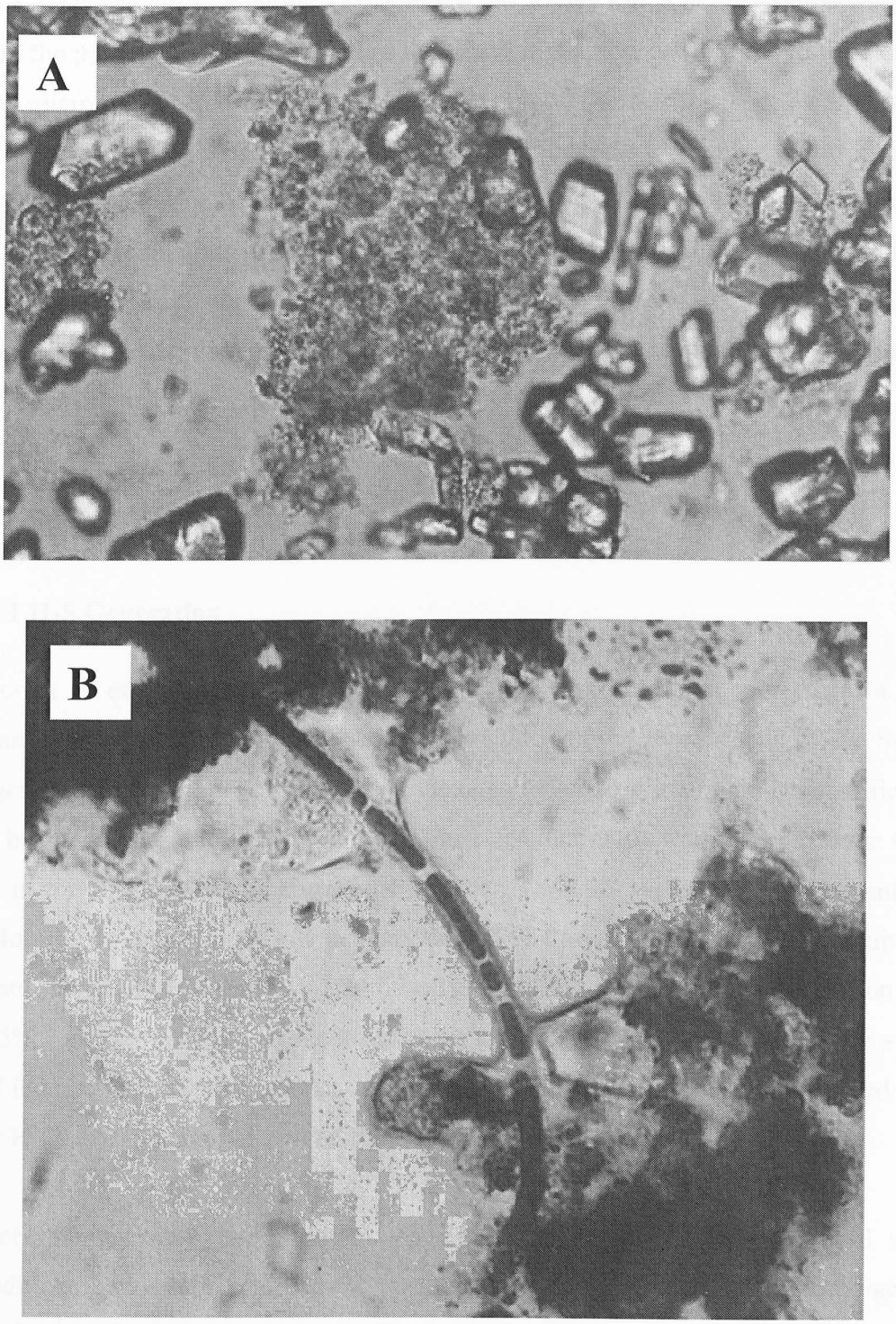


Figure 4. 18- Photographs of samples taken from the packing material in the bioscrubbers as seen under a light microscope. A: Bacterial flock approximately 150 μm in diameter, surrounded by sulfur crystals. B: Gram positive bacterial species, possibly *Thiobacillus* 1.5 μm in diameter.

4.3.2.3 *Thiobacillus* growth: At the end of the 30-day run, a liquor sample was taken from the packing material, stained as described in Section 3.4.15, and viewed under a light microscope. Had there been a proliferation of *Thiobacillus* species, they would have been instantly recognisable as resembling filaments containing sulfur granules, or 'strings of sulfur beads'. The photograph A in Figure 4.18 clearly shows an absence of filamentous or bead-like microorganisms; instead only sulfur crystals can be seen. A search through several samples did show one set of organisms that resembled a description of the filamentous *Thiobacillus* species (Photo B), but they had by no means proliferated.

4.4 DISCUSSION

4.4.1 H_2S Generation

In order to establish a hybrid H_2S Generator, it was important to perform a mass balance of the sulfur that would be recycled through the three stages of the Sulfate Digestion Process. By researching previous studies into primary sludge acidification for heavy metal solubilisation and pathogen destruction, it was found that the target pH in the influent sludge should be between 1.5-2.0. A titration experiment was performed on a sample of raw primary sludge to find the mass of sulfate required to create such a pH, and this was established at 5 g/l SO_4 for a sludge concentration of 2-2.65%. Consequently, two identical completely methanogenic reactors were set up and fed raw primary sludge at an initial HRT of 40 days. This was then reduced to 20 day HRT over a period of several months.

After running these two digesters equally as methanogenic reactors until stable conditions had established, it was possible to statistically verify that the digestion efficiency in both reactors was nearly identical. Once sulfate had been added to the feed of Reactor S to establish sulfidogenic conditions, there was an immediate response in terms of a pH increase in the reactor, probably due to the production of NaOH. In addition, sulfidogenesis levels reached maximum levels within a week. Similar efforts by Forrest (1999) and Hobson *et al.* (1994) to try to establish sulfidogenic conditions in a methanogenic reactor failed, with no significant sulfide production for months after initial sulfate addition. Only after seeding the reactor

with a source of SRB did H_2S production commence in the latter study. Although this suggests the importance of seeding a reactor with an established SRB population, this cannot be sufficiently verified since no control was available to compare the effects of seeding with non-seeding in this study. The anaerobic environment in the afore mentioned studies will not have been identical to the current project. Similarly, some authors in the literature report an immediate increase in H_2S production upon sulfate addition (Ueki *et al.*, 1988). It is probably wiser to conclude that should sulfide production in a stable, fully anaerobic reactor not begin within the first HRT after sulfate supplementation, seeding of the sludge should be considered.

Overall, a measured COD:SO_4 ratio of 5.45:1 caused almost 30% of the electron flow to be directed towards sulfidogenesis, and the CH_4 concentration to be 52.5% in the biogas. The chemical scrubber maintained the headspace sulfide concentration below 0.1%, which was lower than reported values that normally cause sulfide inhibition in a reactor. The percentage sulfate reduction was 100% throughout the run, with residual SO_4 concentrations below 20 mg/l in the effluent. This indicates that sulfate levels at this ratio were not in excess, with the possibility of increasing the ratio even further. A further reaction to sulfate reduction is a statistically significant increase in the effluent TS and VS concentration compared to Reactor M (as confirmed by the Wilcoxon Signed-Rank test).

On the other hand, the difference in TCOD and supernatant effluent quality in terms of residual VFA concentrations is insignificant (and in both cases is of a high standard), suggesting that digestion efficiency had not in fact been compromised in Reactor S. There are two probable explanations for this; the first may be the precipitation of metals as metal sulfides from the production of H_2S contributing to higher overall effluent TS concentrations. A confidential report published in 1998 at WRc plc. (personal correspondence with the author, John Hobson), assessing heavy metal content of UK sewage sludge found the total concentration of 6 heavy metals (Cd, Cr, Cu, Hg, Ni, Pb) to be less than 1000 mg/kg ds, and calcium and magnesium to be ~3500 mg/kg ds each. If the average RMM of the former six heavy metals is taken as 116, and those of Ca and Mg are 40 and 24, respectively, then it is possible to calculate the maximum contribution of these metals as metal sulfides to the final TS concentration. Equation 4.3 represents the Molar reaction of a heavy metal with

hydrogen sulfide; a conversion into mass coefficient yields equation 4.4a, 4.4b and 4.4c:

$M^{2+} + HS^- \rightarrow MS\downarrow + H^+$ (eq. 4.3)

$116M^{2+} + 33\ HS^- \rightarrow 148MS + 1H^+$ (eq. 4.4a)

$40Ca^{2+} + 33\ HS^- \rightarrow 73CaS + 1H^+$ (eq. 4.4b)

$24Mg^{2+} + 33\ HS^- \rightarrow 56MgS + 1H^+$ (eq. 4.4c)

Where;

M = Heavy Metal with a RMM of 116

Taking the influent sludge TS concentration as 20 g ds/l, and assuming it contained 1000 mg M/kg ds and 3500 mg Ca and Mg /kg ds, then the total concentration of M, Ca and Mg would be (20+70+70) 160 mg/l. Assuming all the heavy metals precipitated out as metal sulfides as in equation 4.3, the resulting total mass of MS, CaS and MgS precipitate would be approximately 320 mg/l. This figure would account for less than 23% of the discrepancy between the TS concentration in the effluent of Reactor M and S. Since this assumes complete precipitation of the metals, in Reactor S and none in Reactor M, this value will be an overestimate of the true value.

The second possibility as to the differences in TS effluent concentration may be down to reactor kinetics. The solid fraction of COD entering and leaving a reactor can be divided into particulates and biomass, whereas the soluble fraction may be divided into substrate and products (Eastman and Ferguson, 1981). Taking the solid fraction first, in a steady state CSTR the degradation of particulate matter during the hydrolysis stage was previously described in Chapter 2 as following a First Order Kinetic model. The mass balance for particulate degradable COD was presented in equation 2.12. Since it can be assumed that hydrolysis is the rate limiting step in anaerobic digestion of sewage sludge (due to the lack of accumulation of VFA's in either digester), and that SRB and MPB do not take part in the initial stages of anaerobic digestion (hydrolysis), we would expect K_h to be similar in both the methanogenic and sulfidogenic digesters. As a result, effluent degradable particulate

COD should be similar in both reactors as well, which agrees with the results we obtained. Concerning the higher effluent TS concentration in Reactor S, this may be explained by the biomass fraction of the solids. The mass balance of organisms in a reactor can be written according to equation 4.5 (adapted from Eastman and Ferguson, 1981):

$$X_{b,e} = \frac{Y(\mathrm{COD}_{ipdeg} - \mathrm{COD}_{epdeg} + S_i - S_e) + X_{b,i}}{1 + (b \cdot HRT)} \qquad \text{(eq. 4.5)}$$

Where;

$\mathrm{COD}_{epdeg.}$ = Effluent degradable particulate COD (kg/m^3)

$\mathrm{COD}_{ipdeg.}$ = Influent degradable particulate COD (kg/m^3)

$X_{b,e}$ = Effluent bacterial biomass (kg/m^3)

$X_{b,i}$ = Influent bacterial biomass (kg/m^3)-can be assumed as negligible

S_e = Effluent substrate COD (kg/m^3)

S_i = Influent substrate COD (kg/m^3)

b = Cell decay rate (d^-)

Y = Biomass yield (kg X_b/kg S)

We can see that $X_{b,e.}$ depends on the yield (Y) of biomass from the available COD substrate. Hence, if Reactor S had bacteria with higher biomass yields, this would be reflected in the higher effluent TS concentration, which was indeed the case. We also saw how both Reactors M and S had similar effluent SCOD concentrations as measured by the VFA concentration. From the relationship established in equation 2.16, higher Yield coefficients for SRB but with similar SCOD substrate uptake rates as MPB would mean higher growth rates for SRB. Indeed, according to a comprehensive literature research by Rinzema and Lettinga (1988b), the authors found that "sulfate reducers generally combine a higher maximum specific growth rate with a lower half-saturation constant". Differences in Y between SRB and MPB can therefore contribute to higher TS and VS concentrations in the effluent, providing that the HRT in both reactors is the same.

The Y for mesophilic SRB and MPB growing on $H_{2.}$ or acetate are higher for the former. Listed in Widdel (1988) are Y-values for SRB in the range of 0.7-1.4 g

VSS/mol acetate for *Desulfovibrio baarsii* and 11.2 g VSS/mol acetate for *Desulfobacter postgatei,* both in the presence of sulfate and thiosulfate, respectively. The average yield of all types of SRB listed is 4.3 ± 3 g VSS/mol H_2 or acetate. On the other hand, the range of Y-values for MPB's is much lower at 0.7-2.2 g VSS/mol H_2. as a minimum, and 1.6-3.2 g VSS/mol acetate as a maximum for *Methanosarcina barkeri* (average of all MPB listed is 1.5 ± 0.9 g VSS/mol). Table 4.4 summarises Y-coefficient values for different bacterial species, and reflects the fact that generally speaking, SRB seem to have higher yields than MPB for the same substrates.

In their research, Hobson *et al* (1994) who originally looked at the Sulfate Digestion process found that in the completely sulfidogenic digester supplemented with calcium sulfate in the feed, the carbon balance indicated that carbon was being fixed in the sludge. The expected ratio of 1.75 volumes of CO_2 for every volume of H_2S produced (see equation 2.23) was not met and remained much lower throughout the run. Carbon fixation as inorganic carbonate was excluded since the addition of acid to the sludge did not release any more carbon dioxide. However, the authors speculated that this fixation contributed to lower measured solids reduction than had been expected. From the evidence presented in this work, it is proposed that the 'missing' carbon may have been fixed as SRB bacterial cells due to their higher yields.

It is possible to use the data from the literature and Reactors M and S to determine whether or not the theory of higher yields for SRB is plausible. According to equation 4.5, if we assume an average Y-coefficient of 0.04 g VSS/g COD of substrate for MPB's (obtained by averaging the lower-values of Y in Table 4.4), a TCOD depletion of 19.8 g/l, an HRT of 20 days, and b of 0.05 d^{-1}, this would give the steady state concentration of MPB's in Reactor M of 0.40 g VSS/l. This is approximately 4.0% of the TS concentration in the reactor. Similarly, according to methanogenic TCOD destruction in Reactor S (electron flow of 71% towards methanogenesis of a total of 18.7 g/l COD destroyed), the MPB concentration using the same parameters for HRT, Y and b, is 0.27 g VSS/l in the effluent, or 2.4% of the effluent TS concentration. In order to contribute to the higher TS concentration in the effluent of Reactor S, the VSS concentration of the SRB's must equal approximately 1.4 g/l. We know that the proportion of TCOD destroyed by SRB amounts to 5.42

g/l. Therefore, the equivalent Y-value for the SRB's in Reactor S is 0.51 g VSS/g COD substrate. This figure is significantly higher than the Yield coefficients reported for MPB's, but only ⅔ higher than the upper-end of the Y-coefficient reported in Table 4.4 for the SRB *D. vulgaris* growing on H_2.

Table 4. 4- Selected bacterial species and their range of yields (adapted from Rinzema and Lettinga, 1988b)

Bacteria	Substrate	Yield (g VSS/mole substrate)	Yield (g VSS/g COD substrate)
Sulfate Reducers			
Desulfovibrio sp.	H_2	0.85	0.05
D. vulgaris	H_2	1.0-2.5	0.06-0.16
D. gigas	H_2	1.75-2.0	0.11-0.13
D. desulfuricans	H_2	1.75-2.0	0.11-0.13
Desulfobacter postgatei	Acetate	2.56-4.8	0.04-0.07
Desulfotomaculum acetoxidans	Acetate	5.52-7.55	0.09-0.12
Desulfobulbus propionicus	Propionate	3.67-4.9	0.03-0.04
Methane Producers			
Methanosarcina bakerei	H_2	1.6-2.18	0.10-0.14
Methanobrevibacter arboriphilus	H_2	0.64	0.04
Methanobacter hungatei	H_2	0.2	0.01
Methanobacterium sp.	H_2	0.6	0.04
M. concilii	Acetate	1.13-1.16	0.017-0.018
M. barkeri	Acetate	1.56-3.2	0.02-0.05
Methanosarcina sp.	Acetate	3.2-4.5	0.05-0.07
Acetogens			
Syntrophobacter wolinii enrichment culture	Propionate	2.8	0.02

It must be noted that the tabulated values are for pure cultures of the bacterial species growing on relatively simple substrates, so could well be higher for mixed cultures of SRB growing on more complex substrates. Consequently, the theory of higher yield

coefficients of SRB contributing to higher TS concentrations in the effluent remains plausible, even more so if the contribution to final TS concentration from metal precipitation is taken into account, as this will bring the yield coefficient of SRB down closer to the literature values.

The results from this part of the project imply that sulfate reduction does not impede the overall digestion process in terms of solids stabilisation. Although the aims of anaerobic digestion are to reduce the amount of solids for disposal, in all probability it is the higher yields of SRB compared to MPB that cause the solids concentrations of sulfidogenically-treated sludges to be higher than methanogenically treated sludges. Any further decrease in the COD:SO_4 ratio is expected to cause more of the electron flow to be directed towards SRB, thus increasing the effluent solids concentration even further. However, since the supernatant effluent quality and degree of stabilisation are comparable to methanogenic sludges, recycling of semi-sulfidogenically treated sludges onto land could be beneficial in terms of supplying more fertiliser volume, and should not be any more detrimental to the environment.

4.4.2 Sulfide Oxidation

The novel design for the H_2S bioscrubbers was built and tested for its sulfide oxidation efficiency. At the start of experimentation, the nature of the recirculation system of liquid and gas through the two compartments of the bioscrubber created pressure differences between different sections of the bioscrubber. A lack of pressure regulation resulted in the aeration of the connected H_2S Sulfide Generator, which ultimately led to its shut-down. Once the pressure problems were resolved, it was seen that sulfide oxidation occurred instantly in the air-lean bioscrubber, with a concomitant decrease in pH of the liquor to pH 4 in the first 5 days of operation, and sulfate concentration of 3.5 g/l. This shows that both chemical and biological oxidation of the sulfide was occurring, since chemical oxidation of H_2S completely to sulfate is not spontaneous. However, after this initial period, sulfide continued to be oxidised at a high average efficiency of 92%, but sulfate production ceased right to the end of the run, indicating a possible shut-down of the sulfur oxidising micro-organisms. It was not clear why this had occurred, although it did coincide with the

period after a high sulfide concentration of 4.5% was introduced into the bioscrubber. This may have resulted in the inhibition of the micro-organisms, which were never able to re-establish themselves. It was also thought that introducing fresh activated sludge liquor to the bioscrubbers every week to replace the liquor that was bled off would have provided a source of nutrients for the micro-organisms. However, since a nutritional evaluation of the needs of the bacteria was not carried out, it is possible that the micro-organisms became nutrient deficient.

The sample analysis under the light microscope revealed a lack of proliferation of filamentous *Thiobacilli*, which would be expected in sulfur-rich environments, supporting the argument that the micro-organisms that were responsible for the initial sulfur oxidation to sulfate in the first few days of the experimental run became inhibited and were washed out over time.

Overall, a more in-depth investigation is required to optimise the liquid flow and bleed rates, as well as the gas residence times and maximum sulfide concentrations tolerated by sulfur oxidising bacteria.

4.5 SUMMARY

A concentration of 5 g/l SO_4 as H_2SO_4 added to raw primary sludge at a concentration of 2-2.65% reduced the pH of the sludge down to 1.85. This concentration was equivalent to a COD:SO_4 ratio of 5.2:1, which is necessary for adequate sulfur to be passed around the three stages of the Sulfate Digestion process. When a stable methanogenic reactor was fed sulfate at the required ratio, sulfate reduction commenced almost immediately after initial sulfate addition. The addition of sodium sulfate increased the pH in the reactor, probably due to the production of NaOH as sulfate was reduced and H_2S removed from the system. This may ultimately influence the competition between SRB and MPB for certain substrates due to the favouring of SRB at higher pH's.

A measured COD:SO_4 ratio of 5.45:1 resulted in approximately 30% of the electron flow towards sulfidogenesis, with complete reduction of the added sulfate. The use of

a Hybrid H_2S Generator negatively affected the concentration of solids in the final effluent, although the supernatant effluent quality and TCOD concentrations were comparable to a methanogenic reactor. The most likely explanation for this was demonstrated as a combination of metal precipitation and the higher Y-coefficient for SRB (calculated at 0.51 g VSS/g COD substrate) compared to those of MPB, rather than a reduction in digestion efficiency. The prospects of using hybrid anaerobic digestion as a means of solids stabilisation of primary sludge therefore remains viable.

Concerning the H_2S Bioscrubber, it was shown that the novel design for a 2-compartment bioscrubber had the potential of oxidising sulfide into sulfate. The experiment carried out was not successful in achieving full oxidation of the incoming sulfide to sulfate, although the sulfide concentrations leaving the bioscrubbers were substantially reduced. Unfortunately, this was accounted for by chemical oxidation of the H_2S to elemental sulfur only for a substantial part of the experimental run, and only minimal biological sulfur oxidation to sulfate was observed. More investigations are necessary on this part of the project to optimise the conditions for complete sulfide oxidation to sulfate.

Due to the substantial time that would be required to fully investigate both the H_2S Bioscrubber and H_2S Generator, it was decided to concentrate on just one aspect of the 3-stage Sulfate Digestion process, which was the H_2S Generator.

CHAPTER 5

EFFECT OF SULFURIC ACID ON HYBRID SULFIDE GENERATION

5.1 INTRODUCTION
5.2 PREPARATORY STUDIES & RESULTS
5.3 REACTOR RESULTS
5.4 DISCUSSION
5.5 SUMMARY

5.1 INTRODUCTION

The initial stage in the theory behind the Sulfate Digestion process was to solubilise heavy metals and destroy pathogens from raw primary sludge using sulfuric acid. In the previous chapter, the source of sulfate used was sodium sulfate in order to start-up a hybrid H_2S Generator, but this had the effect of slightly increasing the biosolids concentration in the final effluent. In this part of the project, in order to mimic the original purpose of the Sulfate Digestion process, the source of sulfate was switched to sulfuric acid. Initially, a small-scale test to investigate the effect of sulfuric acid on the hydrolysis of raw primary sludge was carried out. Also, due to the likely event of a pH-drop upon sulfuric acid addition to the reactor, an adapted Anaerobic Toxicity Assay (ATA) was performed on the seed sludge from Reactor S to ascertain the acid-tolerance limits of a full-scale reactor.

In this Chapter, the operation of a total of four daughter hybrid H_2S Generators, all seeded from the original Reactor S is described. The completely methanogenic Reactor M was kept running as a control. Of the four H_2S Generators, three were run at COD:SO_4 ratios equal to, and either side of the theoretical ratio of 5.2:1 established in Chapter 4 (the ratio necessary to produce enough H_2S to pass onto the next two stages of the 3-stage Sulfate Digestion process). The final reactor was run at the

absolute limit of the acid tolerance established in the afore mentioned batch-test. All reactors were tested for their sludge digestion efficiency in terms of solids, COD and VFA destruction, sulfate reduction and biogas production. The final effluent from each reactor was also tested for its particle size distribution.

5.2 PREPARATORY STUDIES & RESULTS

The purpose of the first experiment (described in Section 5.2.1) was to investigate the effect of sulfuric acid on the hydrolysis of primary sewage sludge in terms of TS, VS, TSS and VSS into SCOD and VFA's. Dilute and concentrated sulfuric acid has been used for decades in industry as a means of hydrolysing lignocellulosic material from the pulp and paper, wood and cotton industries to produce ethanol from the released sugars. Concentrated acid disrupts the hydrogen bonding between cellulose chains, and once decrystallised, forms a homogeneous gelatin with the acid. Subsequent dilution with water results in a complete and rapid hydrolysis to glucose (Office of Transport Technologies, 2003). In terms of acid-hydrolysis of sewage sludge, most of the literature so far has focused on the acid-leaching of sludge to promote pathogen destruction and/or metal solubilisation in primary, activated or treated sludge (Jenkins *et al.*, 1981;Wozniak *et al.*, 1984; Blais *et al.*, 1992a; Tyagi *et al.*, 1997; Kitada *et al.*, 2001). Only one study was found, undertaken by Tyagi *et al.*, (1997) to investigate the effect of acid on the hydrolysis of solids, TOC and the nutrient value of a mixed primary and activated sludge. Sulfuric acid production was initiated by indigenous sulfur-oxidising bacteria in shake-flask experiments containing different concentrations of solids and S^0 to bring the pH down to approximately 2. The study found a substantial solids reduction for all the concentrations studied, but the nutrient content in terms of Nitrogen, Phosphorus and Potassium remained intact. No reports have been found on the effects of sulfuric acid on other organic portions of primary sewage sludge, such as COD and VFA's. It can be expected, judging from extensive research on lignocellulosic material (raw primary sludge can contain up to 40% w/v cellulose (Pohland, 1962)), acid hydrolysis of sludge should increase the soluble organic fraction, especially acetic acid.

The second experiment performed (Section 5.2.2) was an ATA, which normally looks at the adverse effect of a toxin on the final and most critical stage of anaerobic digestion which is methanogenesis (Owen *et al.*, 1979). A fixed volume of seed sludge was anaerobically transferred to serum bottles and inoculated with a biomedia solution containing essential nutrients and vitamins. An easily degradable 'spike' containing acetate and propionate is used as a substrate, and then a toxin is added in different concentrations to different bottles to see its inhibitory effect on methanogens by measuring methane production levels. Using sulfate in the form of sulfuric acid as terminal electron acceptor for SRB has the disadvantage of the associated pH-lowering effect. The pH in the digester may fall to inhibitory levels if the acid concentration in the feed is too high. An ATA experiment was adapted in order to find the inhibitory concentration of sulfuric acid on anaerobic digestion.

5.2.1 Hydrolysis of Raw Sludge With Sulfuric Acid

A portion of fresh screened primary sludge (TS ~ 2.5 %) was sifted though a 0.075 mm diameter sieve in order to homogenise the sludge and prevent any 'lumps' from creating errors in the solids measurements (i.e final particle size of sludge < 0.075mm diameter); the final sludge volume was approximately 3L. A 5 ml supernatant sample was filtered with a 0.45µm Nalgene™ syringe filter and used to measure SCOD and VFA's in the liquor (see Tables 5.1 and 5.2 for results). The sludge was then divided equally into four conical flasks and allowed to settle for several hours. Different volumes of supernatant were removed from each flask to achieve a range of solids concentrations. The sludge from each flask was divided into five, 125 ml McCarty bottles with a volume of 60 ml in each bottle. One of the bottles from each set was used as a control. After sealing with a rubber bung, 10ml of the sludge was removed by syringe from each bottle and tested for TS, VS, TSS and VSS. TCOD measurements were not taken since these should remain constant throughout the experiment (no loss from the pool of COD is expected, only transfer from TCOD to SCOD). 10 ml of different concentrations of H_2SO_4 were added to each bottle by syringe to give the final SO_4 concentrations shown in Table 5.3, and which cover the range of concentrations used later in the lab-scale reactors. No additions were made to the control bottles. Each bottle was shaken vigorously and left at room temperature. 10 ml samples from each bottle were then taken and tested again for

solids, SCOD and VFA's and pH after 1 hour and 24 hours, with the results corrected for any natural degradation by subtracting the results from the control bottles.

Table 5. 1- Initial solids concentrations in the raw primary sludge. Average results from five bottles.

Bottles	TS (g/l)	VS (g/l)	TSS (g/l)	VSS (g/l)
1A-1E	25.4 ± 0.4	19.3 ± 0.4	19.2 ± 0.4	16.3 ± 0.4
2A-2E	14.6 ± 0.3	10.8 ± 0.3	10.9 ± 0.1	9.2 ± 0.1
3A-3E	9.0 ± 0.7	6.8 ± 0.6	6.6 ± 0.5	5.6 ± 0.5
4A-4E	4.9 ± 0.4	3.7 ± 0.3	3.5 ± 0.3	3.0 ± 0.3

Table 5. 2- Initial VFA and SCOD concentrations in the raw primary sludge. Results based on duplicate measurement of the supernatant liquor.

Bottles	Acetate (mg/l)	Propionate (mg/l)	Iso-butyrate (mg/l)	Butyrate (mg/l)	Iso-valerate (mg/l)	Valerate (mg/l)	SCOD (mg/l)
All	0±0	2±0	0±0	0±0	1±1	0±0	198±0

Table 5. 3- Mass of SO_4 added as H_2SO_4 to different bottles containing various solids concentrations of raw primary sludge.

SO_4 concentration as H_2SO_4 (g/l), Bottle				
1.75, 1A	3.50, 1B	5.36, 1C	7.00, 1D	0, 1E (control)
1.75, 2A	3.50, 2B	5.36, 2C	7.00, 2D	0, 2E (control)
1.75, 3A	3.50, 3B	5.36, 3C	7.00, 3D	0, 3E (control)
1.75, 4A	3.50, 4B	5.36, 4C	7.00, 4D	0, 4E (control)

For analysis of the results, it should be noted that the addition of H_2SO_4 may distort the VS measurement. Since H_2SO_4 is volatile, its loss would add to the VS measurement, thus decreasing the final apparent VS concentration. In addition, it was of interest to look at the portion of particulate matter solubilised as a result of H_2SO_4 addition. Since TS and VS measurements do not differentiate between particulate and

soluble fractions of the sludge, TSS and VSS measurements were used instead as a basis of judging solids hydrolysis of the raw sludge. By comparing TSS, VSS, SCOD and pH concentrations in each bottle 1 hour and 24 hours after initial H_2SO_4 addition, it was found that there was no significant difference in the results for any solids/SO_4 combination (results not shown). This indicates that hydrolysis of the solids was probably instantaneous, and that the length of time the sludge was exposed to the acid had no effect on increasing the chemical solubilisation rate. Figure 5.1 represents the overall destruction of TSS at the end of the test (24hrs). It is possible to see that at the same solids concentration, an increase in sulfate concentration increased the amount of TSS solubilised.

The relationship between TSS concentration and destruction is not linear for any of the sulfate concentrations used. The best-fit line for the data was a logarithmic curve, with R^2 values generally higher than 90% for three of the curves, indicating a good fit. It is possible to find a general relationship for predicting TSS solubilisation from H_2SO_4 by combining the equations of the curves from each graph according to Table 5.4 to obtain the graph presented within the Table. To predict TSS solubilisation from the addition of H_2SO_4, the values for TSS and SO_4 concentration can be substituted into equation 5.1.

$$\text{TSS Destroyed} = (1.4024 * \text{Ln}[SO_4] + 0.1054) * \text{Ln}[\text{TSS}] + (-2.102 * \text{Ln}[SO_4] + 0.7001) \quad \text{(eq. 5.1)}$$

Where;

$[SO_4]$ = SO_4 added to the primary sludge as H_2SO_4 ($g.l^{-1}$)

[TSS] = Initial Total Suspended Solids concentration of the primary sludge ($mg.l^{-1}$)

This relationship is restricted to domestic primary sewage with TSS concentrations between approximately 5-25 g/l, and particle size <0.075 mm in diameter. Due to the changing nature of domestic sewage both geographically and seasonally, such a relationship would best be derived on a batch-by-batch basis, but may be used as a guide to predict solubilisation levels.

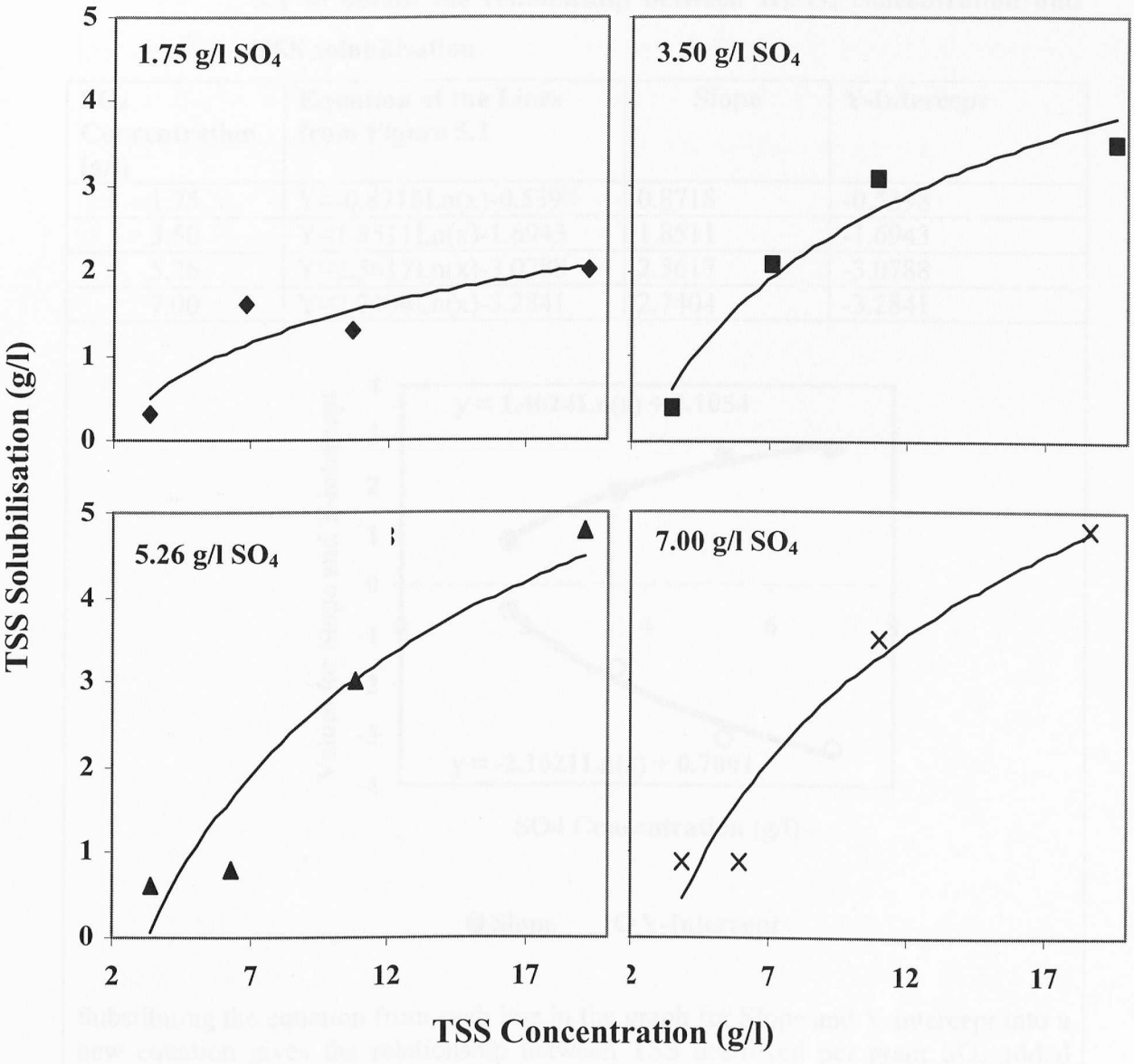
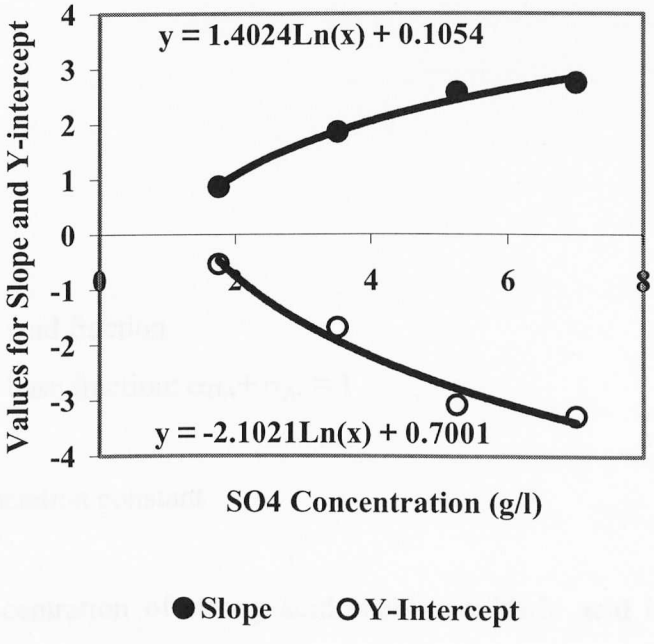


Figure 5. 1- Graphs showing the effect of different concentrations of SO_4 as H_2SO_4 on the destruction of the TSS in primary sludge.

Table 5. 4- Combining the equation of the lines for each graph from Figure 5.1 to obtain the relationship between H_2SO_4 concentration and TSS solubilisation.

SO_4 Concentration (g/l)	Equation of the Lines from Figure 5.1	Slope	Y-Intercept
1.75	Y=-0.8718Ln(x)-0.5398	0.8718	-0.5398
3.50	Y=1.8511Ln(x)-1.6943	1.8511	-1.6943
5.26	Y=2.5617Ln(x)-3.0788	2.5617	-3.0788
7.00	Y=2.7404Ln(x)-3.2841	2.7404	-3.2841



Substituting the equation from each line in the graph for Slope and Y-Intercept into a new equation gives the relationship between TSS destroyed per gram SO_4 added (equation 5.1).

Similarly, VSS destruction as a result of H_2SO_4 addition followed the same trend as the TSS. The relationship was analysed in the same manner as described for TSS, and the following correlation was obtained (eq. 5.2):

$$VSS\ Destroyed = (1.2402 * Ln[SO_4] + 0.0557) * Ln[VSS] + (-1.5815 * Ln[SO_4] + 0.5302) \quad \text{(eq. 5.2)}$$

Where;

[VSS] = Initial Volatile Suspended Solids concentration of primary sludge ($mg.l^{-1}$)

The relationship between amount of acid and organic solids solubilisation into SCOD is highly affected by the final pH of the sludge, which in turn is affected by the initial sludge buffering capacity. In wastewater systems, the buffering capacity is expressed as the calcium carbonate equivalent (see section 3.4.2). The fractional composition of the calcium carbonate into HCO_3^- and carbonic acid is dependent on the pH of the solution as shown in equations 5.3 and 5.4 below.

$$\alpha_{HA} = \frac{[H^+]}{[H^+] + K_a} \qquad \text{(eq. 5.3)}$$

$$\alpha_{A^-} = \frac{K_a}{[H^+] + K_a} \qquad \text{(eq. 5.4)}$$

Where;

α_{HA} = Conjugate acid fraction

α_{A-} = Conjugate base fraction; $\alpha_{HA} + \alpha_{A-} = 1$

$[H^+]$ = 10^{-pH}

K_a = Acid dissociation constant

When a molar concentration of strong acid such as sulfuric acid is added to the solution containing the conjugate acid-base pair, both react completely to give HA. Once the molar concentration of each conjugate acid-base pair after acid addition is known, the Henderson-Hasselbach equation can be used to predict the pH of the final solution (equation 5.5, Harris (1997)).

$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right) \qquad \text{(eq. 5.5)}$$

Where;

pK_a = -log K

$[A^-]$ = Conjugate base concentration (M)

[HA] = Conjugate acid concentration (M)

However, when considering raw primary sludge, the bicarbonate alkalinity equivalent is low at only approximately 100-200 mg/l. The main source of buffering in this case is from the sludge solids themselves (Sreekrishnan *et al.*, 1993; Tyagi *et al.*, 1997) so that using the bicarbonate equivalent to estimate pH would give an extremely over-estimated (low) pH value. A more accurate method of final pH estimation for primary sludge would be to combine the relationship between sulfate concentration with the solids concentration. The experimental results are illustrated in Graph A of Figure 5.2. It shows similar results to those obtained in Section 4.2.1 of Chapter 4, where the higher the solids concentration the greater the amount of acid required to bring the pH down to the same level as for a sludge with a lower solids concentration. The lowest pH obtained was 1.63 for a VSS and SO_4 concentration of 3.9 and 7.01 g/l respectively. Equation 5.6 can be used to predict the pH drop for different VSS concentrations with sulfuric acid derived from Figure 5.2.

$$pH = (-1.6123*Ln[VSS]+1.6608)*Ln[SO_4]+(3.1124*Ln[VSS]-1.6674) \quad \text{(eq. 5.6)}$$

From Graph B in Figure 5.2, it is possible to see that the amount of SCOD produced per gram VSS solubilised depends on the pH of the solution. More COD release occurs for the sludge at the lower VSS concentration (3.0 g/l) than those at the higher concentration (9.2 g/l) for the same pH. In terms of the proportion of SCOD released as VFA's, previous studies looking at the hydrolysis of ligno-cellulosic material found that acetate constituted the largest fraction of the VFA's released. The results in Table 5.5 indicated that the highest concentrations of acetate were released from the primary sludge with the highest SS concentration. This was expected since there is a larger availability of potentially-soluble solids. The amount of acetate released at each solids concentration was independent of the amount of H_2SO_4 added, except for the sludge with the lowest SS concentration. However, even for the sludge with the highest TSS and acid concentration (19.2 & 7.01 g/l respectively), the final acetate concentration in the liquor was 32 mg/l, constituting only 4% of the total 800 mg/l SCOD released. This indicates that the majority of SCOD released was not in the form of short-chain VFA's but probably higher molecular weight organic compounds, soluble nitrogen- and phosphate-compounds.

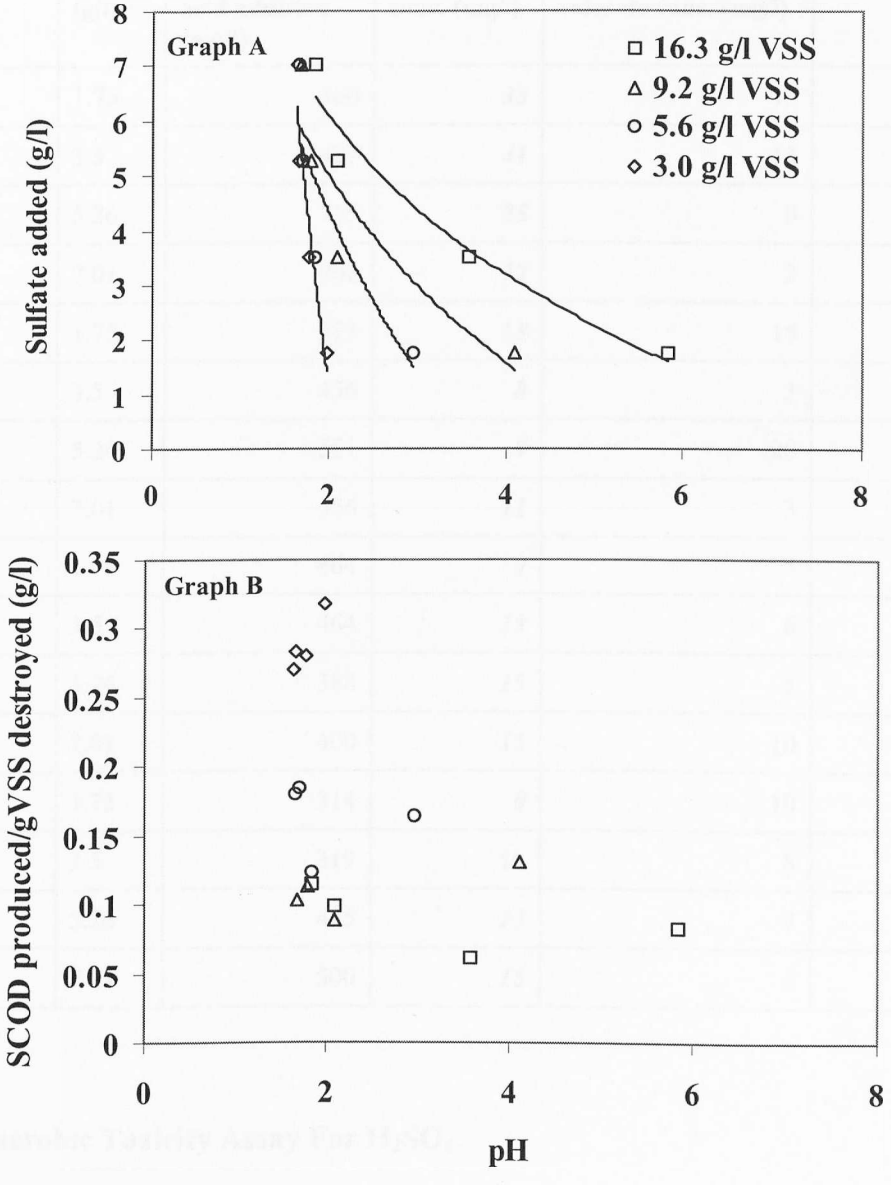


Figure 5. 2- **Graph A: Effect of sulfate concentration on the pH of the primary sludge at different solids concentrations. Graph B: SCOD produced per gram VSS destroyed as a function of pH.**

Table 5. 5- SCOD released as VFA's in raw primary sludge

Initial TSS concentration (g/l)	H_2SO_4 added (g/l)	Final SCOD in liquor after acid addition (mg/l)	Final acetate conc. (mg/l)	Sum of final propionate to n-valerate conc. (mg/l)	SCOD as acetate (%)
19.2 ± 0.4	1.75	360	***38***	13	11
	3.5	429	***41***	13	10
	5.26	715	***25***	0	4
	7.01	802	***32***	2	4
10.9 ± 0.1	1.75	373	***15***	15	4
	3.5	456	***8***	3	2
	5.26	551	***9***	20	2
	7.01	556	***11***	3	2
6.6 ± 0.5	1.75	464	***9***	4	2
	3.5	464	***13***	6	3
	5.26	388	***15***	5	4
	7.01	400	***15***	10	4
3.5 ± 0.3	1.75	314	***0***	10	0
	3.5	319	**10**	8	3
	5.26	405	***13***	7	3
	7.01	500	***15***	5	3

5.2.2 Anaerobic Toxicity Assay For H_2SO_4

Seed sludge from Reactor S was used, which was tested for VSS concentration according to Standard Methods. Clean, empty 125 ml McCartney bottles were flushed with a 30% CO_2:70% N_2 gas mix to drive out any oxygen. 50ml of the sludge was added to eight sets of duplicate bottles while being purged with the oxygen-free gas. No biomedia was added in this case so as to replicate the natural buffering capacity of the sludge in the large-scale reactors. The bottles were quickly sealed with a rubber bung, cable tied and placed upside down in a water bath to reduce chances of gas leakage and to maintain the temperature at 35°C (±1°C). The bottles were left overnight so that any residual COD could be exhausted. After 'zeroing' each bottle by

removing excess gas with a 20 ml glass syringe, 1 ml of a 'spike' containing 32.5 mg acetate and 13.25 mg propionate as sodium salts was injected into seven sets of duplicate bottles to give a final concentration of approximately 1200 mg/l COD equivalent. lml deionised water was injected into the eighth set to act as a control.

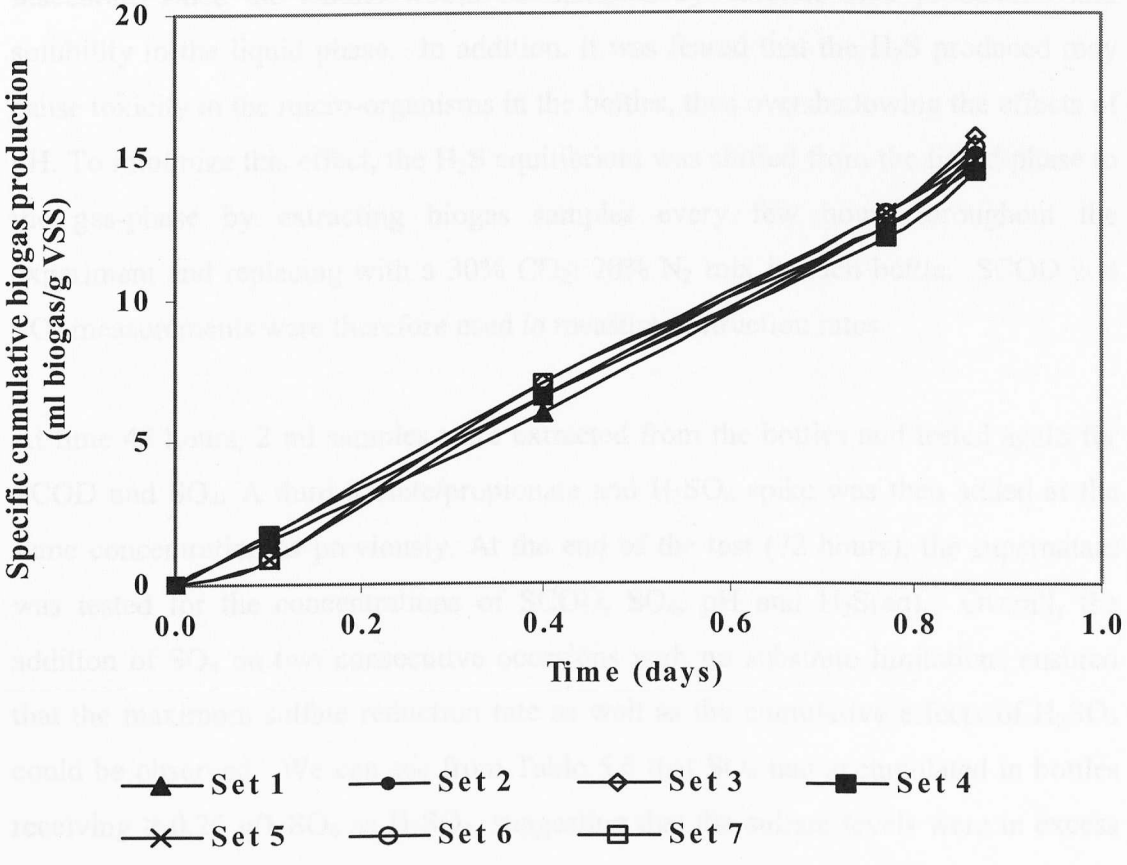


Figure 5. 3- Specific cumulative biogas volumes produced in each set of bottles after initial substrate spike (no SO_4 addition).

Cumulative biogas was measured several times during the first day to ensure that the same initial substrate utilization rates were apparent in all the bottles. Average biogas volume produced from the control set was subtracted from the results of all the bottles. It is possible to see from Figure 5.3 that the specific biogas production rates were near to identical in all the bottles. After 24 hours, a further 1 ml spike of the

acetate/propionate mix was added to the test bottles along with lml spike of various concentrations of H_2SO_4 to give the following final volumetric SO_4 concentrations: 0g/1, 0.05 g/1, 0.15 g/l, 0.25 g/l, 0.35 g/1, 0.46 g/l and 0.55 g/1. The bottles were shaken vigorously, 5ml samples extracted and immediately tested for initial SCOD concentration. Since a GC-TCD was not available at this stage in the project to measure methane concentrations, using cumulative biogas volumes would be inaccurate since the results would be distorted by variable H_2S production and solubility in the liquid phase. In addition, it was feared that the H_2S produced may cause toxicity to the micro-organisms in the bottles, thus overshadowing the effects of pH. To minimize this effect, the H_2S equilibrium was shifted from the liquid-phase to the gas-phase by extracting biogas samples every few hours throughout the experiment and replacing with a 30% CO_2: 70% N_2 mix in each bottle. SCOD and SO_4 measurements were therefore used to measure destruction rates.

At time 48 hours, 2 ml samples were extracted from the bottles and tested again for SCOD and SO_4. A third acetate/propionate and H_2SO_4 spike was then added at the same concentration as previously. At the end of the test (72 hours), the supernatant was tested for the concentrations of SCOD, SO_4, pH and H_2S(aq). Overall, the addition of SO_4 on two consecutive occasions with no substrate limitations ensured that the maximum sulfate reduction rate as well as the cumulative effects of H_2SO_4 could be observed. We can see from Table 5.6 that SO_4 had accumulated in bottles receiving $\geq$ 0.25 g/1 SO_4 as H_2SO_4, suggesting that the sulfate levels were in excess for such a short 24 hour period. In comparison, the bar-chart from Figure 5.4 shows the levels of SCOD destruction from each set of bottles at the two time intervals. It is clear that after the addition of the initial substrate spike, there was no significant difference in the SCOD destruction levels (between 76-77%) for any of the bottles compared to the one receiving no H_2SO_4. Overall, although SO_4 levels were in excess in bottles receiving 0.25-0.55 g/1 SO_4, at this stage no inhibitory effects are apparent. Upon addition of the final substrate and H_2SO_4 spike, sulfate levels decreased to background levels for bottles receiving $\leq$0.15 g/l SO_4, and the final pH remained above 6.5.

Table 5. 6- Average residual SO_4 levels after 48 and 72hrs of spiking with a VFA substrate and H_2SO_4 in duplicate bottles.

Bottle Set	Volumetric SO_4 conc. from spike (mg/l)	Residual SO_4 conc. in bottle 48 hrs after 2nd spike (mg/l)	Residual SO_4 conc. in bottle 72 hrs after 3rd spike (mg/l)	End pH at 72 hrs	End sulfide concentration at 72 hrs (mg/l)
1	0	21± 0	21± 0	6.60± 0.01	0.3± 0.0
2	50	21± 0	23± 2	6.57± 0.01	1.4± 0.1
3	150	26± 2	28± 2	6.51± 0.01	3.2± 0.1
4	250	66± 2	60± 3	6.47± 0.01	8.2± 0.1
5	350	137± 4	187± 7	6.36± 0.02	12.6± 0.4
6	460	156± 8	365± 9	6.26± 0.05	18.0± 1.0
7	550	400± 9	580± 7	6.05± 0.04	15.3± 0.8

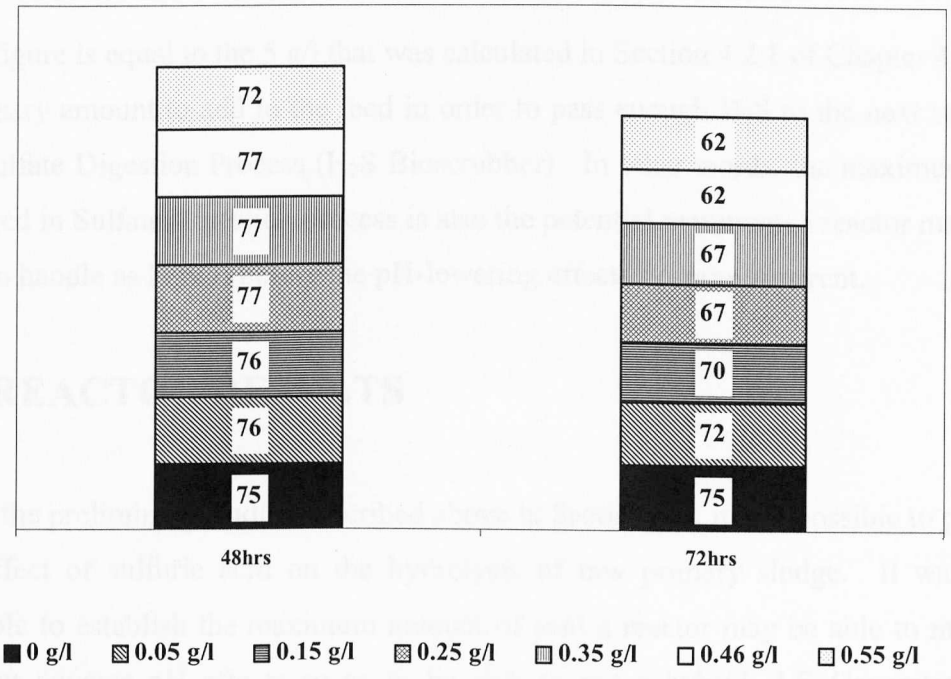


Figure 5. 4- Bar-chart representing SCOD destruction levels after 48 and 72 hours of spiking with a substrate and H_2SO_4 mix. Legend refers to concentration of SO_4 in each set of bottles after spiking.

For the bottle receiving 0.25 g/l SO_4, although the residual sulfate concentration was above background levels (0.060 g/l compared to 0.021 g/l), it still decreased below the background level of the previous spike, indicating that SRB were able to cope with consecutive spikes of H_2SO_4 at this concentration. For all other bottles, the residual SO_4 increased after the second spike and the pH decreased. The final sulfide concentration in the aqueous phase remained below 20 mg/l in all bottles, which is lower than literature values reported to cause sulfide toxicity to microorganisms (see Table 2.3). Consecutive applications of a sulfate spike within a 24hr period reduced the SCOD destroyed in all bottles due to the inhibitory effects of low pH. The maximum consecutive level at which the SRB were able to reduce the sulfate to repeatable levels was 0.25 g/1, above which background levels from previous spikes began to accumulate. Translating this figure into a large-scale CSTR reactor, the addition of a maximum 0.25 g/l SO_4 as H_2SO_4 in an 8L reactor equals:

0.25 g/l SO_4 x 8L = 2.0 g SO_4 as H_2SO_4 per reactor, which equates to 5 g SO_4 /L sludge feed/day

This figure is equal to the 5 g/l that was calculated in Section 4.2.1 of Chapter 4 as the necessary amount to add to the feed in order to pass enough H_2S to the next stage of the Sulfate Digestion Process (H_2S Bioscrubber). In other words, the maximum SO_4 required in Sulfate Digestion process is also the potential maximum a reactor may be able to handle as H_2SO_4 before the pH-lowering effects become apparent.

5.3 REACTOR RESULTS

From the preliminary studies described above in Section 5.2, it was possible to predict the effect of sulfuric acid on the hydrolysis of raw primary sludge. It was also possible to establish the maximum amount of acid a reactor may be able to manage without adverse pH effects so as to be able to run a hybrid H_2S Generator with sulfuric acid in a controlled manner. The set-up for the hybrid H_2S Generators receiving H_2SO_4 in the feed was described in Section 3.3 of Chapter 3. Reactor M continued to be fed as was the case in the previous part of the project in Chapter 4 to maintain methanogenic conditions. The source of sulfate in the feed of Reactor S was

switched from sodium sulfate to H_2SO_4. To avoid confusion, Reactor S with a sulfuric acid feed will be referred to as Reactor A from now on. In order to prevent any 'shock' to the reactor from sudden pH changes due to acid addition, the amount of sulfate added as sulfuric acid was reduced to 1/3 of the original concentration, and the HRT was doubled. Once stable conditions had been established, the effluent from Reactor A was stored in sealed plastic bottles and placed in a water bath (35°C ±1) until enough sludge had been collected to start up Reactors B, C and D. At this stage in the project, a GC-TCD was available for biogas analysis.

5.3.1 Start-up Results Of Reactor A With H_2SO_4

It was expected that the substitution of sodium sulfate by sulfuric acid in Reactor A would have some effect on the stability of the reactor in terms of pH and alkalinity changes. In order to minimize the stress on the reactor, the HRT was increased to 40 days and the concentration of added sulfate reduced to 1.8 g/l (as opposed to 5 g/l as was the case when sodium sulfate was being added). This is equivalent to 0.09 g sulfate/total reactor volume/day for a 20 day HRT, or 0.045 g/total reactor volume/day for 40 day HRT. This concentration is close to the minimum level of acid tested in the sulfuric acid ATA (see Table 5.6), which showed no negative effects on the degradation of a substrate spike. The reactor was run at this HRT for one week with no adverse effects on solids destruction or sulfate reduction. On day 10, the HRT was reduced to 27 days, and then 20 days on day 16. Graph A in Figure 5.5 shows the decrease in the pH and alkalinity of the reactor as the HRT was decreased. This was to be expected as a result of an increase in percent acidification and a relative reduction in the mineralisation of organic nitrates to the buffer NH^+_4.

However, a comparison with Graph B in Figure 5.5 shows that a combination of decreasing the HRT and increasing the amount of acid added to the reactor per day resulted in the accumulation of SCOD in the effluent from a stable 500 mg/l (HRT 40 days) to 1400 mg/l during the 20 day HRT period. Testing of the supernatant for VFA's attributed over 90% of this COD to acetate. At the same time, the specific TCOD destroyed (g TCOD/g VS) also decreased drastically from an initial maximum of 1.3 g/g to a low of 0.4 g/g by day 22, indicating some inhibitory effect on anaerobic

digestion. Such a build-up of acetate in the reactor would contribute significantly to the decrease in pH and alkalinity.

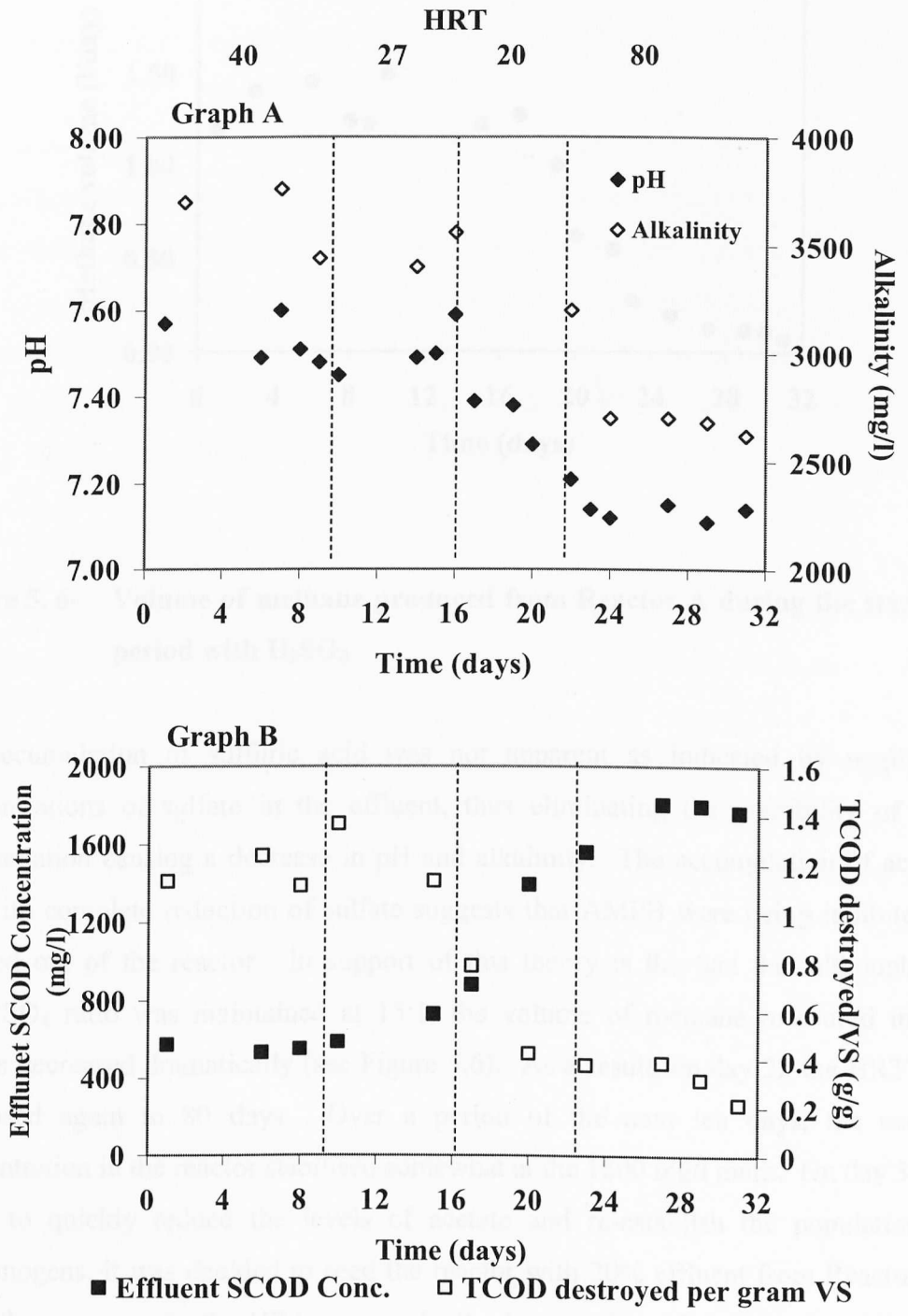


Figure 5. 5- Effect of sulfuric acid on initial performance of Reactor A. Graph A: Reactor pH and alkalinity; Graph B: Effluent SCOD concentration and specific TCOD destruction. Dotted lines indicate HRT (above Graph A) during the period between the lines.

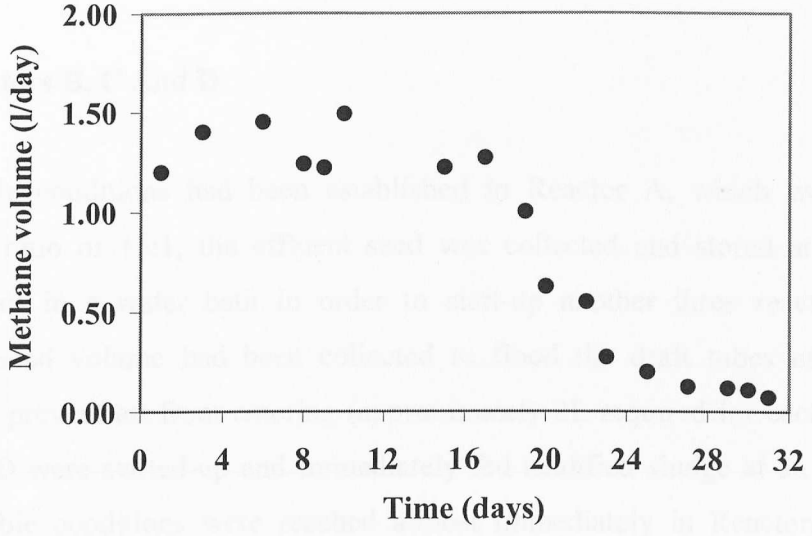


Figure 5. 6- Volume of methane produced from Reactor A during the start-up period with H_2SO_4

An accumulation of sulfuric acid was not apparent as indicated by negligible concentrations of sulfate in the effluent, thus eliminating the possibility of acid accumulation causing a decrease in pH and alkalinity. The accumulation of acetate but with complete reduction of sulfate suggests that AMPB were being inhibited or washed out of the reactor. In support of this theory is the fact that although the COD:SO_4 ratio was maintained at 15:1, the volume of methane measured in the biogas decreased dramatically (see Figure 5.6). As a result, on day 22 the HRT was increased again to 80 days. Over a period of the next ten days, the acetate concentration in the reactor stabilised somewhat at the 1800 mg/l mark. On day 32, in order to quickly reduce the levels of acetate and re-establish the population of methanogens, it was decided to seed the reactor with 20% effluent from Reactor M. Over the next month, the HRT was gradually decreased to 20 days again while still supplementing the feed with sulfate. By day 83, the SCOD concentration in the reactor had reduced to approximately 300 mg/l. Reactor A had been running for several weeks in an efficient manner (as Reactor S) prior to changing the HRT and source of sulfate to sulfuric acid. The most likely reason for this instability in the reactor is that the change of HRT from 40 days to 20 days in a short space of time,

which may have been too rapid to allow for the growth of a sufficient number of slow-growing MPB to cope with the higher COD load.

5.3.2 Reactors B, C And D

Once stable conditions had been established in Reactor A, which was run at a COD:SO_4 ratio of 15:1, the effluent seed was collected and stored at mesophilic temperatures in a water bath in order to start-up another three reactors. Once sufficient seed volume had been collected to flood the draft tubes of three new reactors to prevent air from entering (approximately 2L required for each), Reactors B, C and D were started-up and immediately fed acidified sludge at an HRT of 20 days. Stable conditions were reached almost immediately in Reactors B and C, indicated by lack of VFA accumulation in the effluent as well as complete sulfate reduction. In contrast, Reactor D did not stabilise as will be discussed in Section 5.3.3. A summary of the feed and sulfate concentrations entering each reactor can be found in Table 5.7. In order to clarify the extent of sulfate limitation in a reactor, Barber *et al.* (2000) formulated the idea of Rate Limiting Factors (RLF) for several sulfidogenic reactions (see Table 5.7). Since each electron donor requires a stoichiometric amount of electron acceptor, it is possible to quantify the amount of sulfate required to degrade a concentration of a certain substrate with sulfate as an electron acceptor. The form of the equation to calculate the RLF factor of a reaction is presented in equation 5.7.

$$RLF = \frac{\left(\frac{[D]}{[A]}\right)}{\left(\frac{MW_d M_d}{MW_a M_a}\right)} \qquad \text{(eq. 5.7)}$$

Where;

RLF = Rate Limiting Factor
D = Electron donor concentration (mol/L)
A = Electron acceptor concentration (mol/L)
MW = Molecular weight
M = Stoichiometric molar requirement

d = electron donor

a = electron acceptor

The sulfate concentration is considered increasingly rate-limiting as RLF values rise above 1. If the RLF = 1, neither electron donor nor electron acceptor are rate-limiting, while values < 1 indicate that the electron donor is rate-limiting. Freese (2001) found in her study treating sulfate-rich tannery wastewater in an ABR that, at the lowest COD:SO_4 ratio of 1:1, the degradation of acetate through to butyrate was substrate-limited with RLF values of 0.4. At the higher COD:SO_4 ratios, acetoclastic sulfidogenesis was generally the most sulfate-limited reaction. Literature values to predict sulfate limitation in anaerobic reactors are varied and will depend highly on reactor configuration, substrate type, temperature etc.

Table 5. 7- Stoichiometric sulfidogenic reactions used to calculate RLF values (Reactions 1-5 from Freese, 2001; Reaction 6 adapted from Metcalf and Eddy, (1979)).

Reaction	Donor/SO_4^{2-} ratio
1) $Ac^- + SO_4^{2-} \rightarrow HS^-$	0.625
2) $Pr^- + 1.75\ SO_4^{2-} \rightarrow 1.75\ HS^-$	0.440
3) $Pr^- + 0.75\ SO_4^{2-} \rightarrow 0.75\ HS^- + Ac^-$	1.028
4) $Bu^- + 2.5\ SO_4^{2-} \rightarrow 2.5\ HS^-$	0.367
5) $Bu^- + 0.5\ SO_4^{2-} \rightarrow 0.5\ HS^- + 2Ac^-$	1.833
6) $H_2SO_4 + 1.75CH_{1.9}O_{0.3}N_{0.24} \rightarrow H_2S + 1.75CO_2 + 1.03H_2O + 0.42NH_3$	0.85

Rinzema *et al.* (1988b) quoted vales of 100-200 mg/l SO_4 in the bulk liquid to pose no sulfate limitation, while Barber *et al.* (2000) found that values between 100-500 mg/l in an ABR posed a degree of sulfate limitation on aceticlastic sulfidogenesis. Carepo *et al.* (2002) found that sulfate limitation in pure cultures of three *Desulfovibrio* species began at concentrations between 100-1000mg/l. With such diverse values quoted, using an RLF value is a useful way of normalising substrate and sulfate concentrations, allowing comparisons to be made despite substrate type. Since a complex wastewater is being treated in this project, a further sulfidogenic reaction

was included, which takes into account the approximate sludge formula presented by Metcalf & Eddy (1979) (see section 2.6.1).This is presented as equation 6 in Table 5.7. The RMM of sludge in the equation was calculated as 83.46. The form of the modified Rate Limiting Factor for Primary Sludge (RLF-PS) is presented in equation 5.8.

$$RLF-PS=\frac{\left(\frac{[PS]/83.46}{[SO_4^{2-}]/96.06}\right)}{\left(\frac{83.46\times M_{PS}}{96.06\times M_{SO_4^{2-}}}\right)} \qquad \text{(eq. 5.8)}$$

Where;

RLF-PS = Rate Limiting Factor for Primary Sludge

[PS] = Bioavailable primary sludge concentration, normally considered as 60% of total ($g.l^{-1}$)

$[SO_4^{2-}]$ = Sulfate concentration ($g.l^{-1}$)

83.46 = Molecular weight of sludge

96.06 = Molecular weight of SO_4^{2-}

M = Stoichiometric molar requirement

The COD:SO_4 ratio at which enough sulfate is theoretically available to destroy all the organic COD by sulfidogenesis is 0.67, as calculated from equation 2.18 $\left(\frac{96gSO_4^{2-}}{64gO_2}=0.67\right)$. Therefore, to confirm the accuracy of results generated from the RLF-PS equation, the RLF-PS value of a sludge with a COD:SO_4 ratio of 0.67 was calculated. The TS:COD ratio used was 0.77 g/g assuming all the sludge in this case was bio-available. The RLF-PS value generated was 1.3, close to the expected RLF-value of <1.0 at which substrate concentration is limiting. In order to include only the bio-available portion of a real influent sludge, results obtained for Reactor M in Chapter 4 show that a conservative value of 60% of the influent primary sludge COD can be considered bio-available. The RLF-PS values were calculated for all the sulfidogenic reactors and these are presented in Table 5.8. As we can see, all the

sulfidogenic reactors are expected to be sulfate limited at the COD:SO_4 ratios they will be running at.

Table 5. 8- Summary of conditions in Reactors M-D. RLF-PS values calculated assuming 60% biodegradability of influent sludge COD.

Reactor	Sulfate feed conc. (g/l)	Feed strength (g COD/l)	COD:SO_4 ratio	Sulfate concentration in reactor upon feeding (g/l)	Theroretical RLF-PS values
M	0	22±5	_	0.00	_
A	1.47	22±5	15:1	0.07	21
B	2.35	22±5	9:1	0.12	13
C	4.33	22±5	5:1	0.22	7
D	7.15	22±5	3:1	0.36	4

5.3.3 pH and Alkalinity In The Reactors

From Figure 5.7, it is possible to see the pH of the sludge in Reactor D which was fed sulfate at a higher concentration than the limit established in the ATA for H_2SO_4 (Section 5.2.2) of a volumetric concentration of 0.25 g/l. A steady decrease in reactor pH was apparent as soon as H_2SO_4 addition commenced, so that after less than 2 retention times, the pH had fallen to 6.2 and effluent sulfate concentrations had steadily accumulated to over 800 mg/l, confirming the batch test results that such a concentration in the reactor is unsustainable. In contrast, the pH in Reactors M and A remained similar at an average of 7.02 (±0.1) throughout the run. The average pH in Reactors B and C was 7.35 (±0.10) and 7.9 (±0.34) respectively.

In Chapter 4, where sodium sulfate was used as a source of sulfate in Reactor S at a similar concentration as that in Reactor C, it was hypothesised that the production of sodium hydroxide according to equation 4.1 may have caused the increase in reactor pH. In this case, the lack of sodium sulfate suggests the reason for such an increase in pH may be attributed to increasing levels of bacterial sulfate reduction. SRB activity produces approximately two moles of alkalinity per mole of sulfate reduced. The exact amount of alkalinity varies with the structure of the electron donor. For

example, sulfate reduction with acetate as the electron donor produces the alkalinity shown in equation 5.9:

$$CH_3COO^- + SO_4^{2-} + H^+ \rightarrow H_2S + 2HCO^-_3 \quad \text{(eq. 5.9)}$$

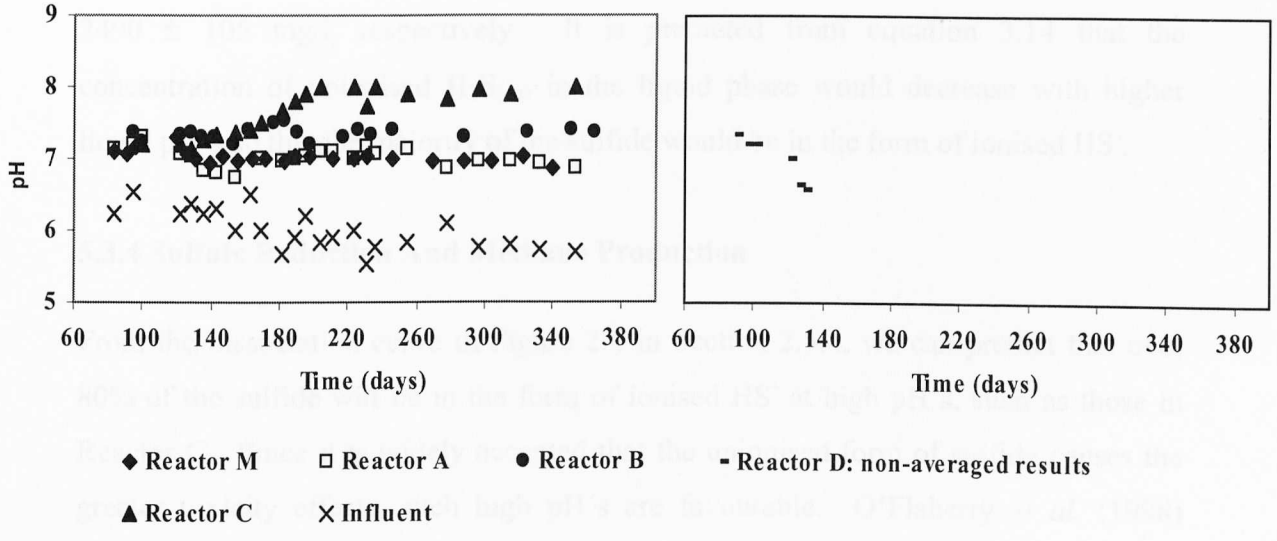


Figure 5. 7- pH of effluent in Reactors M-D. Points for Reactor M-C represent 5-point averages.

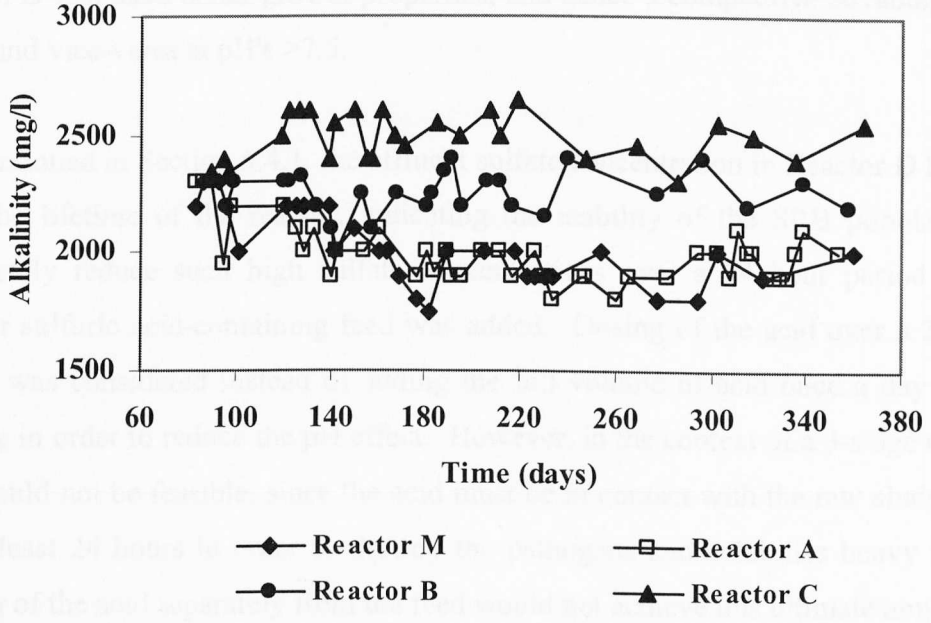


Figure 5. 8- Alkalinity measured in Reactors M to C.

This theory is supported by the fact that Total Alkalinity (which include hydroxide, carbonate and bicarbonate alkalinity) in each reactor increased, in comparison to Reactor M as the amount of sulfidogenesis increased as shown in Figure 5.8. Average alkalinity levels were similar in Reactors M and A, at 2015 ± 150 mg/l and 2005 ± 120 mg/l, respectively, while those of Reactors B and C were 2245 ± 95 mg/l and 2490 ± 105 mg/l, respectively. It is predicted from equation 3.14 that the concentration of unionised $H_2S_{(aq)}$ in the liquid phase would decrease with higher liquid pH's so that the majority of the sulfide would be in the form of ionised HS^-.

5.3.4 Sulfate Reduction And Methane Production

From the dissociation curve in Figure 2.4 in Section 2.5.4, we can predict that over 80% of the sulfide will be in the form of ionised HS^- at high pH's, such as those in Reactor C. Since it is widely accepted that the unionised form of sulfide causes the greater toxicity effects, such high pH's are favourable. O'Flaherty *et al.* (1998) looked at the growth kinetics of a number of sulfate and non-sulfate adapted sludges, and found the pH optima for MPB to be between 7.0 and 7.5, and SRB in the range of 7.5-8.5. Adjusting the pH of the sludges to either side of these optima resulted in a decrease in the μ_{max} for both species. Such pH optima will mean that at pH's below 7.0, MPB will have better growth properties, and hence a competitive advantage over SRB, and vice-versa at pH's >7.5.

As mentioned in Section 5.4.1, the effluent sulfate concentration in Reactor D built up over the lifetime of the reactor, indicating the inability of the SRB population to completely reduce such high sulfate concentrations over a 24 hour period before another sulfuric acid-containing feed was added. Dosing of the acid over a 24 hour period was considered instead of adding the full volume of acid once a day during feeding in order to reduce the pH effect. However, in the context of a 3-stage reactor, this would not be feasible, since the acid must be in contact with the raw sludge feed for at least 24 hours in order to destroy the pathogens and solubilise heavy metals. Dosing of the acid separately from the feed would not achieve this ultimate aim.

Another option would have been to run the CSTR as a continuously fed reactor instead of a batch-fed reactor to 'spread' the acid volume over a period of time. This

configuration was tested early in the project, but due to the small-scale nature of the reactors, narrow feed lines and rate of feeding, created many problems in terms of blockages of the feed sludge lines. As a consequence, Reactor D was decomissioned early in its lifetime, and results for Reactor D are not included from here on. In contrast to Reactor D, complete sulfate reduction was apparent in Reactors A, B and C, with no significant difference in the effluent concentrations as seen from Figure 5.9.

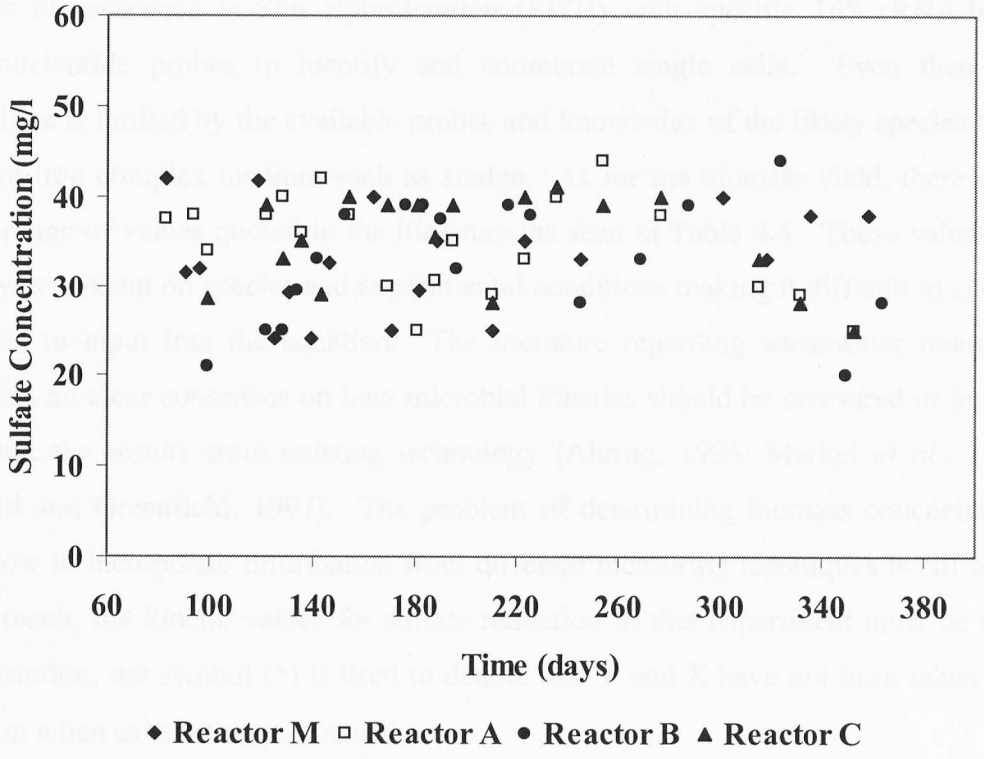


Figure 5. 9- 5-point-average effluent sulfate concentration in Reactors M-C.

By following the concentration of sulfate from the time of feeding in each reactor, it was possible to determine μ_{max} and K_s for sulfate in each reactor according to equation 2.17. Samples of sludge of 5 ml volume were extracted via syringe from the feed inlet of each reactor before and straight after feeding and every 0.5-1 hour intervals from thereon until stable effluent sulfate concentrations were apparent. The sludge was filtered through 0.45µm Nalgene™ filters and sulfate concentrations determined colorimetrically. The VSS concentration was averaged from daily VSS measurements made 7 days prior to this test. The experiment was repeated three days in a row, the results pooled and kinetic parameters calculated by a non-linear function analysis

software as described in Appendix D. The raw data can be found in Table E1 of Appendix E.

However, there are some limitations to measuring μ_{max} and K_s using this method. Firstly, the rate of substrate utilisation r_{su} from equation 2.17 is dependent on the biomass concentration X and the biomass yield Y. It is not possible to measure the mass of the proportion of SRB in the sludge using the conventional method described in section 3.4.9. One of the few possibilities available to measure this value would be to use Fluorescence In-Situ Hybridization (FISH) with specific 16S rRNA-based oligonucleotide probes to identify and enumerate single cells. Even then, the technique is limited by the available probes and knowledge of the likely species to be present in a complex medium such as sludge. As for the biomass yield, there are a wide range of values quoted in the literature, as seen in Table 4.4. These values are highly dependent on species and experimental conditions making it difficult to choose a value to input into the equation. The literature regarding wastewater treatment contains no clear consensus on how microbial kinetics should be measured or how to interpret the results from existing technology (Ahring, 1995; Merkel *et al.*, 1999; Pollard and Greenfield, 1997). The problem of determining biomass concentration and how to incorporate information from different measuring techniques is sill open. As a result, the kinetic values for sulfate reduction in this experiment must be used with caution; the symbol (*) is used to denote that Y and X have not been taken into account when calculating μ_{max} and K_s.

Table 5. 9- Kinetic parameters in full-scale Reactors A-C regarding sulfate reduction. * Derived from adapted equation 2.17-see Section 5.3.4 for explanation. $^{\Delta}$ See Appendix D for further explanation.

Reactor	μ_{max*} (d^{-1})	Standard Error of μ_{max*},$^{\Delta}$ (d^{-1})	Proportion of Variance Explained $^{\Delta}$ (R^2)	K_{s*} (g/l)	μ_{max*}/K_{s*} ratio
A	0.471	0.174	0.0833 (8.33%)	0.0947	4.97
B	0.965	0.116	0.4644 (46.44%)	0.0925	10.43
C	1.493	0.159	0.3996 (39.96%)	0.0756	19.75

Table 5.9 presents the results of the test to determine sulfate reduction kinetic parameters in Reactors A-C. The low "Proportion of Variance Explained" values are indicative of high scatter of the raw data around the estimated μ values. This is mainly because the results are based on the average of three separate trials for each reactor. From the Standard Error of the μ_{max*} values, we can see that the results for each reactor do not overlap, so that each reactor has a distinct μ_{max*} value.

The K_{s*} values for all three reactors are similar, although in Reactor A, which was fed extremely limiting sulfate concentrations, the K_{s*} is the highest indicating a low affinity for sulfate, while the corresponding μ_{max*} is low. This result is reversed when looking at Reactor C which was fed higher sulfate concentrations. By comparing the μ_{max*}/K_{s*} ratios, we can see that the SRB in Reactor C would have a greater competitive advantage for substrates than those in Reactor A. The fact that the same effluent sulfate concentrations were apparent in all three reactors contradicts the kinetic findings, since we would have expected higher residual sulfate concentrations in Reactors A and B as a result of the lower sulfate affinity.

These contradictions may be explained by the fact that the amount of time required for sulfate levels to reach approximately 50 mg/l in Reactor A and B was about 8 hours after initial feeding. To reach background levels of approximately 30 mg/l required a further 16 hours before the next feed, which would be sufficient time for any slow-growing SRB with lower K_{s*} values to completely reduce the sulfate to threshhold levels. In contrast, these background levels were reached within the 8 hour period for Reactor C, as explained by the lower K_{s*} values of the faster-growing SRB involved during the period of initial feeding.

The biogas composition and volume produced from each reactor was also measured regularly. The sulfide produced was scrubbed from the biogas of Reactors A-C in a zinc acetate solution so that headspace sulfide concentrations were maintained below 1% in all reactors. As expected, as the sulfate levels in the feed increased from

Reactor M to C, the percentage methane in the biogas decreased while H_2S levels increased as shown in Figure 5.10. However, due to the increasing VS concentrations from Reactor M to C (as we will see in Section 5.5.5), which may not all consist of active bacterial biomass, reporting of specific total bigas production per day (L biogas/gVSS/day) may not be accurate. Biogas yield per unit mass substrate added (L biogass/gVSS added) would be a better value to use.

The precipitated zinc sulfide was dried, weighed and averaged out over the number of days it was collected. To calculate equivalent litres of H_2S produced per day, equation 3.3 was used. CH_4 and CO_2 biogas volumes collected were added to the calculated H_2S volumes to give the specific total biogas production per day. The values were 0.48, 0.41, 0.25 and 0.19 L/gVSS added/day for Reactor M, A, B and C, respectively. To note, it was seen from equations 2.7 to 2.10 in Chapter 2 that both the volume of CH_4 and H_2S produced per gram of COD destroyed were identical. This means that the total volume of biogas produced should be equal regardless of the degree of sulfidogenesis if COD destruction levels were the same. The significantly lower values for Reactors B and C compared to the methanogenic reactor indicate that the levels of organic matter destrucion in these reactors were reduced.

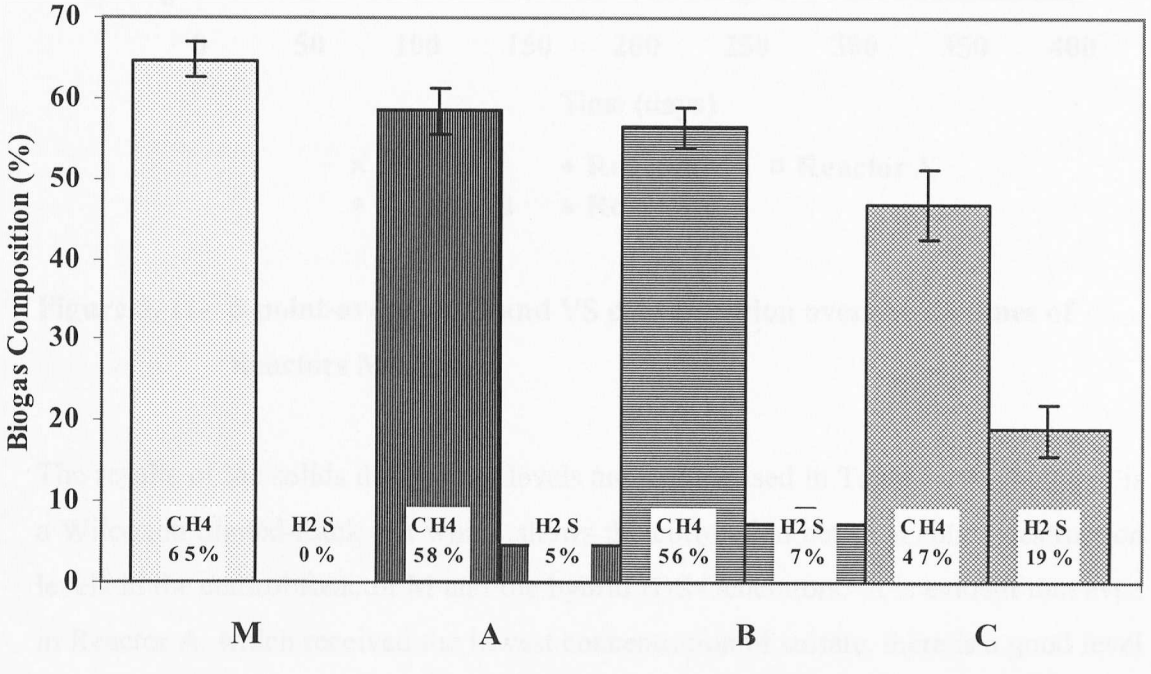


Figure 5. 10- Average biogas composition in reactors M-C over the lifetime of

the reactors. Total biogas volume includes CH_4, CO_2 and H_2S.

5.3.5 Total And Volatile Solids Destruction

Figure 5.11 shows the 5-point-average effluent TS concentrations in Reactors M to C throughout their lifetimes. A similar trend was found in the case of VS destruction levels. As the influent concentration fluctuated towards the end of the run, the effluent solids remained relatively stable in Reactors M and A compared to Reactors B and C, which fluctuated to a greater degree.

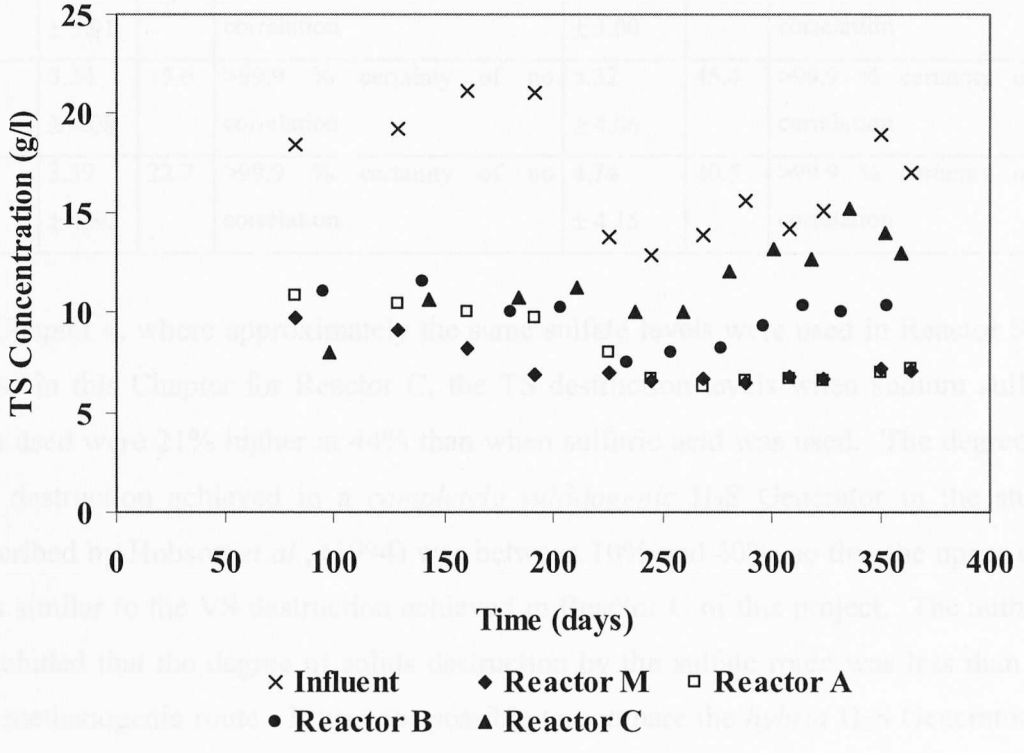


Figure 5. 11- 5-point-average TS and VS concentration over the lifetimes of Reactors M-C

The results of the solids destruction levels are summarised in Table 5.10. Included is a Wilcoxon Signed-Rank test which shows the correlation between solids destruction levels in the control Reactor M and the hybrid H_2S Generators. It is evident that even in Reactor A, which received the lowest concentration of sulfate, there is a good level of certainty that there is a significant difference between the control and sulfidogenic

reactor in terms of solids destruction, and this certainty increases with increasing sulfidogenic conditions.

Table 5. 10- Summary of TS and VS Destruction levels in Reactors M-C.

Reactor	Average TS Destruction		Wilcoxon Signed-Rank test for significance of TS destruction in correlation with Reactor M	Average VS Destruction		Wilcoxon Signed-Rank test for significance of VS destruction in correlation with Reactor M
	g/l	%		g/l	%	
M	7.64 ± 3.42	51.0	_	6.82 ± 3.85	58.3	_
A	7.00 ± 3.91	46.6	90-95% certainty of no correlation	6.46 ± 3.00	55.2	>99.9% certainty of no correlation
B	5.34 ± 4.08	35.6	>99.9 % certainty of no correlation	5.32 ± 4.06	45.4	>99.9 % certainty of no correlation
C	3.39 ± 4.80	22.7	>99.9 % certainty of no correlation	4.74 ± 4.15	40.5	>99.9 % certainty of no correlation

In Chapter 4, where approximately the same sulfate levels were used in Reactor S as those in this Chapter for Reactor C, the TS destruction levels when sodium sulfate was used were 21% higher at 44% than when sulfuric acid was used. The degree of VS destruction achieved in a *completely sulfidogenic* H_2S Generator in the study described by Hobson *et al.*, (1994) was between 10% and 40%, so that the upper end was similar to the VS destruction achieved in Reactor C of this project. The authors concluded that the degree of solids destruction by the sulfate route was less than by the methanogenic route. It was not possible to compare the *hybrid* H_2S Generator in the afore mentioned study to any of the reactors in this chapter since the study by Hobson *et al.* (1994) used a combination of calcium sulfate and sulfuric acid rather than purely sulfuric acid.

Starting on day 289, a particle size distribution (PSD) test was performed on the effluent from each reactor on three separate days as described in Section 3.4.10. This was done to gain insight in to the degree of hydrolysis of the particulate matter within in each reactor to help explain differences in the effluent solids. From Table 5.11, it is

possible to see that there is an overall decrease in VSS on a mass basis for the sum of the larger particle sizes (≥0.075mm diameter).

However, the level of destruction decreases with increasing sulfidogenic conditions. For particle sizes <0.075mm, the difference in VSS destruction on a mass basis is much more pronounced, so that in Reactor C, there is only a 6% difference in mass of VSS in the effluent and the influent raw primary sludge. This calculation was made without taking into account the effect of sulfuric acid on the influent sludge.

Table 5. 11- Average concentration (mg/l) of different particle sizes of raw sludge and the effluent of reactors M-C. 'd'=diameter. Triplicate samples.

Particle Size	Raw		Reactor M		Reactor A		Reactor B		Reactor C	
(mm)	VSS	VSS/ TSS	VSS	VSS/ TSS	VSS	VSS/ TSS	VSS	VSS/ TSS	VSS	VSS/ TSS
D ≥1.000	839±34	0.86	199±15	0.86	240±18	0.90	361±38	0.87	355±52	0.89
0.850≤d<1.000	61±3	0.84	17±1	0.84	41±1	0.89	35±3	0.89	38±1	0.84
0.710≤d<0.850	177±9	0.89	22±2	0.86	42±2	0.89	37±1	0.87	40±2	0.85
0.600≤d<0.710	88±7	0.80	29±2	0.83	43±1	0.84	45±4	0.86	48±2	0.84
0.500≤d<0.600	53±2	0.82	32±1	0.83	78±1	0.84	72±2	0.86	78±3	0.82
0.425≤d<0.500	64±4	0.80	43±1	0.78	35±2	0.86	49±3	0.86	53±2	0.84
0.300≤d<0.425	346±28	0.84	89±2	0.75	106±7	0.81	125±8	0.87	135±5	0.84
0.180≤d<0.300	356±32	0.81	195±3	0.73	180±12	0.80	192±8	0.86	208±6	0.83
0.106≤d<0.180	498±48	0.77	201±3	0.69	185±6	0.78	217±3	0.85	235±9	0.82
0.075≤d<0.106	319±17	0.74	201±3	0.64	138±5	0.79	168±6	0.77	181±3	0.73
Sum d ≥0.075	2801±152	0.82	1028±16	0.78	1089±24	0.84	1266±41	0.86	1371±54	0.83
Reduction			**63%**		**61%**		**55%**		**51%**	
<0.075	5729±132	0.66	3072±98	0.58	3721±102	0.64	4110±113	0.68	4453±155	0.72
Reduction			**35%**		**21%**		**13%**		**6%**	
Sum of all particles	8530	0.70	4100	0.62	4810	0.68	5376	0.72	5874	0.74
			52%		**44%**		**37%**		**32%**	

The acid hydrolysis test in Section 5.2.1 describing the relationship between VSS hydrolysis and sulfuric acid concentration added (equation 5.1) predicted a significant amount of solubilisation of the influent VSS. As a result, effluent VSS levels of the smaller particle sizes that were tested under the acid hydrolysis test (<0.075 mm diameter) should decrease from Reactor A to C, assuming the K_h of hydrolysis was not affected. Table 5.12 compares the predicted influent VSS concentrations after acid addition to a primary sludge of 5.7 g/l VSS and particle size <0.075 mm diameter, to the effluent VSS concentrations in Reactors M to C.

It is evident that although the predicted influent VSS concentrations of the smaller particles is lower in the sulfidogenic reactors compared to Reactor M, effluent VSS concentrations increased from Reactor A to C, with Reactor C showing a positive increase in particle numbers compared to the influent. Although the number of smaller particle sizes entering the Hybrid H_2S Generators may indeed be lower after acid addition, a possible disruption in the hydrolysis stage could mean an accumulation of smaller particles that have broken down from larger particles. This would strongly contribute to the low TS and VS destruction levels experienced in all three sulfidogenic reactors. Amongst the different particle sizes in the ranges ≥0.075mm diameter, the majority of particles in the raw and digested sludges were in the range ≥1.000mm. The greatest conversion of VSS to soluble or smaller-sized products occurred at the particle size range of $0.710 \leq d < 0.850$ where it was at 87% for Reactor M, 76% for Reactor A, 79% for Reactor B, and 77% for Reactor C. From the VSS/TSS ratio, it is clear that the organic fraction of the larger paricles is high but similar for all the sludges tested, so that the effects of increasing sulfidogenic conditions is negligible on the organic content of these larger particles.

Table 5. 12- Comparison of expected influent VSS concentration (particle sizes <0.075 mm diameter) after sulfuric acid addition (interpolated from equation 5.2), with actual effluent VSS concentrations from PSD test in Reactors M-C.

Reactor	Expected influent VSS conc. after acid addition (g/l)	Actual effluent VSS conc. (g/l)	% Reduction in VSS conc. (%)
M	5.73	3.07	46
A	4.78	3.72	22
B	4.60	4.11	11
C	4.25	4.45	-5

The overall trend is for the organic content of the particles to decrease with decreasing particle size regardless of degree of hybridity. As for the smaller particles (< 0.075 mm diameter), apart from Reactor M, the organic content of the particles increases

with increasing sulfidogenic conditions compared to the raw sludge. Overall, the majority of all the sludges, digested or raw, consisted of fine material smaller than 0.075 mm in diameter.

5.3.6 Total And Soluble COD Destruction

Figure 5.12 shows TCOD destruction levels over the lifetime of the reactors. This follows the trend of the solids destruction levels, so that the effluent TCOD concentration increased with increasing sulfidogenic conditions.

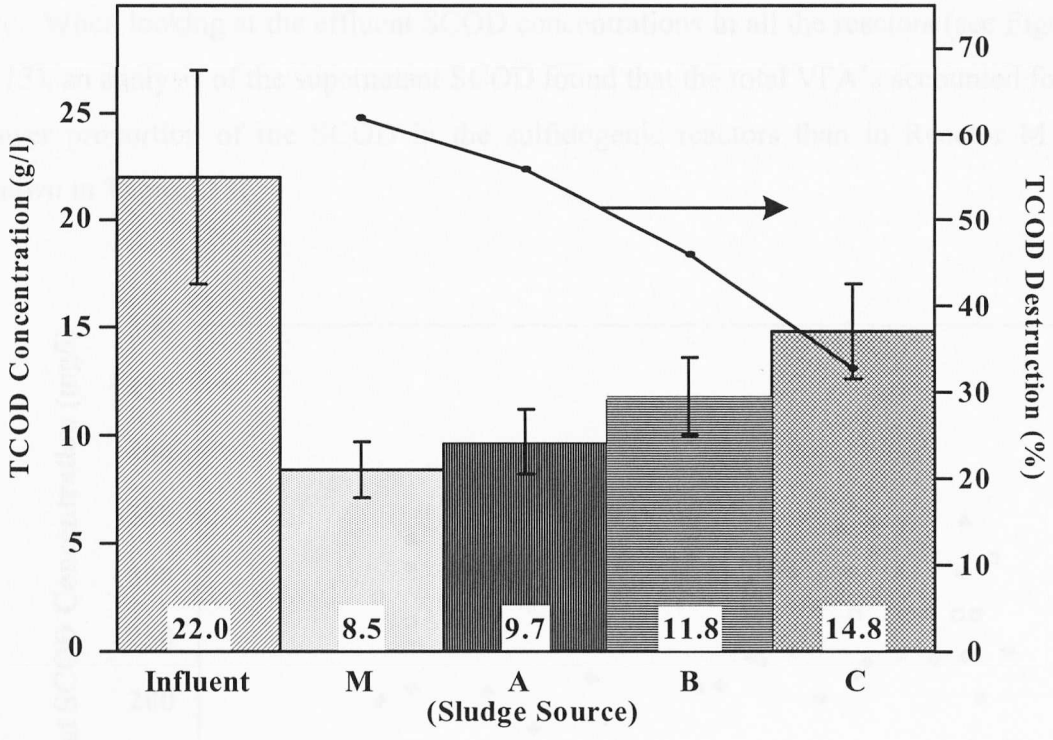


Figure 5. 12- Average influent and effluent TCOD concentration and destruction in Reactors M-C over the lifetime of the reactors.

From equation 2.9 in Chapter 2, we calculated that 64g of O_2 (COD) are required to reduce 34g H_2S to 96g SO_4^{2-}. By knowing the amount of sulfate reduction taking place in each reactor, it is possible to calculate the electron flow toward sulfidogenesis over the stable lifetime of the reactors. These were 7±1% for Reactor A, 13±3% for Reactor B and 33±5% for Reactor C of the TCOD destroyed.

Rinzema *et al.* (1988b) also concluded from their literature research that, for a

carbohydrate-containing wastewater which fractionates into acetate and hydrogen in a 67:33% ratio respectively during acidogenesis, and where SRB do not play a role in acetate oxidation, SRB can at most only oxidise 33% of the available COD which is in the form of H_2. The literature value for complete sulfate reduction of the COD was at least 2 g/g. In this project, although Reactor C was running at a higher ratio of 5:1, the maximal sulfidogenesis figure of 33% was attained, most likely due to the complexity of the wastewater and ability of SRB to degrade other long-chain substrates, not just H_2. In fact, Fukui *et al.* (2000) demonstrated that over 80% of the substrates used by SRB in municipal sludge digesters are as yet unknown higher chain compounds which may include amino acids, ethanol, aromatic compounds, pyruvate etc. When looking at the effluent SCOD concentrations in all the reactors (see Figure 5.13), an analysis of the supernatant SCOD found that the total VFA's accounted for a lower proportion of the SCOD in the sulfidogenic reactors than in Reactor M as shown in Table 5.13.

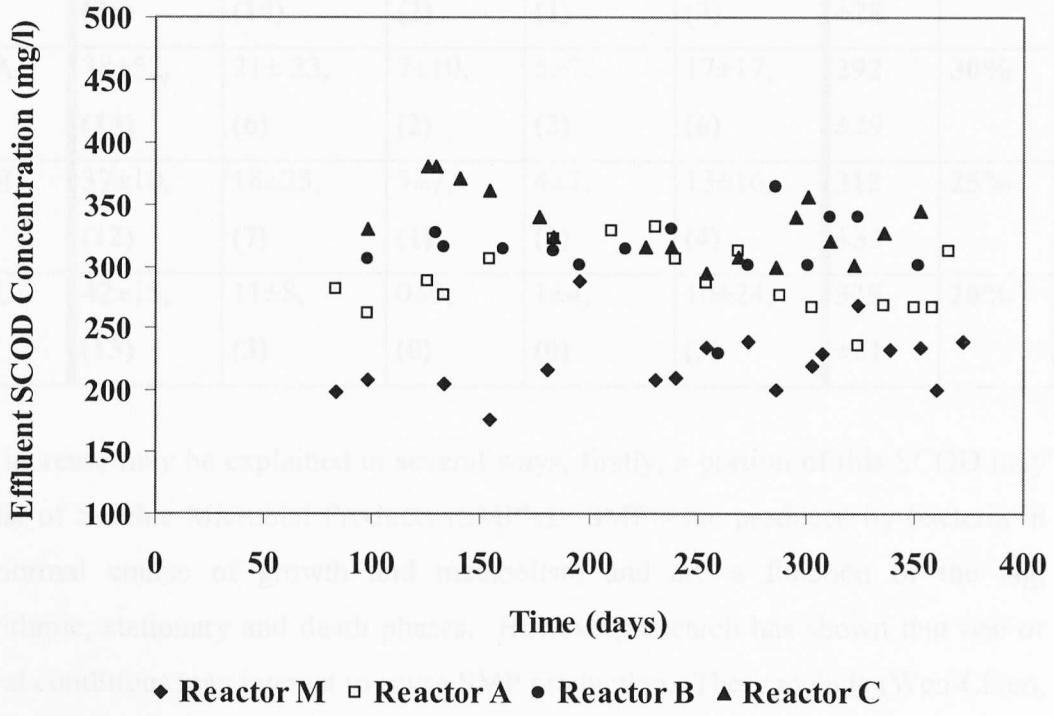


Figure 5. 13- SCOD concentrations in the effluent of Reactors M-C.

In fact, of all the VFA's, the propionate concentration in the final effluent supernatant showed the most significant decrease in proportion to increasing sulfidogenic

conditions. This corroborates previous findings by several authors who showed that SRB were mainly responsible for propionate uptake under sulfidogenic conditions (Fukui *et al.*, 2000; O'Flaherty *et al.*, 1998; Rinzema *et al.*, 1988b). We can also see that the non-VFA portion of the sludge increased in proportion to sulfidogenic conditions.

Table 5. 13- SCOD as VFA's in the influent and from Reactors M-C.

	VFA Concentration in mg/l ±SD , (% of Total VFA's)						
Sludge Source	Acetate	Propionate	Iso-butyrate	Butyrate	Iso-Valerate	**Total SCOD (mg/l)**	**SCOD as VFA**
Influent	379±143, **(23)**	365±144, **(22)**	21±10, **(1)**	34±19, **(2)**	36±14, **(2)**	**1668 ±535**	**50%**
M	59±47, **(26)**	32±43, **(14)**	3±7, **(2)**	3±6, **(1)**	8±13, **(3)**	**226 ±28**	**47%**
A	38±52, **(13)**	21± 23, **(6)**	7±10, **(2)**	5±7, **(2)**	17±17, **(6)**	**292 ±29**	**30%**
B	37±10, **(12)**	18±23, **(7)**	5±7, **(1)**	4±7, **(1)**	13±16, **(4)**	**312 ±33**	**25%**
C	42±15, **(13)**	11±8, **(3)**	0±0, **(0)**	1±4, **(0)**	10±24, **(3)**	**325 ±21**	**20%**

This increase may be explained in several ways; firstly, a portion of this SCOD may consist of Soluble Microbial Products (SMP's). SMP's are produced by bacteria in the normal course of growth and metabolism and are a function of the lag, logarithmic, stationary and death phases. However, research has shown that one or several conditions may interact to cause SMP production. These include (Wen-Chien, 1993):

- Starvation,
- Substrate-accelerated death by addition of a carbon source after prolonged period of starvation,
- Unavailable essential nutrients,

- Increased concentration of carbon source,
- Maintainance of a concentration equilibrium after dilution,
- Relieving environmental stress.

Factors such as lack of availability of essential nutrients or sulfide toxicity are an unlikely cause for SMP production in this case, since similar sulfidogenic levels were experienced in Reactor S without any negative consequences in terms of VFA degradation. Also, none of the reactors accumulated any VFA's in this part of the project which would have been the case had methanogenesis or sulfidogenesis been affected. A possible source of the excess SCOD may not only be SMP's, but the same soluble SCOD that was apparent during the acid hydrolysis test (see Section 5.2.1). The fraction of SCOD increased in proportion to acid concentration added to the raw sludge. However, the residual SCOD concentrations in Reactor C for example, whose feed was receiving 4.33g/l SO_4, equals less than half of that produced upon additon of 5.36 g/l SO_4 to the same influent sludge concentration (see Table 5.5). Pletschke *et al.*, (2002) had demonstrated an increase in the activity of the enzyme ATPS in sulfidogenic reactors by a factor of 10-20 times, which could also contribute to the higher SCOD measurements in the sulfidogenic reactors.

5.4 DISCUSSION

Initially, the effect of sulfuric acid on the solubilisation of raw primary sludge was explored to show that the relationship between solids concentration and destruction was logarithmic in nature with respect to the amount of acid added. Two equations were established to predict the amount of TSS or VSS solubilised from a certain concentration of acid. However, the relationship is restricted to raw primary sludge with a TSS concentration between 5-25g/l and particle size <0.075mm in diameter. Moreover, the amount of SCOD released depends on the final pH of the solution. Although it was expected that a large proportion of this SCOD would be in the form of VFA's, this was contradicted by results which showed that at a maximum, only 11% was in the form of acetic acid, with the mean figure being only 4%. The ability of sulfuric acid to hydrolyse a portion of the raw sludge before feeding did not give any advantages to the sulfidogenic reactors in terms of lowering the final solids concentration in the effluent. Indeed, results of the PSD test showed that with

increasing sulfidogenic conditions, there is not only an accumulation of fine material (<0.075mm diameter), but also an increase in the proportion of their organic content. In contrast, the large diameter organic material is only slightly negatively affected by increasingly sulfidogenic conditions. Although there is an overall reduction in VSS on a mass basis compared to the raw primary sludge, the methanogenic reactor out-performs all the other reactors. These results contrast research findings by Dunn, (1998) who observed an enhancement in the overall particulate matter breakdown in a sulfate-reducing ponding system treating tannery wastewater. This difference may be as a result of the variation in reactor configuration where, in the afore mentioned study, sludge in a ponding system would have experienced cyclical up-welling from anaerobic to anoxic zones. This is not the case in a CSTR configuration. Also, there was no report of which category size of particles a reduction was observed, so that an overall enhancement maybe disguised by a large enhancement in only one category compared to another.

By following the sulfate degradation rate over time in each reactor, kinetic values were obtained for sulfate reduction. These values are able to give an idea of SRB activity in each reactor in competition for MPB for substrates. Taking H_2 as an example of a substrate which constitutes the ultimate source for competition between the two groups of bacteria, the total H_2 uptake by HSRB and HMB can be described by a two-term Monod equation (eq. 5.10):

$$\mu = \mu_{max} \frac{S}{K_{s,\,HSRB} + S} \times \frac{S}{K_{s,\,HMPB} + S} \qquad \text{(eq. 5.10)}$$

Combining equation 5.10 with equation 2.19 gives equation 5.11:

$$\mu = \mu_{max} \frac{S}{K_{s,\,HSRB} + S} \times \frac{SO_4^{2-}}{K_{so_4^{2-},\,HSRB} + SO_4^{2-}} \times \frac{S}{K_{s,\,HMPB} + S} \qquad \text{(eq. 5.11)}$$

Where;

μ = Specific biomass growth rate on H_2 (d^{-1})

μ_{max} = Maximum specific cell growth rate on H_2 (d^{-1})

K_s = Half saturation constant for H_2 ($mg.l^{-1}$)

S = Substrate concentration, in this case H_2 ($mg.l^{-1}$)

SO_4^{2-} = Sulfate concentration ($mg.l^{-1}$)

HSRB,HMBP = Hydrogenotrophic -SRB and -MPB population, respectively

We can see from equation 5.11 that the specific biomass growth rate of HSRB on H_2 is dependent on the sulfate concentration when sulfate is not limited. The fact that the sulfate concentration entering Reactor A is extremely low restricts the growth of a substantial SRB population, as seen with the low μ_{max} value. In addition, the low sulfate affinity in Reactors A and B means that substrate uptake by SRB is further restricted through sulfate limitation. A high sulfate concentration must be present before SRB are able to utilise substrates. For example, the K_s value determined by Visser *et al.* (1996) for suspended ASRB derived from a UASB reactor treating a sulfate-rich acetate feed at a COD:SO_4 of 1:1 was lower than any in Table 5.9 at 0.018 g/l. This lower value is most likely due to the unlimited sulfate concentrations found in the reactor. In previous studies it was shown that the inhibition of sulfate reduction in sulfate-rich anaerobic environments resulted in an increase in the hydrogen partial pressure and methane production rates to levels found in methanogenic environments (Lovley *et al.*, 1982; Mizuno *et al.*, 1998). This demonstrates that when sulfate concentrations are relatively high, as in Reactor C, HSRB would inhibit methane production by lowering the hydrogen partial pressure below a threshold level of 10^{-6} atm. which is necessary for hydrogen utilization by HMB.

The inhibition of methane production at low H_2 partial pressures is due to the decrease in the energy yield from methane production. Reported μ_{max}/K_s values for H_2 are nearly always higher for HSRB than HMPB indicating that the former should outcompete the latter (Robinson *et al.*, 1984; Widdel, 1988; Lovley *et al.*, 1982). It must be noted that pH can have a dramatic effect on the growth rate of bacteria as was shown by the investigations of Visser *et al.*, (1996) where for example, the acetate μ_{max} of ASRB increased from 0.109 d^{-1} at pH 7 to 0.120 d^{-1} at pH 8. The pH in the Reactors A, B and C in this project were 7.02, 7.35 and 7.9 respectively. The higher pH values in Reactors B and C are therefore expected to encourage higher SRB

growth rates, which may have contributed to the high μ_{max*} values. Suffice to say at this point that Chapter 6 will deal with the kinetic aspects of sulfidogenic and methanogenic substrate uptake in more detail.

Interestingly, although the results for sulfate kinetic values, along with the low supernatant SCOD levels show that the hybrid H_2S Generators were not experiencing inhibitory conditions at the methanogenic/sulfidogenic levels, the overall effluent solids concentration *do* suggest a decrease in digestion efficiency. Inhibition of the final stages of digestion are normally a result of MPB inhibition owing to several possible factors. These include biomass washout, mass-transfer rate limitation of substrates to bacteria, or accumulation of inhibitory substances such as fatty acids or ammonia (from high levels of Kjedahl-N). All these factors would decrease methanogenic activity, resulting in rising residual VFA concentrations, especially of acetate, butyrate and propionate (Parkin *et al.*, 1990: Maillacheruvu *et al.*, 1993). Rising VFA levels would eventually inhibit the hydrolysis of polymers and acetogenesis of longer-chain VFA's to acetate (Angelidaki, 1992). The lack of acetate accumulation suggests that the MPB, who are mainly responsible for acetate degradaton even under low COD:SO_4 ratios (Fukui *et al.*, 2000; Polpasert *et al.*, 1995; Ueki *et al.*, 1992), have not been inhibited other than by lack of substrate.

Other important factors contributing to inhibition are high sulfide levels or sulfide precipitation of essential metals (Fe is crucial for methanogenesis (Isa *et al.*, 1986)). We can exclude these two factors by looking at Reactor S of Chapter 4. Reactor S was run at a similar COD:SO_4 ratio as Reactor C of 5.45:1, and sulfide concentrations were maintained at similar levels in both due to the action of the chemical sulfide scrubbers. Had sulfide inhibition or essential metal precipitation resulted in MPB inhibition, we would have seen evidence of this in Reactor S in terms of VFA accumulation and lower TCOD destruction levels than the control reactor. As this was not the case, these factors can be excluded.

In Chapter 4, it was discussed how despite differing solids destruction levels, the TCOD destruction levels in Reactor M and S were nearly identical. This was explained by the fact that the Yield of SRB in the sulfidogenic Reactor S may have

been greater, thus contributing to the higher effluent solids concentration in this reactor, as represented by equation 4.6. The TCOD destruction levels remained similar since these are dependent on the hydrolysis rate constant, K_h.

We would expect K_h to be the same in both methanogenic and sulfidogenic reactors on account of SRB and MPB not taking part in the hydrolysis stage. When looking at the results of this Chapter where the sulfidogenic reactors were fed sulfuric acid, this trend of higher TS but comparable TCOD effluent levels is not apparent. Instead, the efficiency in both the solids and the TCOD destruction was negatively affected by increasing levels of sulfuric acid concentrations in the feed. Supported by the results of the PSD test, an initial conclusion is that the K_h in Reactors A to C is lower than that of Reactor M. The effect of a decrease in K_h would mean a proportionate increase in effluent degradable particulate COD (COD_{epdeg}) as described in equation 4.5, as indeed the results demonstrated. A knock-on effect of this lower TCOD degradation rate is that $X_{b,e}$ (effluent bacterial biomass) decreases. This theory is also supported by the fact that the biogas yield per gram VSS added decreased from Reactor A to C, meaning that MPB and SRB growth rates and numbers had decreased from the lower amount of soluble substrate being released from the hydrolysis stage.

The micro-organisms involved lower-down the anaerobic digestion chain in Reactors A-C cannot grow as efficiently as those in Reactor M due to substrate inavailability from the lack of particle hydrolysis. The development of a certain concentration of a group of bacteria in a reactor over time is dependent on the specific growth rate and substrate affinity of the bacteria, as well as the initial concentration of biomass in the reactor. If the growth rate μ is affected at some stage during the lifetime of the reactor from substrate deprivation, then biomass concentration would also decrease. Since the measured soluble COD, VFA and sulfate in the effluent supernatant was comparable to the methanogenic reactor, toxic inhibition of methanogenic and sulfidogenic organisms can be excluded.

It has already been established that the hydrolysis stage follows First Order kinetics. However, very little research has been carried out concerning inhibition factors affecting the initial stage of anaerobic digestion of municipal primary sludge. The

literature that has been found has looked at the effects of temperature, pH and VFA concentration on K_h of solid biowaste digestion. Veeken *et al.* (1999) initially looked at the effect of temperature on biowaste hydrolysis, and concluded that inhibition was related to VFA concentration and not temperature. Later, Veeken *et al.* (2000) looked at the separate effects of pH and VFA's on the hydrolysis inhibition of biowaste based on research by Llabres-Luengo *et al.* (1988), who proposed that the hydrolysis rate was considered proportional to the substrate volatile solids and biomass concentrations, but inversely proportional to the VFA concentration. In other words, hydrolysis kinetics followed a VFA inhibition model. Veeken *et al.* (2000) found that the hydrolysis rate was in fact dependent on the pH and not on VFA concentration between pH's 5–7, and VFA concentrations up to 30 g COD/l.

However, VFA and pH are inexorably linked since VFA's control the pH via chemical equilibrium. Although the waste used in both experiments was solid biowaste, the same principles apply as to wastewater digestion. From these literature findings, if we assume that pH was the inhibiting factor for the hydrolysis of sewage sludge, batch feeding of acidified primary sludge may cause the pH to plummet to inhibitory levels for the first few minutes after feeding, from which the hydrolytic microorganisms could not recover. Using equations 5.3-5.5 to test this theory, we can see from Table 5.14 that due to the high alkalinity and pH already in the reactors, the pH in the reactors immediately after feeding will only reduce to slightly lower-than-normal levels, and is not enough to cause any kind of inhibition. The reason for the decrease in the K_h, therefore, remains unclear at this stage.

Table 5. 14- Expected pH in Reactors A-C immediately after batch feed. Refer to equations 5.3-5.5 for calculations.

Reactor	Reactor pH	Volumetric SO_4 conc. (M)	Alkalinity (M)	H^+ conc. (M)	Before H_2SO_4 addition		After H_2SO_4 addition		Expected pH after batch feed
					[HA] fraction (M)	[A^-] fraction (M)	[HA] fraction (M)	[A^-] fraction (M)	
A	7.05	0.0007	0.0329	8.91E-08	0.0055	0.0274	0.0062	0.0267	6.98
B	7.35	0.0013	0.0368	4.47E-08	0.0034	0.0334	0.0046	0.0322	7.20
C	7.90	0.0023	0.0408	1.26E-08	0.0011	0.0397	0.0034	0.0374	7.39

5.5 SUMMARY

The preliminary studies undertaken before running a further four hybrid H_2S Generators (Reactors A, B, C and D) at different COD:SO_4 ratios clarified the boundaries of sulfidogenic anaerobic digestion with sulfuric acid. The maximum amount of sulfuric acid that could be reduced without negative pH effects was determined in small-scale batch tests, and established as 5 g/l. Indeed, when Reactor D was fed sulfuric acid at the higher concentration of 7.15 g/l, sulfate accumulated, and the pH in the reactor dropped after less than 2 retention times. Equations to predict the extent of SCOD solubilisation and final pH as a function of suspended solids and sulfate concentration in the feed were also formulated from small-scale batch tests. The equations were used to show that the initial hydrolysis of the primary sludge with acid gave no advantages to the sulfidogenic reactors in terms of lowering the effluent suspended solids concentrations.

In contrast, the effect of increasing acid concentration in the feed caused an accumulation of organic matter in the form of VSS of the smallest diameter particle-size range tested (<0.075mm). This accumulation negatively affected effluent TCOD and solids concentrations. The reason for this was attributed to a decrease in the extent of hydrolysis of these smallest particles. The degree of degradation of the largest particles (diameter $\geq$ 0.075mm) was not significantly different from one reactor to the next. A knock-on effect of the decreased hydrolysis rates equal to or less than the reduction in solids concentration was the reduction in the total biogas yield as a possible result of lower MPB and SRB growth rates caused by substrate deprivation.

Although hydrolysis inhibition is normally attributed to either unfavourable pH or high VFA concentrations due to toxicity inhibition of the final stages of methanogenesis, neither of these factors were apparent in any of the reactors. Sulfide

toxicity was excluded as a factor since low sulfide concentrations were maintained thanks to chemical scrubbing of the H_2S. Also, a comparison with Reactor S from Chapter 4 which was running at a similar COD:SO_4 ratio as Reactor C showed no negative effects on anaerobic digestion due to sulfide production. In addition, had MPB or SRB been exposed to any type of toxicity to cause inhibition, SCOD effluent concentrations in the form of VFA's would have been substantially higher than those experienced, and sulfate concentrations would have risen as a result of SRB-shutdown.

Comparative sulfate reduction kinetic values that were measured experimentally showed that that μ_{max*} for sulfate reduction was highest in Reactor C, which was receiving the highest acid concentration in the feed. This was explained by the possibility that Reactors A and B were relatively more sulfate limited than Reactor C (as demonstrated by the low RLF-PS value), causing the growth rates of SRB in the former reactors to be lower. K_{s*} values, on the other hand, did not differ greatly from one reactor to the next, although Reactor C did have a slightly lower value than the others, possibly due to a different set of SRB being active during the initial stages of feeding. The limitations of trying to measure kinetic values in complex wastewaters were identified, namely the determination of X and Y values for the accurate calculation of μ_{max} and K_s values.

Overall, results from this part of the project revealed that only the initial stages of anaerobic digestion were negatively affected in the hybrid H_2S Generators in proportion to increasing sulfate concentrations in the feed. This inhibition did not seem directly related to digestion performance in the final stages of sludge digestion, namely methanogenesis and sulfidogenesis.

CHAPTER 6

KINETIC STUDIES

6.1 INTRODUCTION
6.2 HYDROLYSIS OF PRIMARY SLUDGE VSS BY FERMENTATIVE BACERIA
6.3 BIOCHEMICAL METHANE POTENTIAL ASSAYS
6.4 KINETIC PARAMETERS OF SRB & MPB FROM LAB-SCALE REACTORS
6.5 DISCUSSION
6.6 SUMMARY

6.1 INTRODUCTION

In the previous two chapters, the digestion efficiency of several hybrid H_2S Generators running at different COD:SO_4 concentrations was investigated. Results showed that with increasing sulfuric acid concentrations in the feed, the solids and TCOD destruction efficiency decreased compared to a fully methanogenic reactor. On the other hand, the addition of sulfate as a salt had no negative effects on the TCOD destruction efficiency. Following on from these findings, this Chapter investigated the various kinetic parameters controlling sludge digestion in each lab-scale reactor using small-scale assays.

The first investigation looked at the VSS hydrolysis of particles by fermentative bacteria in order to establish whether or not the type of sulfate used (acid or salt) had any effect on the hydrolysis rate constant K_h. The method and results of this experiment are described in Section 6.2. In Section 6.3, a brief description of Biochemical Methane Assays (BMP's), which are the foundation of all the small-scale assays used henceforth, is given. A preliminary study to determine nutrient

requirements of the sludge in these assays was first established and the results used to carry out kinetic studies on the seed sludge from the lab-scale reactors. The purpose of these studies was to compare μ_{max} and K_s values of MPB and SRB on various substrates during the final stages of digestion, and these are described in Section 6.4.

6.2 HYDROLYSIS OF PRIMARY SLUDGE VSS BY FERMENTATIVE BACTERIA

The literature research presented in Chapter 2 explained how the process of particulate hydrolysis was the result of break down of complex organic compounds by extra-cellular enzymes released from fermentative bacteria. The rate at which particle hydrolysis occurs is widely accepted to follow the First Order Kinetic model. The purpose of this hydrolysis test was to determine the K_h operating in each lab-scale reactor. All three hybrid H_2S Generators tested in Chapter 5 showed an increase in final solids and TCOD concentration in the effluent in proportion to increasing sulfuric acid concentration in the feed. The cause for this was hypothesised to be a decrease in the K_h of the smallest sized VSS particles tested, as demonstrated by their accumulation in the final effluent. In contrast, Chapter 4 results showed no detrimental effects on sludge digestion efficiency if sodium sulfate was used as a source of sulfate.

Hydrolytic enzymes, like all enzymes, are specific for a particular reaction due to their unique amino acid sequence, which results in a specific three-dimensional structure. The active site on the enzyme is the portion that interacts with a substrate, so that any substance that blocks or changes the shape of the active site affects the activity of the enzyme. Possible reasons as to why an active site may be altered include (Ferscht, 1985):

1. pH: Changes in pH alter the state of ionization of charged amino acid side chains such as -COOH and NH_2. As the pH decreases, an enzyme will gain H^+ ions so that eventually enough side chains will be affected to disrupt the enzyme's shape. Likewise, as the pH is raised, the enzymes will lose H^+ ions and eventually lose its

active shape. Each enzyme will have its own pH optimum, so that deviation from that pH denatures the enzyme.

2. Temperature: Generally speaking, increasing the temperature accelerates enzyme activity since more kinetic energy is available for the molecules to undergo a reaction. Above the optimum temperature, the kinetic energy of the enzyme and water molecules is so great that the conformation of the enzyme molecules is disrupted. Denaturing of proteins occurs around temperatures of 40-50°C, although some can withstand much higher temperatures.

3. Salt Concentration: Salts can widely affect a range of enzyme properties including stability, solubility and activity. At zero concentrations, charged amino acid side chains will attract, causing the enzyme to denature and form an inactive precipitate. At high salt concentration on the other hand, normal interactions of the charged groups will be blocked, again causing the enzyme to precipitate. Nitrate Reductase activities, derived from mildly halophitic *Desulfovibrio desulfuricans* species showed that K^+ and Na^+ in high concentrations in both ionic and non-ionic buffers have an activation effect, while the sulfate anion caused activation only in phosphate buffer. This indicates some dependence of enzyme activity on ionic strength. On the other hand, Ca^{2+} and Mg^{2+} caused periplasmic nitrate reductase activity to decrease (Bursakov, *et al.*, 1997). The authors speculated that the enzyme may undergo changes in the polarity, hydration and charged state to denature the molecular configuration of the enzyme.

4. Activators and Inhibitors: Molecules other than the substrate may interact with an enzyme so that an activator or inhibitor regulates the rate of the reaction. A substance which tends to unfold the enzyme, such as an organic solvent or detergent, will act as an inhibitor. Many inhibitors react with the side chains in or near the active site to change its shape or block it.

From the results of the previous two Chapters, it was speculated that the difference in sulfate source (acid or salt) may have had an effect on hydrolytic enzyme activity to cause the difference in observed digestion efficiency between Reactor S and Reactors A-C. The following section describes the method used to determine the differences, if any.

6.2.1 Method To Determine Primary Sludge VSS Hydrolysis By Fermentative Bacteria

Towards the end of the lifetime of Reactors M-C, seed sludge was collected over a number of days, placed in sealed plastic bottles and stored in a water-bath at 35°C ±1°C. On the day of the experiment, each seed sludge was tested for VSS concentration. Clean, empty 125ml McCarty bottles were flushed with a 30% CO_2:70% N_2 gas mix to drive out oxygen. For seed sludge from Reactor M, only one set of triplicate bottles was used, while for seed sludge from Reactors A-C, three sets of triplicate bottles were used. All bottles were filled with 50ml of sludge and 20ml anaerobic biomedia (see Appendix B) while being purged with the oxygen-free gas. The bottles were quickly sealed with a rubber bung, cable tied and placed upside down in a water bath (maintained at 35°C ±1°C). To each bottle was then added 2 ml of sodium molybdate to give a final volumetric concentration of 4 mM; sodium molybdate is a known inhibitor of SRB. Since it was of interest in this case to look at the activity of fermentative bacteria, the inhibition of SRB was not expected to affect fermentative bacterial activity. The reason for inhibiting SRB was to prevent the reduction of sulfate into sulfide, which may lead to sulfide toxicity in the bottles receiving a high sulfate concentration (e.g. seed from Reactor C). The bottles were left for 48 hours until no biogas production could be detected, indicating that any residual COD had been exhausted.

On the day of the test, fresh primary sludge which had been sieved to particle sizes < 0.075 mm in diameter was tested for VSS and TCOD concentration. The sludge was sieved in order to homogenise the sludge and prevent any 'lumps' from affecting the results. 8 ml of this sludge was anaerobically injected into each bottle by syringe. Immediately afterwards, to one set of triplicate bottles containing sulfidogenic seed, a 2 ml solution of sodium sulfate was added, to the second set a 2 ml solution of H_2SO_4 and to the third set 2 ml de-ionised water. The triplicate set of bottles containing methanogenic seed received 2ml deionised water only. The concentration of sulfate added to the relevant bottles was maintained at the COD:SO_4 ratio found in the lab-scale reactors. After vigorous shaking, 5 ml samples of the mixed solution from each bottle was immediately extracted and tested for VSS concentration. Samples were also taken once a day for 5 days.

6.2.2 Results For Primary Sludge VSS Hydrolysis By Fermentative Bacteria

The VSS and TCOD concentration of the sifted primary sludge was 14.8 g/l and 20 g/l respectively. At this COD concentration, the maximum volumetric sulfate concentration necessary in the bottles was 0.44 g/l to match the highest sulfate concentration in the lab-scale Reactor C. In Figure 5.4 of Chapter 5, it was seen that at the similar volumetric concentration used of 0.46 g/l, no significant difference in SCOD destruction was observed compared to the other volumetric concentrations after the first substrate/sulfate spike. In this case, the additional presence of buffer solution meant that digestion inhibition as a result of a decrease in the pH in the bottles in this experiment was excluded.

Figure 6.1 shows the specific VSS concentration in the seed sludge derived from each large-scale reactor over time when water was added (Control), and when sulfate in the form of sodium sulfate or sulfuric acid was added. It can be seen that the rate of degradation of particulate matter is all cases greatest in Reactor M. The difference in the rate of degradation between the seed sludge receiving water or sodium sulfate (Graph A and B) is not great, but is significantly lower in the three sludges receiving sulfuric acid.

From equation 2.12, plotting $\ln(COD_{pdeg})$ vs. t will give a straight line for a first order reaction with the slope of the line equal to K_h. In this experiment, COD_{pdeg} concentration was assumed to be equivalent to VSS concentration. Figure 6.2 shows plots of ln(specific VSS) vs. t, from which the K_h values in Table 6.1 were derived.

Comparing the hydrolysis rates for the Control bottles (receiving deionised water) to those receiving sulfate in the form of sodium sulfate, the results are comparable as demonstrated by the confidence intervals; due to the low number of samples (n=3) the confidence intervals were calculated using the t-test rather than the Wilcoxon Rank test. The results indicate no apparent negative effects of adding sulfate as a salt on digestion efficiency. In comparison, the use of sulfuric acid causes a dramatic decrease in the hydrolysis rate constant, most pronouncedly in Reactors B and C, so that the differences between control and sulfuric acid assays were 72%, 87% and 97% for Reactors A, B and C, respectively.

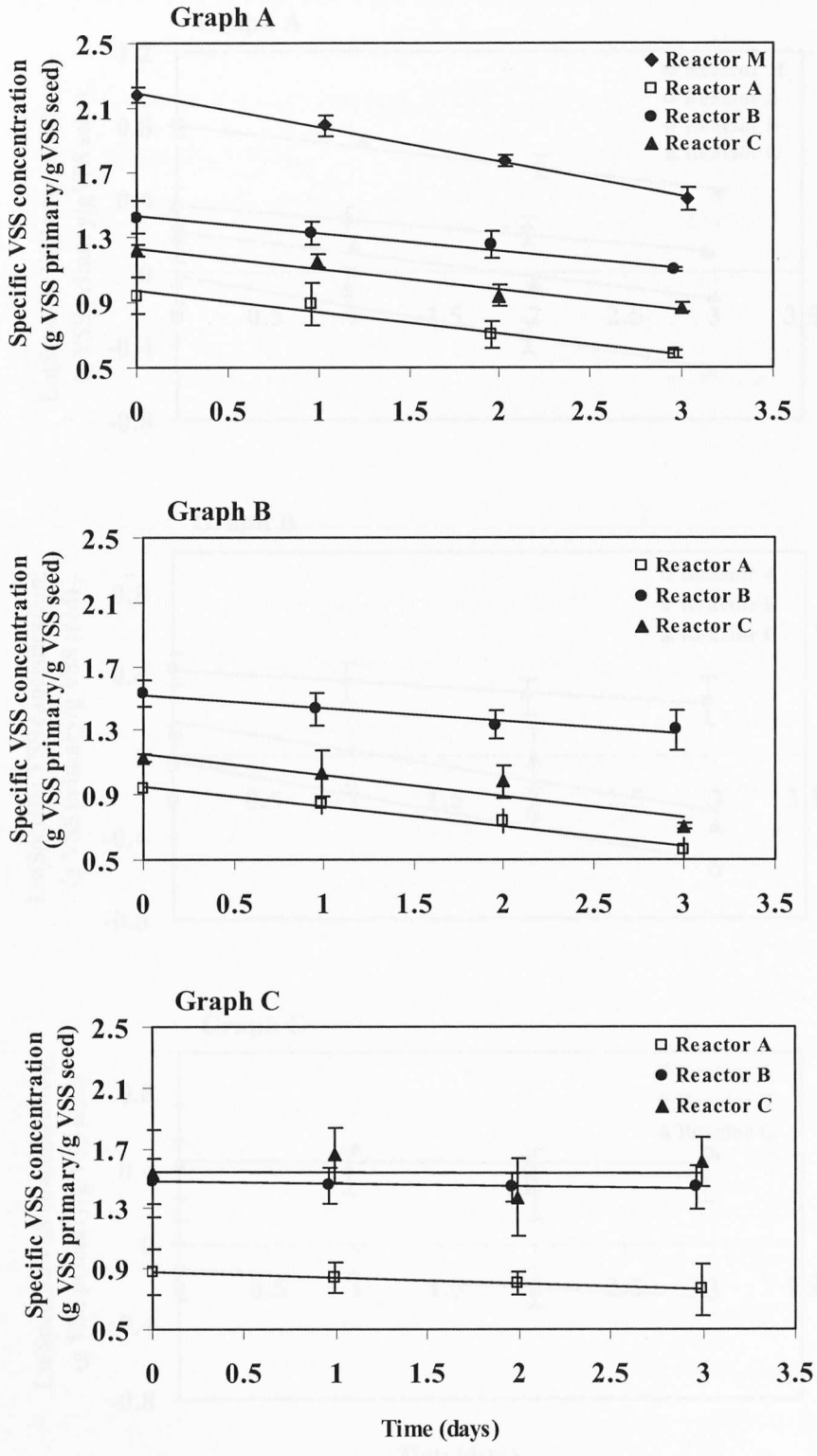


Figure 6. 1- Specific VSS concentration versus reaction time in seed sludge derived from Reactors M-C. Graph A: with deionised water (control); Graph B: with sodium sulfate; Graph C: with sulfuric acid.

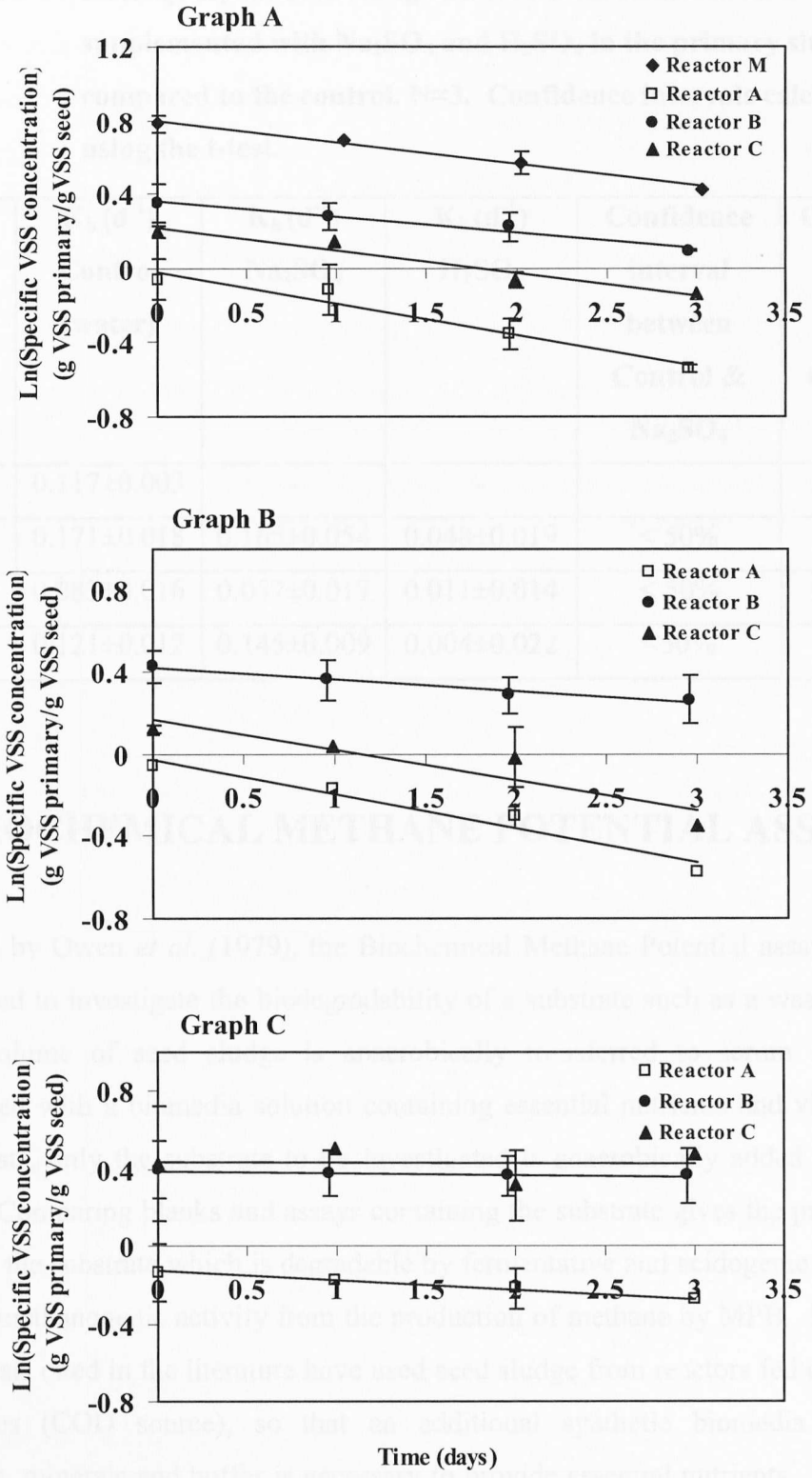


Figure 6.2- The plot of natural logarithm of specific VSS concentration versus reaction time in seed sludge derived from Reactors M-C. Graph A: with deionised water (control); Graph B: with sodium sulfate; Graph C: with sulfuric acid.

Table 6. 1- Average K_h for seed sludge derived from Reactors M-C supplemented with Na_2SO_4 and H_2SO_4 in the primary sludge compared to the control. N=3. Confidence intervals calculated using the t-test.

Reactor	K_h (d^{-1}) Control (water)	K_h (d^{-1}) Na_2SO_4	K_h (d^{-1}) H_2SO_4	Confidence interval between Control & Na_2SO_4	Confidence interval between Control & H_2SO_4
M	0.117±0.003	-	-		
A	0.171±0.018	0.165±0.054	0.048±0.019	< 50%	> 99.9%
B	0.083±0.016	0.057±0.017	0.011±0.014	< 50%	99-99.5 %
C	0.121±0.012	0.145±0.009	0.004±0.022	<50%	> 99.9%

6.3 BIOCHEMICAL METHANE POTENTIAL ASSAYS

Devised by Owen *et al.* (1979), the Biochemical Methane Potential assays (BMP's) were used to investigate the biodegradability of a substrate such as a wastewater. A fixed volume of seed sludge is anaerobically transferred to serum bottles and inoculated with a biomedia solution containing essential nutrients and vitamins. In these tests, only the substrate to be investigated is anaerobically added to the seed sludge. Comparing blanks and assays containing the substrate gives the proportion of COD in the substrate which is degradable by fermentative and acidogenic bacteria, as well as methanogenic activity from the production of methane by MPB. Most serum bottle tests cited in the literature have used seed sludge from reactors fed on synthetic substrates (COD source), so that an additional synthetic biomedia containing vitamins, minerals and buffer is necessary to provide essential nutrients. A synthetic biomedia is not an ideal substitute for BMP assays in this project, since the biomass in the reactors were already adapted to nutrients derived from sewage sludge. A change in nutrient source, type and concentration may have adverse effects on the biomass activity during the tests due to lack of acclimatisation and change in environmental

conditions such as pH. It was hypothesised that adequate nutrients would be available in the seed sludge to exclude the need for a synthetic biomedia in these BMP assays. A Nutrient Deficiency Test was therefore devised accordingly, and is described below. Once optimal conditions for the BMP assays were established, these results were used to devise BMP assays to quantify μ_{max} and K_s of the *mixed* microbial consortia in the lab-scale digesters of Chapter 5.

6.3.1 Nutrient Deficiency Test Method

Seed sludge from Reactor M (Methanogenic Reactor) was chosen in order to simplify the tests by excluding the need for SO_4 addition, which may interfere due to H_2S production. Two synthetic anaerobic solutions were prepared in exactly the same way, except one contained trace elements & vitamins similar to that recommended by Owen *et al.* (1979) while the other did not (blank solution). The composition, concentration and method of preparation of the biomedia can be found in Appendix B. Effluent seed sludge was collected over a number of days after feeding the reactor, placed in a sealed plastic bottle and stored in a water-bath at 35°C ±1°C.

On the day of the test, the VSS of the collected sludge was determined. Clean, empty 125ml McCarty bottles were flushed with the 30% CO_2:70% N_2 gas mix to drive out any air. 50ml of the sludge was added to 18 bottles labelled A-R while still being purged with the oxygen-free gas. To bottles A-F was added 20ml vitamin-rich biomedia, to G-L another 20ml 'blank solution', while bottles M-R received 20 ml of deoxygenated deionised water. The pH of the resulting sludge mixture was carefully measured in each bottle while still purging with gas. The bottles were quickly sealed with a rubber bung, cable tied and placed upside down in a water bath (maintained at 35°C ±1°C) to reduce chances of gas leakage. The bottles were left overnight so that any residual COD could be exhausted. After 'zeroing' each bottle by removing excess gas with a 20ml glass syringe, 2ml of a 'spike' containing glucose was injected into bottles A-C, G-I and M-O using a plastic syringe to give a final volumetric concentration of 1200 mg/l in each the bottles. The rest of the bottles from each set were injected with a 'blank' solution of deionised water to act as controls. The bottles were re-zeroed and gas production measured four times daily using a 20ml glass syringe for the first two days, and then once daily from thereafter until gas production

ceased. The bottles were zeroed after approximately every 15ml of gas production, and this volume analysed for methane content using a GC-TCD. When biogas production ceased in all bottles, the pH in each bottle was measured. The supernatant was also filtered through 0.45µm Nalgene™ filters and tested for residual SCOD and VFA concentration. The total biogas and methane produced, as well as SCOD and VFA concentrations in the control bottles were subtracted from the test bottles in order to take into account residual substrate degradation taking place in the sludge.

6.3.1.1 Nutrient deficiency test results: Figure 6.2 shows average methane production from each set of bottles.

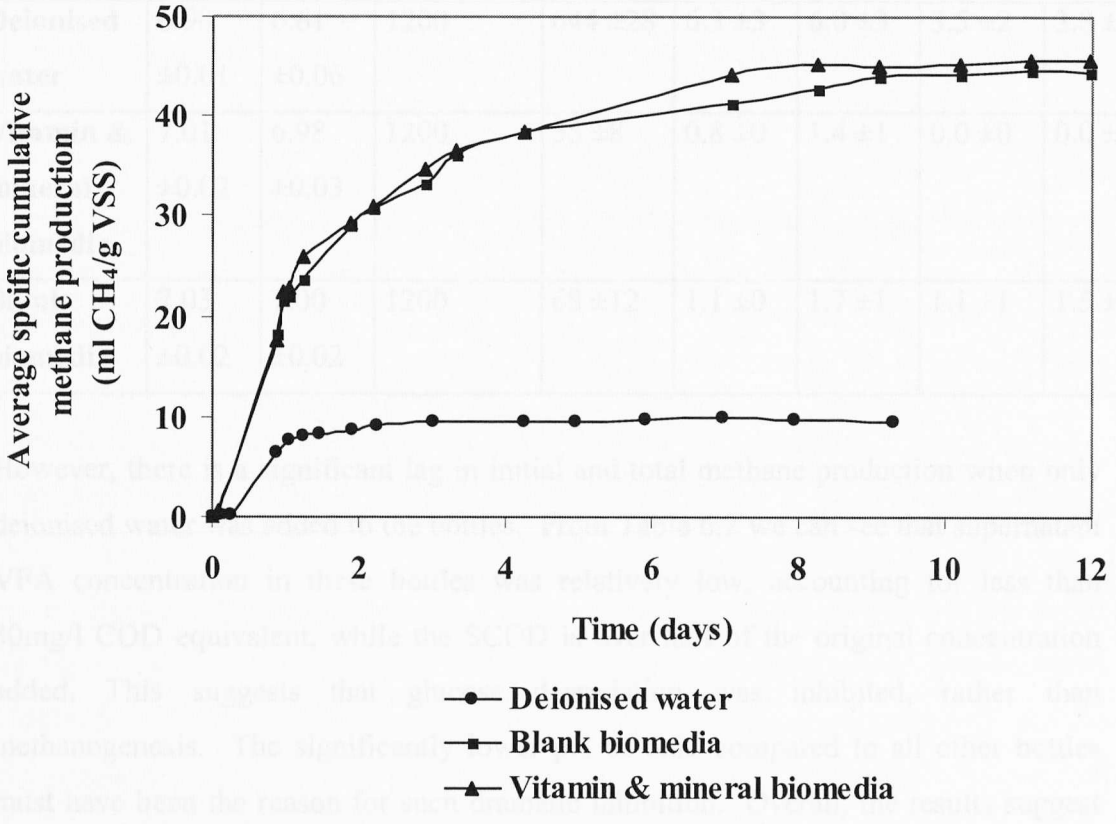


Figure 6. 3- Nutrient Deficiency Test to observe the effect of addition of vitamin-and mineral-rich biomedia, blank biomedia and deionised water on glucose degradation by methanogenic seed sludge. Average based on triplicate samples.

It is possible to see that there is no significant difference in specific methane yield between the bottles supplemented with vitamin- and mineral-rich biomedia or blank

biomedia. This suggests that the effluent sludge contains adequate trace elements since the supplements showed no effect in terms of enhancement, or indeed inhibition of the specific methane yield.

Table 6. 2- Average SCOD, VFA's and pH in triplicate bottles as part of a Nutrient Deficiency Test.

Test Bottle	Start pH	End pH	Initial calculated SCOD conc. (mg/l)	End SCOD conc. (mg/l)	End VFA conc. (mg/l)			
					Acetate	Prop.	N-But.	N-Val.
Deionised water	6.90 ±0.01	6.61 ±0.06	1200	644 ±28	6.3 ±3	6.0 ±3	3.5 ±2	3.8 ±2
Vitamin & mineral biomedia	7.01 ±0.02	6.98 ±0.03	1200	55 ±8	0.8 ±0	1.4 ±1	0.0 ±0	0.0 ±0
Blank biomedia	7.03 ±0.02	7.00 ±0.02	1200	68 ±12	1.1 ±0	1.7 ±1	1.1 ±1	1.5 ±1

However, there is a significant lag in initial and total methane production when only deionised water was added to the bottles. From Table 6.2 we can see that supernatant VFA concentration in these bottles was relatively low, accounting for less than 30mg/l COD equivalent, while the SCOD is over half of the original concentration added. This suggests that glucose degradation was inhibited, rather than methanogenesis. The significantly lower pH of 6.61 compared to all other bottles must have been the reason for such dramatic inhibition. Overall, the results suggest that at this small scale, pH buffering is the crucial factor in maintaining an ideal environment for microbial activity, and that mineral and vitamin concentrations in the supernatant are not at inhibitory levels. Since no apparent negative or positive effects were observed as a result of addition of a vitamin & mineral solution, it was decided to supplement the biomedia with this solution as a precautionary measure.

6.4 KINETIC PARAMETERS OF MIXED MICROBIAL CONSORTIA FROM LAB-SCALE REACTORS

The purpose of these assays was to investigate the effect of different COD:SO_4 ratios on the final stages of anaerobic digestion of primary sewage sludge. The VFA's investigated were those found during the final stages of digestion, namely acetate, propionate, n-butyrate and n-valerate. Although H_2 is an important precursor for methanogenesis and sulfidogenesis, lack of equipment to accurately measure H_2 uptake rates meant that this aspect could not be investigated.

The method behind measuring μ_{max} and the K_s of VFA consists of adding different concentrations of substrate to cover a range that will induce zero-order and first-order kinetics. The concentration of the substrate is measured at time 0 (t_0) and a short time period afterwards (t_x), thus establishing the rate of substrate utilisation at each substrate concentration. Plotting these rates on a curve of substrate utilisation rate vs. substrate concentration will give a classic Monod curve from which the μ_{max} and K_s can be measured.

The advantage of this method is that since the time lapse between t_0 and t_1 is in the region of only 3 to 4 hours microbial growth rates can be considered as negligible. An alternative method would be to add substrate to the seed sludge to induce zero-order kinetics and then follow the degradation rate over a period of several days until first order kinetics could be measured. More importantly, since sulfate is to be added alongside the substrate in the same test, substrate degradation rates will not be representative over the whole period of the test. If sulfate is depleted at a higher rate than the substrate due to the presence of a mix of micro-organisms, no SO_4^{2-} will be present towards the end of the test, thus altering the true value of substrate degradation in the presence of sulfate. As a result, the former method for microbial kinetic analysis was adopted.

As described earlier in Section 5.3.4 on page 145, there are limitations to the kinetic values derived in these experiments due to the inability to measure the biomass concentration X accurately, as well as a lack of information on the Y-values of the

various microbial species involved. As a result, the values derived from these experimental data should be treated with caution; a (*) symbol is used to highlight kinetic values that were derived without the use of Y or X values.

6.4.1 Method To Establish μ_{max^*} And K_{s^*} Of Mixed Microbial Consortia For VFA's

Clean, empty 125 ml McCarty bottles were flushed with the 30% CO_2:70% N_2 gas mix to drive out any air. 50ml of the sludge collected from the lab-scale reactor to be investigated was placed in bottles, and to these was added 20 ml vitamin-rich biomedia while still being purged with the oxygen-free gas. The bottles were quickly sealed with a rubber bung and cable tied. For assays requiring the specific inhibition of SRB, a 1ml spike was injected into the bottle to give a 4mM final concentration of sodium molybdate, and the bottles placed upside down in a water bath (maintained at 35°C). They were left overnight to allow for equilibration of the micro-organisms, and the exhaustion of any residual COD. A 50ml stock solution of 10 mg/ml of the desired VFA and an amount of SO_4 (in the form of H_2SO_4) was formulated to make up a COD:SO_4 ratio such as those found in the large-scale reactor. Different volumes of the stock solution were spiked into duplicate bottles using a syringe to give the following final concentrations in mg/l:

Acetate (COD 1.067 g/g): 1000, 800, 500, 400, 300, 200, 100, 75, 50
Propionate (COD 1.514 g/g): 500, 300, 200, 100, 75, 50
n-butyrate (COD 1.818 g/g): 500, 300, 200, 100, 75, 50
n-valerate (COD 2.039 g/g): 300, 200, 100, 75, 50

A 2 ml sample was immediately extracted for duplicate VFA analysis. This was considered $t_{0;}$ at time t_x, another 2ml sample was removed and similarly analysed. VFA's were analysed using a GC-FID detector. Unfortunately, the detector broke down during sampling of n-valerate assays so that an HPLC detector had to be used instead for these samples. A preliminary test to establish the adequate amount of time to elapse to show significant VFA reduction beyond the Standard Deviation margin of the average results of a duplicate analysis showed t_x to be 3 hours for acetate and 4 hours for propionate, n-butyrate and n-valerate. For the experimental samples, four

samples were collected at t_0 to t_4 at the intervals established above. At t_4, the bottles were immediately removed from the water bath analysed for VSS concentration according to Standard Methods. The results from each bottle were plotted on graphs of Time vs. g VFA/g VSS, and a linear trend-line was drawn. Any results which achieved a linear plot of Time vs. g VFA/g VSS of less than 0.90 were discarded. If results from fewer than four sample bottles (16 data points on Monod curve) could be used, the experiment was repeated again to obtain an adequate number of points. Using the non-linear function analysis software (NLREG) as described in Appendix D, the kinetic parameters of μ_{max} and K_s were derived from the raw data.

6.4.2 Kinetic Data Results Of Mixed Microbial Consortia On VFA's

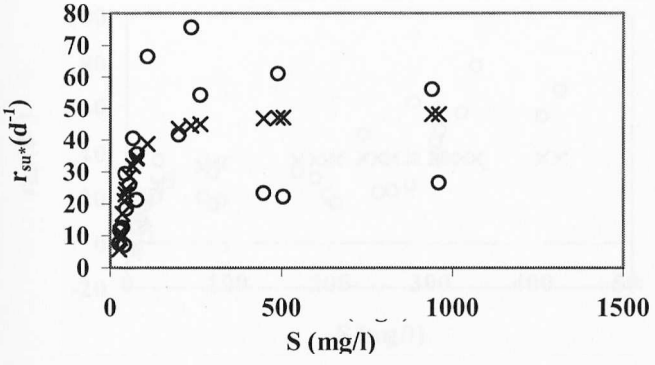
The Monod curves in Figure 6.3 represent the actual experimental data obtained, as well as estimated data obtained via non-linear analysis.

The kinetic constants μ_{max*} and K_{s*} were derived according to the method described in Appendix D. Unfortunately, the linear R^2 values for Time vs. g Valerate/g VSS obtained via HPLC analysis for seed sludge from Reactor C were below 0.90, even after repetition of the experiment. Due to time and budget constraints, it was not possible to repeat the experiment, and therefore these results were excluded. The kinetic data of μ_{max*} and K_{s*}, along with the Standard Error and Proportion of Variance Explained for μ_{max*} as calculated by NLREG software is summarised in Table 6.3. The average Proportion of Variance for all μ_{max*} values calculated was $50.4\% \pm 22\%$.

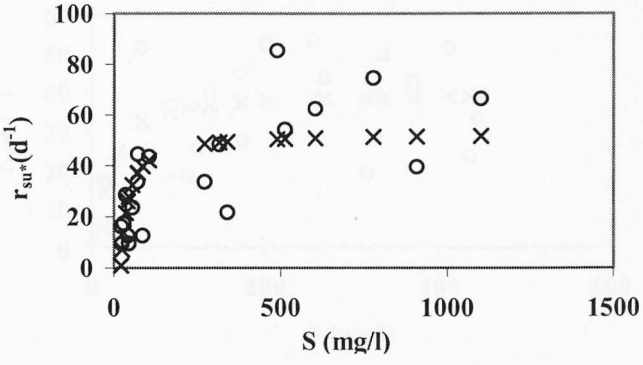
Table 6. 3- Summary of kinetic data for seed sludge from Reactors M-C obtained from small-scale assays. Standard errors of μ_{max*} and Proportion of Variance Explained were derived from non-linear analysis of the raw data by NLREG software (see Appendix D). *See page 145 for explanation.

Reactor Seed Sludge	VFA	Sulfate/ Sodium Molybdate Addition	μ_{max*} (d^{-1})	Standard error of μ_{max*}	Proportion of Variance explained %	K_{s*} ($mg.l^{-1}$)	μ_{max*}/K_{s*}
M	Acetate	–	52.7	5.3	49.4	42.2	1.35
A	Acetate	SO_4	130.1	14.4	49.2	83.1	1.57
B	Acetate	SO_4	118.3	9.4	62.6	98.7	1.20
C	Acetate	SO_4	120.3	10.6	61.6	214.9	0.56
M	Acetate	NaMo	49.4	5.4	47.5	47.3	1.04
A	Acetate	NaMo	79.0	6.2	73.2	94.5	0.84
B	Acetate	NaMo	77.8	3.3	84.3	85.5	0.91
C	Acetate	NaMo	89.7	6.4	66.7	75.6	1.19
M	Propionate	–	40.1	3.4	35.0	22.5	1.79
A	Propionate	SO_4	195.7	14.3	73.7	66.6	2.94
B	Propionate	SO_4	163.5	13.2	60.0	67.1	2.44
C	Propionate	SO_4	101.5	8.3	64.5	51.8	1.96
M	Propionate	NaMo	38.2	3.6	36.00	19.4	1.97
A	Propionate	NaMo	61.8	5.3	17.8	37.7	1.64
B	Propionate	NaMo	53.7	9.8	7.8	139.5	0.38
C	Propionate	NaMo	65.8	12.3	25.8	248.6	0.26
M	Butyrate	–	46.9	3.1	76.6	36.1	1.3
A	Butyrate	SO_4	36.6	2.8	72.3	27.8	1.31
B	Butyrate	SO_4	38.0	3.5	73.8	42.1	0.90
C	Butyrate	SO_4	28.1	2.3	74.3	38.2	0.74
M	Butyrate	NaMo	49.4	5.4	44.7	35.7	1.38
A	Butyrate	NaMo	44.5	4.6	48.8	36.6	1.22
B	Butyrate	NaMo	42.8	2.8	74.2	33.2	1.29
C	Butyrate	NaMo	58.7	5.9	9.9	21.8	2.69
M	Valerate	–	13.5	1.4	26.7	20.1	0.67
A	Valerate	SO_4	13.5	1.0	57.8	22.4	0.60
B	Valerate	SO_4	12.6	1.3	43.0	23.7	0.53
C	Valerate	SO_4	N/A	N/A	N/A	N/A	N/A
M	Valerate	NaMo	15.9	1.3	25.3	16.1	0.99
A	Valerate	NaMo	18.6	1.5	56.0	21.4	0.87
B	Valerate	NaMo	12.9	0.9	15.0	17.0	0.76
C	Valerate	NaMo	N/A	N/A	N/A	N/A	N/A

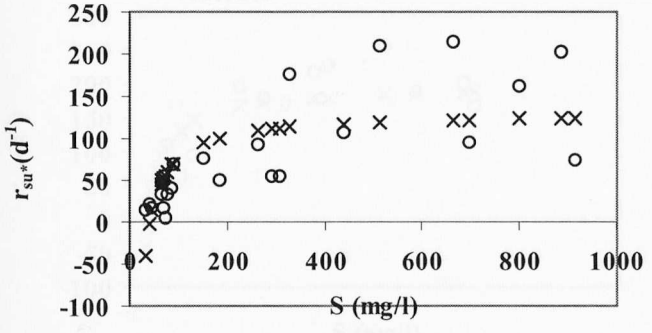
Reactor M Seed + Acetate



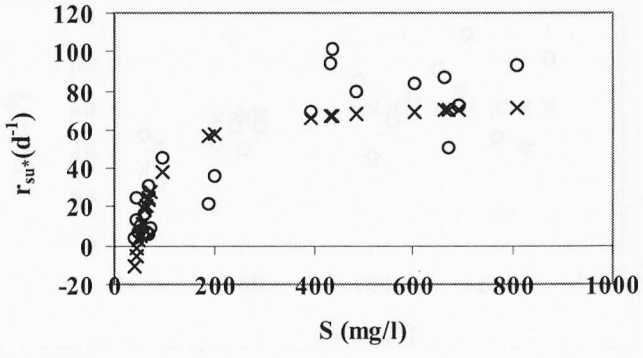
Reactor M Seed + Acetate + Sodium Molybdate



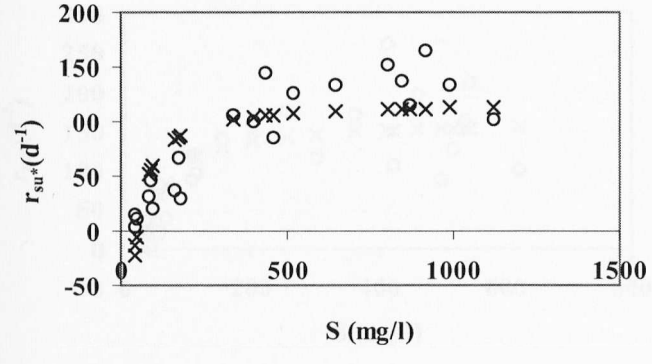
Reactor A Seed + Acetate + Sulfate



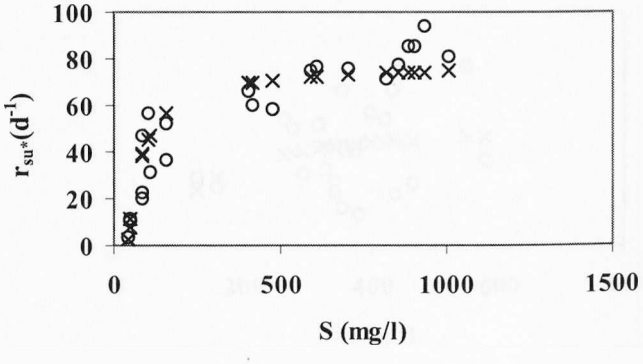
Reactor A Seed + Acetate + Sodium Molybdate



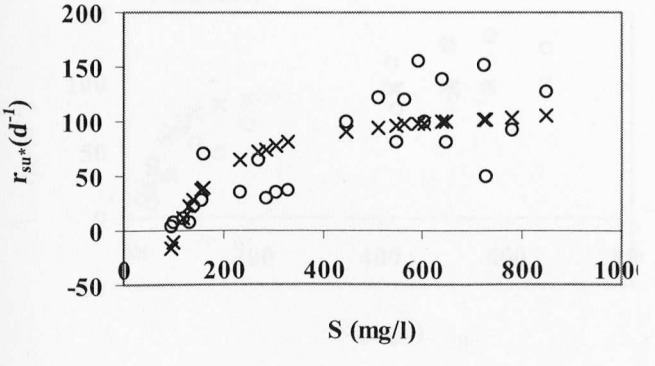
Reactor B Seed + Acetate + Sulfate



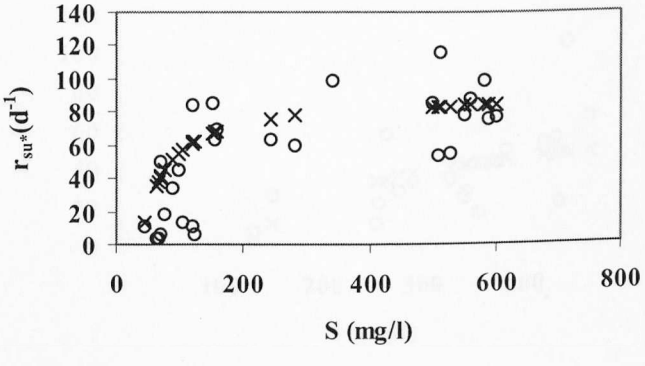
Reactor B Seed + Acetate + Sodium Molybdate

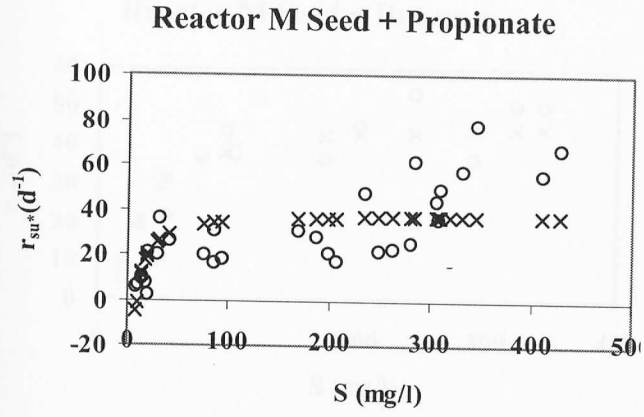


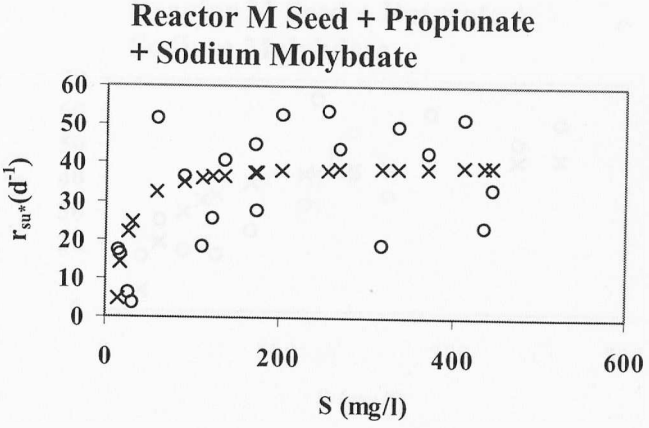
Reactor C Seed + Acetate + Sulfate

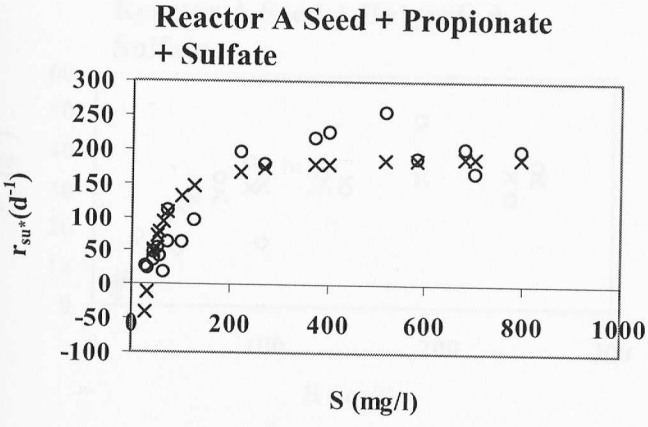


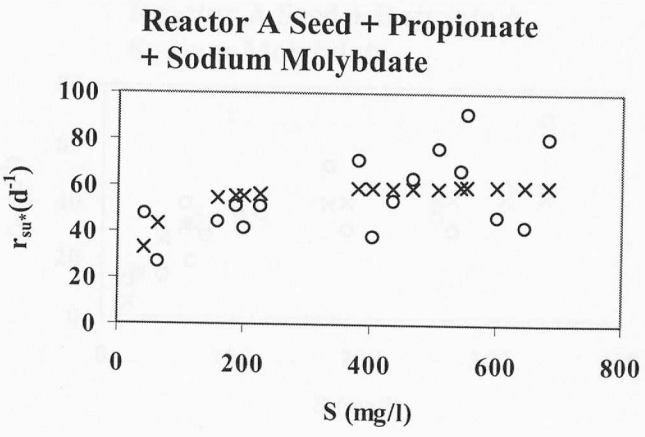
Reactor C Seed + Acetate + Sodium Molybdate

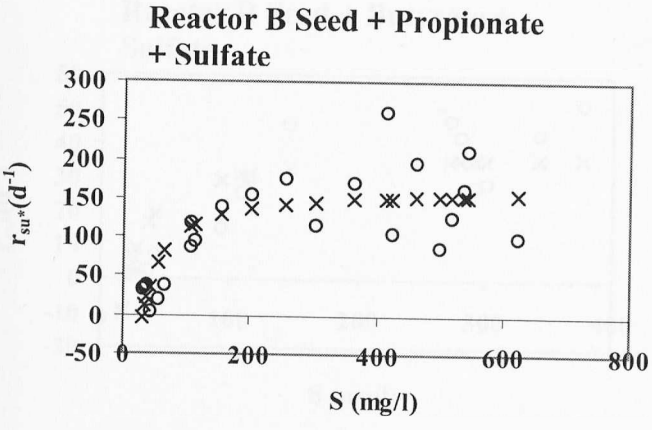


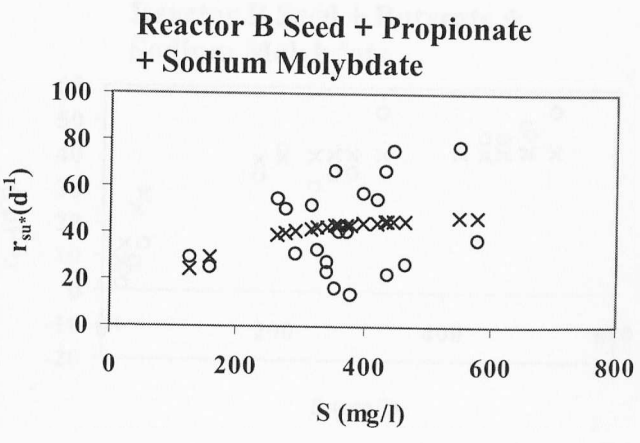


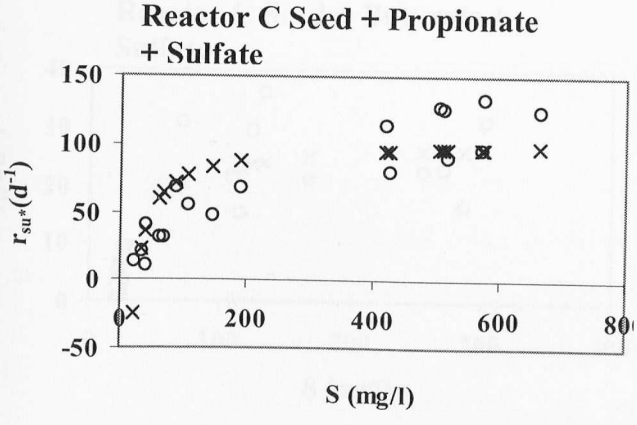


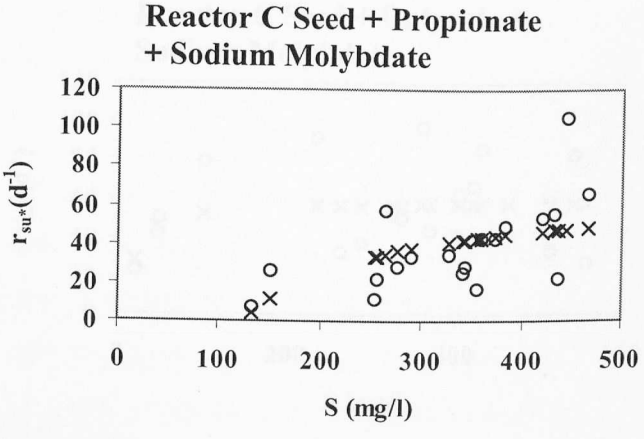






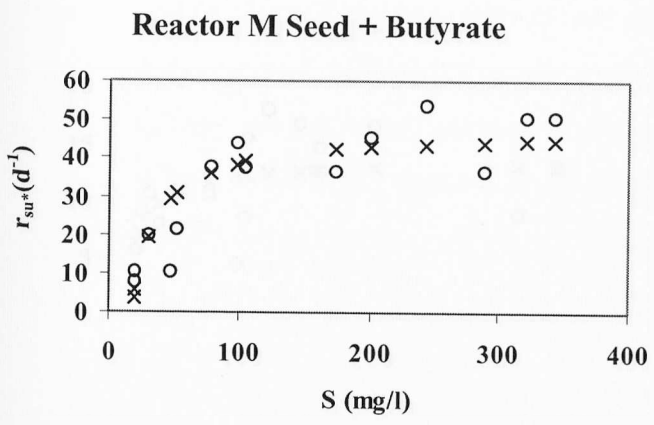


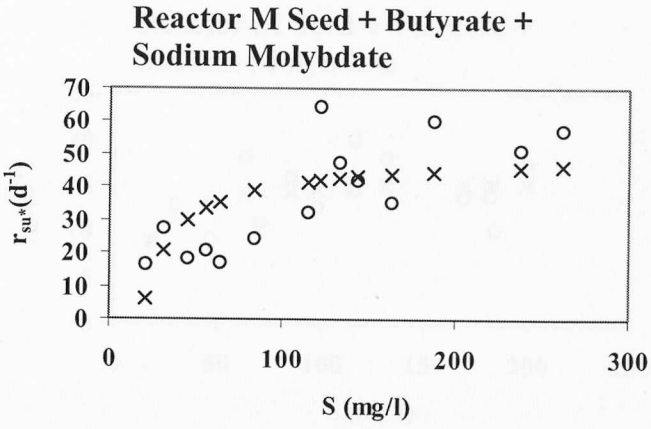


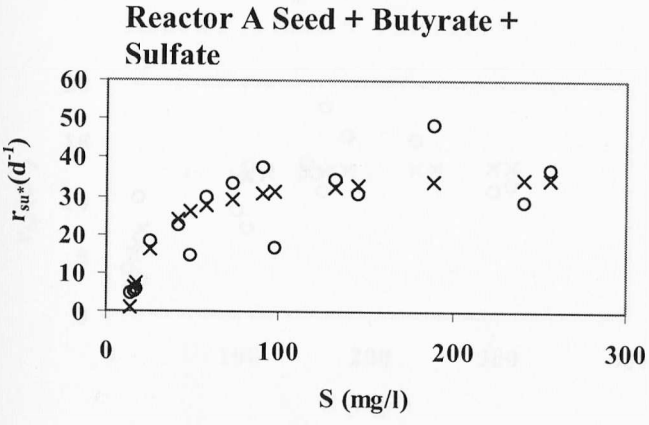


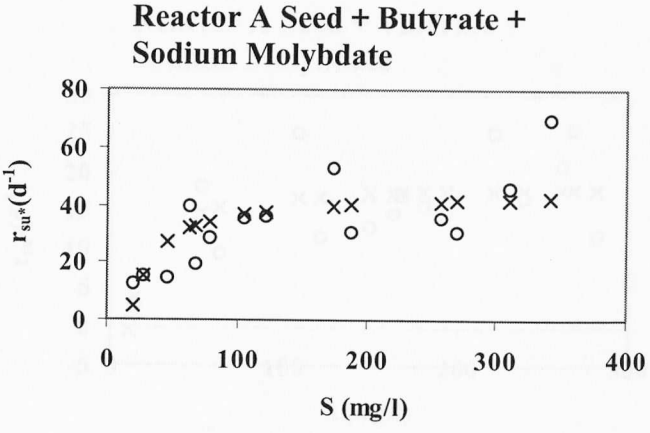
o - Experimental Data

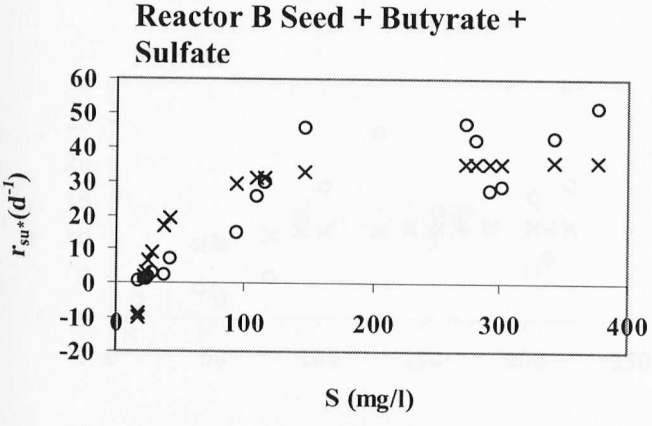
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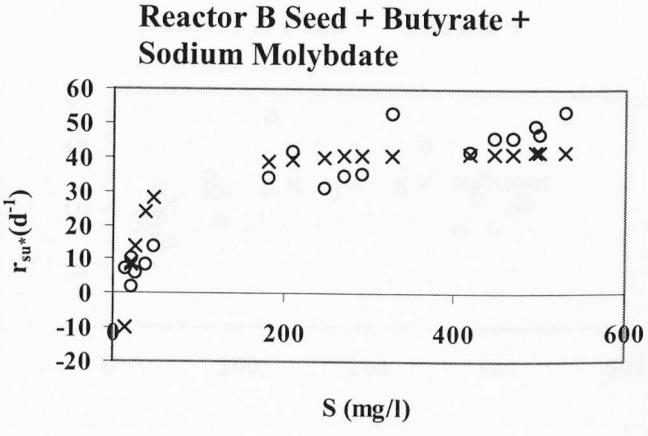


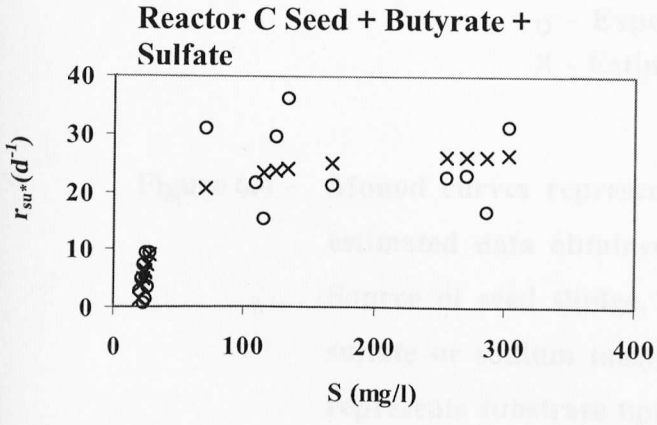


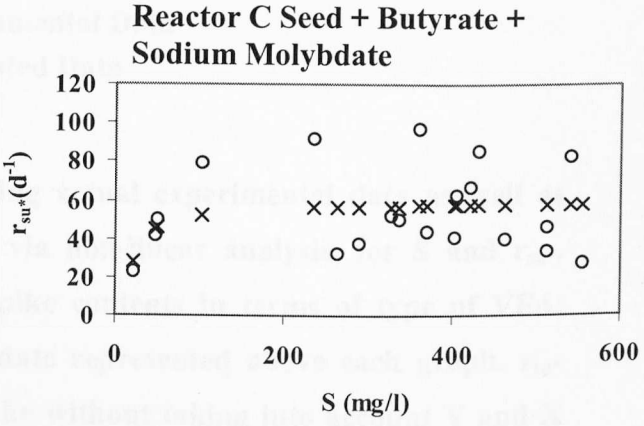












o - Experimental Data

X - Estimated Data

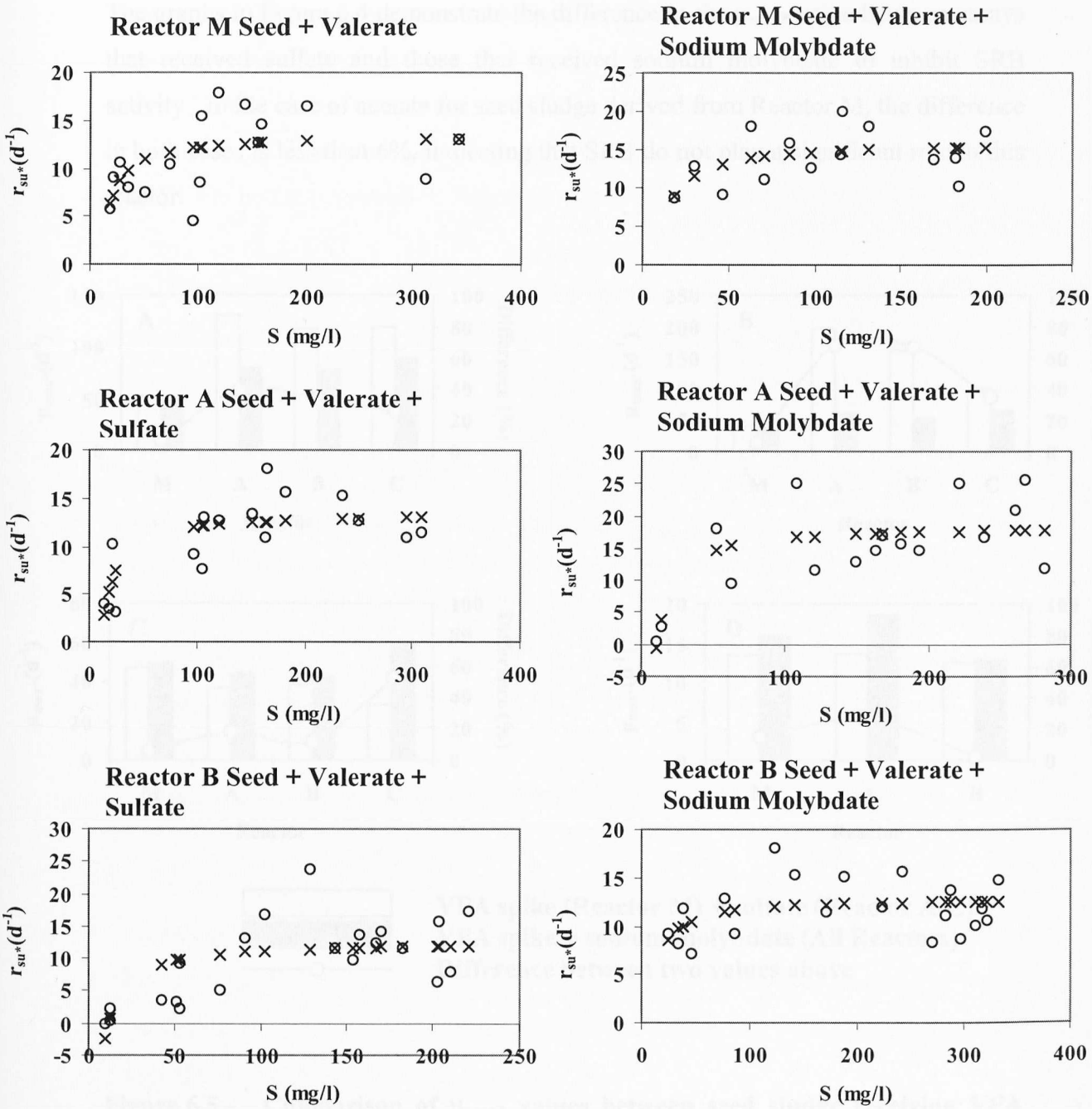


Figure 6.4 – Monod curves representing actual experimental data as well as estimated data obtained via non-linear analysis, for S and r_{su*}. Source of seed sludge, spike contents in terms of type of VFA, sulfate or sodium molybdate represented above each graph. r_{su*} represents substrate uptake without taking into account Y and X from equation 2.17.

The graphs in Figure 6.4 demonstrate the difference in the μ_{max*} value between assays that received sulfate and those that received sodium molybdate to inhibit SRB activity. In the case of acetate for seed sludge derived from Reactor M, the difference in both cases is less than 6%, indicating that SRB do not play a significant role in this reactor.

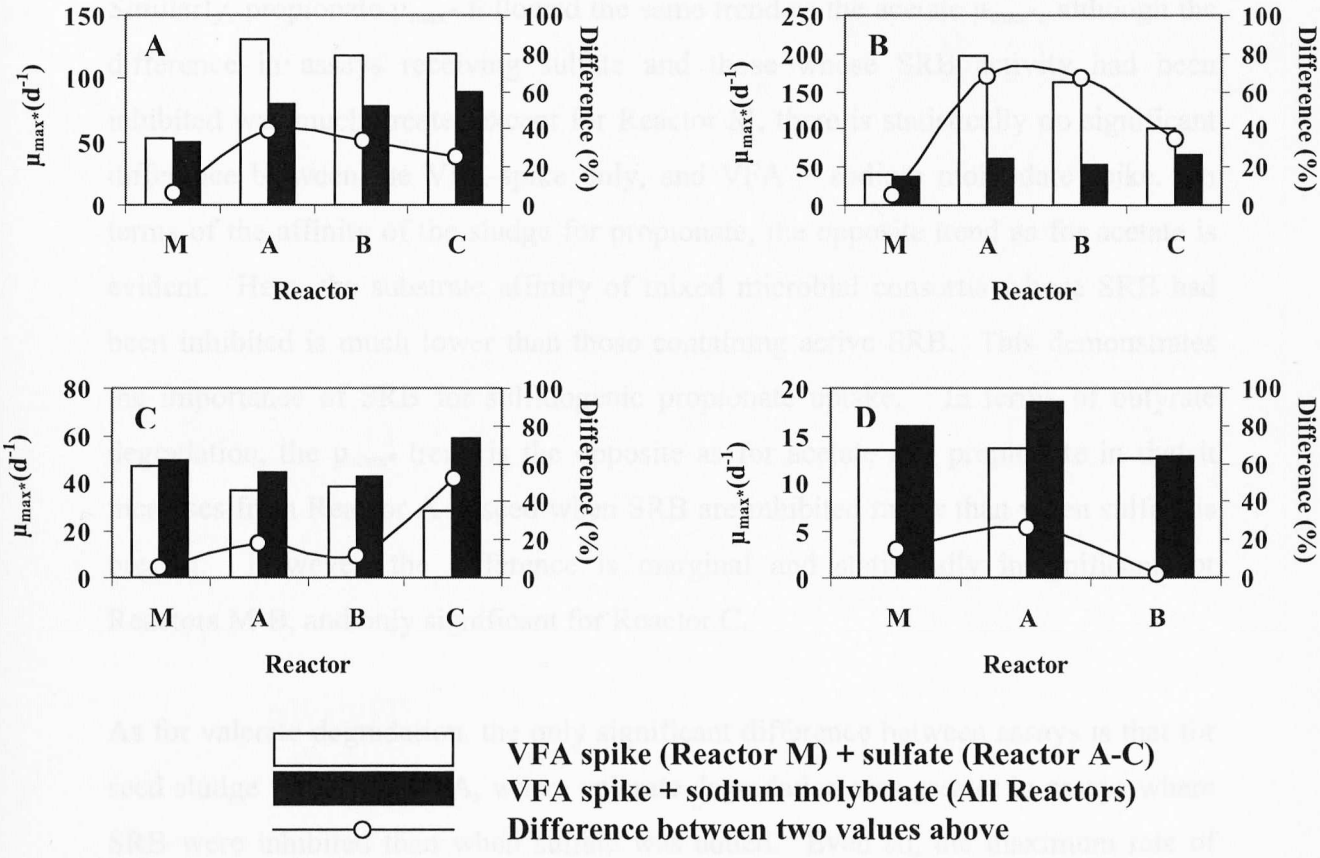


Figure 6.5 - Comparison of μ_{max*} values between seed sludge receiving VFA and sulfate, and VFA + sodium molybdate for SRB, with the difference expressed as a percentage on the secondary Y-axis. Graph A = Acetate spike, Graph B = Propionate spike, Graph C = Butyrate spike, Graph D = Valerate spike.

In contrast, although the μ_{max*} in all assays receiving both acetate and sulfate was higher than those who's SRB had been inhibited (Figure 6.5 - Graph A), the affinity of the mixed microbial consortia for acetate was lower compared to SRB-inhibited

sludge as demonstrated by the increasing K_{s*} values from Reactor A-C in Table 6.3. This shows that is necessary to have a high sulfate concentration in the sludge for SRB to be able to degrade the associated acetate. Reactor C had the highest K_{s*} value of 215 mg/l in the presence of sulfate, which may have resulted from natural selection of SRB that had acclimatised to high sulfate concentrations.

Similarly, propionate μ_{max*} followed the same trend as the acetate μ_{max*}, although the difference in assays receiving sulfate and those whose SRB activity had been inhibited was much greater except for Reactor M, there is statistically no significant difference between the VFA-spike only, and VFA + sodium molybdate spike. In terms of the affinity of the sludge for propionate, the opposite trend as for acetate is evident. Here, the substrate affinity of mixed microbial consortia whose SRB had been inhibited is much lower than those containing active SRB. This demonstrates the importance of SRB for sulfidogenic propionate uptake. In terms of butyrate degradation, the μ_{max*} trend is the opposite as for acetate and propionate in that it increases from Reactor A-C seed when SRB are inhibited rather than when sulfate is present. However, the difference is marginal and statistically insignificant for Reactors M-B, and only significant for Reactor C.

As for valerate degradation, the only significant difference between assays is that for seed sludge from Reactor A, where valerate degradation was greater in assays where SRB were inhibited than when sulfate was added. Even so, the maximum rate of degradation was still low at an average of only 14.5 $d^{-1} \pm 2.3$, which is to be expected due to the long-chain nature of the substrate.

The extent of the importance of SRB activity in the final stages of anaerobic digestion in the sulfidogenic reactors is better expressed by looking at the μ_{max*}/K_{s*} ratios represented in Figure 6.6, which can be used to compare the competitiveness of the bacteria involved in each assay environment for a certain VFA. It is evident that for assays receiving acetate (Reactor M) and acetate + sulfate spike (Reactor A and B), the mixed microbial consortia in these assays were more competitive than those whose SRB activity had been inactivated. On the other hand, Reactor C seed whose source from the lab-scale reactors had been receiving the highest sulfate

concentration, microbial consortia in the assays receiving sulfate were less competitive than those whose SRB had been inhibited, and is a reflection of the low affinity (high K_s) for the acetate in these assays.

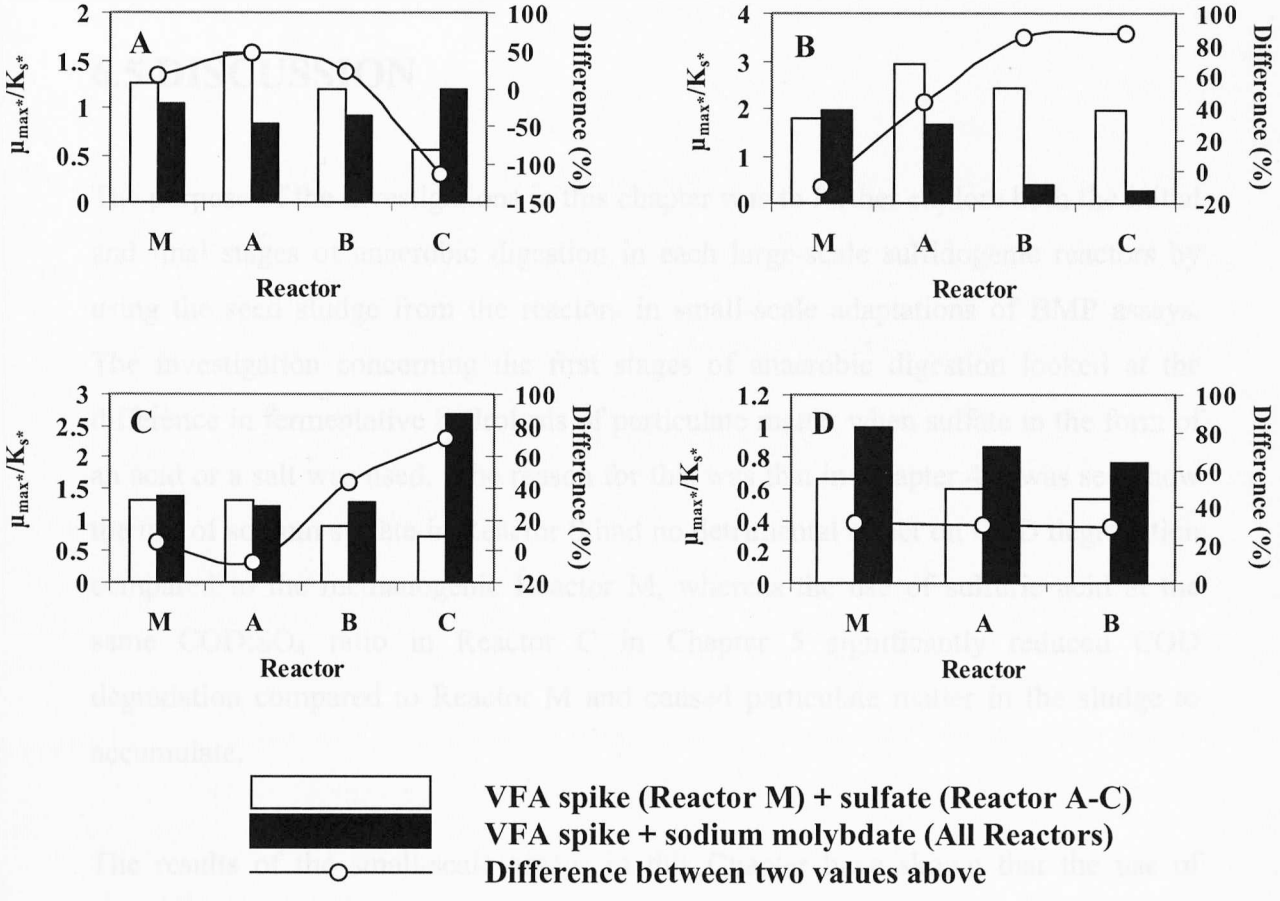


Figure 6.6 - Comparison of μ_{max*}/K_{s*} ratios between seed sludge receiving VFA and sulfate, and VFA + sodium molybdate for SRB, with the difference expressed as a percentage on the secondary Y-axis. Graph A = Acetate spike, Graph B = Propionate spike, Graph C = Butyrate spike, Graph D = Valerate spike.

The μ_{max*}/K_{s*} ratios for propionate shows that in all sulfidogenic reactors, the mixed microbial consortia receiving sulfate in the spike were more competitive for propionate degradation than those whose SRB had been inhibited. The exception is Reactor M, which showed no significant difference between either assay. As for butyrate degradation, there is no significant difference for assays for seed from Reactors M and A. However, with increasing sulfidogenic conditions in the large-scale reactors, those receiving sulfate in the assays were less competitive than those

whose SRB had been inhibited. Graph D in Figure 6.6 shows that valerate degradation is more competitive in reactors who's SRB population had been inhibited, and the difference in μ_{max*}/K_{s*} ratios is consistent in all 3 Reactors whose seed sludge was tested.

6.5 DISCUSSION

The purpose of the investigations in this chapter was to further explore both the initial and final stages of anaerobic digestion in each large-scale sulfidogenic reactors by using the seed sludge from the reactors in small-scale adaptations of BMP assays. The investigation concerning the first stages of anaerobic digestion looked at the difference in fermentative hydrolysis of particulate matter when sulfate in the form of an acid or a salt was used. The reason for this was that in Chapter 4 it was seen how the use of sodium sulfate in Reactor S had no detrimental effect on COD degradation compared to the methanogenic Reactor M, whereas the use of sulfuric acid at the same COD:SO_4 ratio in Reactor C in Chapter 5 significantly reduced COD degradation compared to Reactor M and caused particulate matter in the sludge to accumulate.

The results of the small-scale assays in this Chapter have shown that the use of sulfuric acid does in fact have a detrimental effect on the K_h value, so that the K_h values were reduced by over 70% in seed sludges derived from Reactors A-C. Young *et al.* (1995), investigated ways of making sludge more amenable to anaerobic digestion by the addition of sulfuric acid to a mixture of primary and waste activated sludge to bring the pH down to 1.5, then using Ultra Heat Treatment to autoclave the sludge for 30 minutes at 135°C. The pH of the sludge was raised to 6.5 with sodium hydroxide prior to digestion in a mesophilic anaerobic digester. Results showed that the TS concentration of the sludge was higher than untreated sludge, with a concomitant lower proportion of VS. This was attributed to the addition of sulfuric acid and sodium hydroxide to the sludge prior to digestion, which contributed to the VSS concentration when testing for the solids concentration. As a result, it was not possible to accurately judge the effect of digestion on TS destruction for this sludge. The net specific gas yield of only 48 ml/g VS added compared to 525 ml/g VS added

for non pre-treated sludge lead the authors to speculate that this apparent inhibition of methanogens was due to the formation of toxic compounds during acid hydrolysis treatment. However, it is conceivable that the lower gas yield may be due to similar forces that were acting in the H_2S Generators, so that the lack of particle hydrolysis may have reduced the pool of electrons down to the methanogens.

The inhibition of hydrolysis may be due to a number of reasons. Firstly, as established in the literature research in Chapter 2, the addition of the acid to primary sludge causes the solubilisation of heavy metals into the supernatant. Increasing concentrations of heavy metals as a result of increasing acid addition in bottles containing seed sludge from reactors M-C have caused heavy metal toxicity to the fermentative bacteria. However, this is an unlikely cause in this case, since the production of H_2S in the large-scale reactors is expected to precipitate out most heavy metals. It also does not explain the fact that residual SCOD concentrations in the supernatant remained low, indicating a lack of inhibition of the MPB or SRB.

Other reasons for the inhibition of hydrolysis may be due to the release of some unknown compound as a result of sulfuric acid solubilisation of particulate matter, which may have interfered with the activity of extra-cellular hydrolytic enzymes. Related to this is the possibility of AB inhibition as a result of the release of a toxin. According to Speece (1996), "aceticlastic methanogens are considered to be the most sensitive class. While this is usually consistent, yet some toxicants have actually inhibited the acidogens more than the methanogens. VFA increase and CH_4 decrease indicates methanogen inhibition more than acidogens; however, under the opposite circumstances (VFA low while CH_4 decreases), the acidogens have been inhibited as much or more than the methanogens". If AB were indeed inhibited, the accumulation of intermediate compounds normally broken down by then may have resulted in the inhibition of the fermentative bacteria (an analogy to this would be the inhibition of MPB at high H_2 partial pressures). A final possibility as to why hydrolysis was inhibited is that the easily degradable particles were solubilised first by the sulfuric acid, leaving behind recalcitrant particles that were more difficult to hydrolyse by the bacteria, and are therefore less-energy yielding. As a result, the fermentative bacteria are effectively 'starved' of an energy source.

Overall, the hydrolytic activity did not seem to be permanently inhibited. This was seen with the comparable K_h in the small-scale assays receiving sodium sulfate, even though the seed had been derived from a reactor exposed to sulfuric acid for several hundred days. This suggests that substituting sodium sulfate with sulfuric acid at this stage may restore the hydrolytic activity of the sludge and improve digestion efficiency once more. The results obtained here are in direct contrast to those received of Dunn (1998), who observed an increase in the particulate matter hydrolysis in the sulfidogenic zone of a ponding system, but this may be due to differences in reactor type and kinetics involved. However, the study did observe instances of when the enzyme activity of hydrolytic phosphatases and proteases was inhibited, namely under high sulfate concentration, and pH values between 6.5-7.5.

On the whole, a definite link was established between a decrease in the hydrolysis of primary sludge VSS and sulfuric acid addition, and these results pave the way for further necessary in-depth investigations which are required to determine what precisely causes hydrolytic activity to decrease upon the addition of sulfuric acid.

Although the final stages of anaerobic digestion in the lab-scale digesters seemed to be running efficiently (as demonstrated by a lack of VFA accumulation in the supernatant as well as complete sulfate reduction), the kinetic values derived from the small-scale assays may have been distorted by the apparent inhibition in the particulate hydrolysis stage. This inhibition may have prevented the AcB and AB further down the digestion chain from growing efficiently. This means that the results are only meaningful when looking at these particular digesters, since they give an insight into VFA degradation of acetate through to valerate in the large-scale reactors by MPB and SRB. A comparison of the results with literature data must therefore be used with caution.

The length of time sodium molybdate was used as an inhibitor was minimised since it was possible that genetic mutations/adaptations would occur in the inhibited species, thus altering their metabolic activity. The overall length of inhibition was 12 hours as a maximum; this can be regarded as a relatively long time if considering an average generation time of 0.5 hrs for most microbial species. However, for the purposes of this project, any metabolic changes during this time were considered negligible. In

addition, since a reflection of the kinetic conditions in the lab-scale digesters was desired, the inhibition of only the SRB was deemed adequate since it was seen in Section 5.3.4 that complete sulfate reduction had occurred approximately 8 hours after feeding in the sulfidogenic reactors. It was, therefore, assumed that the lack of an electron acceptor in the form of sulfate for the SRB would prevent them from taking part in the rest of the digestion process in any significant way, thus mimicking their inhibition with sodium molybdate in the small-scale assays.

In the assays with seed sludge from Reactor M, the K_{S*} for acetate when SRB were inhibited was 47 mg/l; slightly higher than the 29 mg/l for AMPB in general cited in Visser *et al.* (1996), but similar to the 50 mg/l value for *Methanosaeta* cited in Pavlostathis and Giraldo-Gomez (1991). The overall results for acetate uptake showed that the rate of acetate oxidation and competitiveness for acetate was higher in the presence of SRB than in their absence for Reactors M-B. The higher substrate affinity also means a higher affinity for sulfate, since each electron donor requires a stoichiometric amount of electron acceptor. This suggests that in Reactors A and B, sulfate limitation may have selected for SRB that were more competitive for acetate. Hence, it was expected that any initial acetate present after feeding would be taken up by SRB until sulfate levels fell below the lower bacterial threshold limit, after which the remaining substrate would be diverted to the methanogens.

As the sulfate concentration increased to the levels in Reactor C, there is a distinct transition where the methanogens involved in acetate oxidation became more competitive to acetate than the mixed microbial consortia. This is demonstrated by the much lower K_{S*} value in assays receiving acetate and sodium molybdate (76 mg/l) compared to those receiving acetate and sulfate (215 mg/l). This result is unexpected, since from a thermodynamic point of view, ASRB are expected to out-compete AMPB, especially at high sulfate concentrations. The reason for this difference may be that when the sulfate concentration becomes less limiting, the competitiveness of different SRB species decreases. As a result, initial acetate uptake in Reactor C would be performed by SRB while sulfate concentrations were still high. As the sulfate concentration decreases, the remaining acetate is taken up by the methanogens. The low acetate affinity in the presence of SRB means that the ASRB affinity for sulfate will also be low. It was seen in the lab-scale reactors that all the sulfate in Reactor C

was reduced to H_2S, and residual levels were negligible (<20 mg/l), meaning that the affinity for sulfate was in fact high. Hence the high K_{s*} value for acetate in the presence of sulfate shows that ASRB are not the main sulfate reducers in this reactor. Various studies have also shown this to be the case in large-scale reactors. For example, in a sulfidogenic municipal sewage treatment plant, a study by Fukui *et al.* (2000) showed that 260 mg/l volumetric sulfate concentration (compared to 220 mg/l in Reactor C) directed less than 20% of acetate towards sulfate reduction. The results obtained here therefore corroborate previous findings that acetate is not a major substrate for sulfate reduction, as discussed at depth in the literature research in Section 2.5.2. The H_2S Generator exhibiting the greatest competitiveness for acetate (high μ_{max*}/K_{s*} ratio) was Reactor A.

In contrast, the results of the small-scale assays for propionate oxidation show that SRB were the main oxidisers of propionate in the presence of sulfate. When sulfate was not present at all, as in Reactor M, kinetic values for SRB-inhibited and non-inhibited sludge were almost identical, indicating that propionate uptake must have taken place by acidogenic bacteria (AB). This was also hypothesised by Gujer *et a.l* (1983), who estimated that up to 15% of methane is derived as a result of propionate degradation by AB in mesophilic anaerobic digesters. As the sulfate concentration increased from Reactors A to C, the non-SRB-inhibited assays showed an increase in μ_{max*} and a concomitant decrease in K_s, whereas when the SRB were inhibited, there was a dramatic decrease in the μ_{max*}/K_{s*} ratios, indicating restricted propionate uptake. Therefore, propionate-degrading SRB had out-competed propiogenic AB in all the sulfate reactors, as was the case in a study by Visser *et al.* (1993), who showed that bacterial activity with propionate was significantly higher in the presence of sulfate than in its absence. Even so, the affinity of AB for propionate was greater to a mixed bacterial consortia containing SRB at any sulfate concentration.

The sulfate concentration at which the mixed consortia were most competitive to propionate (high μ_{max*}/K_{s*} ratio) was equivalent to that found in Reactor A (70 mg/l), and decreased as the sulfate concentration increased. In Reactor C, for example, although the affinity for propionate was higher than in Reactor B in the presence of sulfate, the substrate-uptake rate decreased. This may have been a reflection of the

fact that the digestion efficiency in lab-scale Reactor C had decreased as a result of lack of particulate hydrolysis, thus affecting the SRB further down the chain. Overall, these results for propionate uptake match those obtained by previous authors who investigated the oxidation of this substrate in mesophilic anaerobic digesters, as was summarised in Section 2.5.7.

Concerning butyrate uptake, there was no significant difference in assays receiving sulfate and those who's SRB population had been inhibited in Reactors M and A. However, with increasing sulfidogenic conditions in Reactors B and C, the SRB-inhibited sludge was more active with respect to butyrate uptake than the sludge whose SRB had not been inhibited. This indicates that even after prolonged exposure to sulfate, butyrate-oxidising SRB were not able to out-compete the mixed microbial consortia, due mainly to the lower affinity of SRB-containing sludge for butyrate. Visser *et al.* (1993a), who looked at the degradation of butyrate in 3 UASB reactors also showed that syntrophic butyrate-degrading methanogenic consortia remained present in high numbers even when the reactors were operated at high sulfate concentrations. As for valerate uptake, although it has been reported previously that SRB are able to utilise high-chain VFA's (Fukui *et al.*, 2000; O'Flaherty *et al.*, 1998; Widdel, 1988), the results here show no significant difference between SRB-inhibited and -non-inhibited sludges in Reactors M-B for this substrate. Unfortunately, no results were obtained for Reactor C receiving the highest sulfate concentration in the feed to be able to unequivocally confirm this point.

6.6 SUMMARY

The purpose of experiments performed in this chapter was to look at the kinetics controlling digestion in the lab-scale Sulfate Digestion reactors treating primary sludge at various COD:SO_4 ratios compared to a fully methanogenic digester. It was previously seen in Chapter 5 that an accumulation of particulate matter was evident in the H_2S Generators in proportion to increasing sulfate levels in the feed when sulfuric acid was used. As a result, a small-scale assay experiment was devised to look at whether sulfuric acid indeed has an effect on particulate matter hydrolysis, or whether there was some other unknown factor determining the rate of hydrolysis. The results

of the small-scale assays confirmed the hypothesis that sulfate in the form of sulfuric acid had a detrimental effect on particle hydrolysis compared to sulfate in the form of a salt, which had no significant detrimental effects. Several reasons were put forward as to why this was the case, including the release of toxic substances, or as a result of the hydrolysis of easily-degradable substrates leaving behind more recalcitrant substrates that are less-amenable to hydrolysis and are less energy-yielding to the fermentative bacteria. However, more research is necessary to determine the exact cause of this phenomenon.

The kinetic studies looking at the final stages of digestion were able to successfully determine the K_{s*} and μ_{max*} of the sludge derived from the lab-scale digesters on C2-C5 VFA substrates. The raw data was analysed using non-linear analysis software (NLREG) as described in Appendix D. The results for acetate degradation showed that for a volumetric sulfate concentration of up to 120 mg/l (corresponding to Reactor B), a mixed microbial consortia of ASRB and AMPB were both responsible for acetate uptake. As the concentration increased to 220 mg/l, the K_{s*} value decreased, showing that SRB were not competitive for acetate at high sulfate concentrations. The results for propionate uptake on the other hand, showed that SRB were the main oxidisers of this substrate, and that a mixed consortia in the presence of SRB had a higher μ_{max*} than AB found in a completely methanogenic reactor. However, the affinity of AB for propionate was much higher than a mixed consortia containing active SRB. The reactor in which the microbial consortia was most competitive for acetate and propionate was Reactor A when SRB and sulfate were present.

In terms of butyrate degradation, the small-scale assays showed that for a range of sulfate concentrations, there was no significant difference in assays receiving sulfate and those whose SRB population had been inhibited with sulfate concentrations up to 70 mg/l. As the sulfate levels increased, the butyrate-oxidising AB became more competitive to butyrate uptake than the sludge containing a mixed consortia of AB and SRB. This indicates that even after prolonged exposure to sulfate, butyrate-oxidising SRB were not able to out-compete AB. Finally, concerning valerate as a

substrate, there was no significant difference in its uptake rate at any sulfate concentration, indicating that SRB were not significant users of this VFA.

CHAPTER 7

DISCUSSION AND CONCLUSIONS

7.1 INTRODUCTION
7.2 OBJECTIVES
7.3 DISCUSSION OF THE RESEARCH RESULTS
7.4 CONCLUSIONS
7.5 SUGGESTIONS FOR FUTURE WORK

7.1 INTRODUCTION

The idea for the 3-stage Sulfate Digestion process was developed by Hobson *et al.* (1994), as a novel means of anaerobic sludge digestion; the common thread running through all three stages of the process was the element sulfur. The use of sulfur was intended as a means of destroying pathogens and solubilising metals in its most oxidised form (H_2SO_4), and precipitating out these heavy metals from the feed sludge as metal sulfides in its most reduced form (H_2S). The main aim of this study was to look at the part of the process concerned with anaerobic sludge digestion in a hybrid Sulfide Generator in the presence of sulfate. An investigation into the second part of the Sulfate Digestion process concerned with sulfide oxidation in an H_2S Bioscrubber was also studied.

Previous studies that had explored various aspects of sulfidogenic anaerobic digestion and sulfide oxidation, such as the kinetics and bacteriology, were presented in Chapter 2 as part of the literature review. Using this knowledge, experiments were devised to explore the behaviour of sulfidogenic anaerobic digestion of primary sludge to suit the purposes of the 3-stage process, as well as sulfide oxidation in the bioscrubbers. The work in Chapter 4 was concerned with the start-up of a hybrid Sulfide Generator

using sodium sulfate in the feed, and its behaviour compared to a completely methanogenic reactor. The bioscrubbers were also designed, built and set-up. The main focus was to determine the best layout for the bioscrubbers to avoid problems of pressure differences from one compartment to the next. Preliminary studies into sulfide oxidation were also made.

The next stage then focused solely on the Sulfide Generation process, which led to setting up several more daughter reactors running at various sulfate concentrations. In order to mimic proposed conditions in the 3-stage process, the original sulfate source (sodium sulfate) was changed to sulfuric acid in these reactors. The results of these experiments were described in Chapter 5. The final set of studies looked more closely at the kinetic aspects of the first and final stages of methanogenic and sulfidogenic digestion using the seed sludge from each reactor.

The aim of this Chapter is to bring together all the threads emerging from this research in order to be able to draw clear conclusions about the findings, and any future investigations which may be of relevance to this project.

7.2 DISCUSSION OF THE RESEARCH RESULTS

7.2.1 Start-up Of A Hybrid Sulfide Generator

A preliminary study on the sulfate requirements of a hybrid H_2S Generator showed the required concentrations to be 5 g/l for a 2-2.65% sludge strength. When this concentration was used in the form of sodium sulfate to start-up a sulfidogenic reactor, sulfide production commenced immediately, alongside an increase in reactor pH due to sodium hydroxide production. The chemical scrubber was successful in reducing H_2S concentrations in the biogas to below toxic limits of the micro-organisms in the reactor.

Once the H_2S Generator had been running for approximately 4 HRT's at a 20 day HRT, and stable conditions had been established, a further four daughter reactors

were set up. It was thought that increasing the HRT to 40 days would reduce any shock-effects resulting from substituting sodium sulfate for sulfuric acid in the feed. However, it was seen that decreasing the HRT back to 20 days, with just a 7-day intermediate 27 day HRT, had a detrimental effect on acetate degradation and biogas production, due either to inhibition or wash-out of the AMPB's. It was then necessary to re-seed the reactor with methanogenic sludge, after which stable conditions were re-established. The three daughter hybrid H_2S Generators running at various COD:SO_4 ratios which were started immediately at an HRT of 20 days, established stable conditions in terms of biogas production, sulfate reduction and VFA destruction until the end of the project. These results show that during the start-up of a hybrid H_2S Generators running at a maximum COD:SO_4 ratio of 5:1 , no problems are anticipated when trying to encourage sulfidogenic conditions if sulfate is added in the form of a salt or acid.

7.2.2 Sludge Stabilisation

The use of sulfate as a salt had no detrimental effects on the TCOD destruction of primary sludge in the sulfidogenic reactor compared to the methanogenic reactor at a COD:SO_4 ratio of 5.45:1. This is in line with previous studies that also showed this to be true at even lower ratios, as long as sulfide toxicity was prevented. On the other hand, the solids concentration in the effluent were significantly higher than the control reactor; a result which had not been previously documented. The reason for this apparent contradiction was accounted for by the higher SRB biomass yield which is present in the sludge of the sulfidogenic reactor, but comparatively absent from the methanogenic reactor. A comparison with literature values for biomass yield showed this theory to be plausible.

In contrast, the use of sulfuric acid as a source of sulfate at increasing concentrations in the feed caused both the TCOD and effluent solids to increase, with a concomitant decrease in the specific biogas production levels. This showed that H_2SO_4 gave no advantage to the sulfidogenic reactors in terms of solids solubilisation prior to digestion, even though small-scale assays showed otherwise.

7.2.3 Particle Size Distribution

An analysis of the particle sizes in each sulfuric acid-fed reactor confirmed that there was an accumulation of VSS with increasing sulfuric acid in the feed. On a mass basis, there was an overall decrease in the sum of particles ≥0.075 mm diameter compared to the feed, with a minimum 51% destruction in the reactor receiving the highest sulfate concentration. However, concerning the smaller sized particles < 0.075 mm diameter, there was only 6% decrease in the same reactor. It was speculated that a disruption on the hydrolysis stage of digestion may have caused this decrease in solids digestion.

7.2.4 Sulfate Reduction And VFA Destruction

In all sulfidogenic reactors - whether receiving sulfate as a salt or acid - effluent VFA and sulfate concentrations remained low and comparable to the methanogenic reactor, indicating that SRB and MPB activity had not been negatively affected in any way by sulfidogenic conditions. By looking at the μ_{max*}/K_{s*} ratio for sulfate in the sulfuric acid-fed reactors, it was possible to conclude that with increasing sulfate concentrations in the feed, the greater the competitive advantage of the SRB for substrates; however, the results are subject to the limitation of the inability to accurately measure the biomass yield and biomass fractions in the sludge.

Overall, these results contradicted what would be expected if a digester was not destroying VSS (i.e. digestion efficiency was low), since a lack of VFA or sulfate accumulation implied a lack of inhibition of the final stages of digestion. As a result, the theory that the hydrolysis stage was the only inhibited stage was re-enforced.

7.2.5 Inhibition Of Primary Sludge Hydrolysis

The results from the lab-scale digesters prompted further investigation into why particles were accumulating in the sulfuric acid-fed digester, but not in the sodium sulfate-fed digester which had been running at comparable COD:SO_4 ratios. A small-scale test performed using seed sludge from each reactor and primary sludge supplemented with sulfuric acid at different concentrations showed that with increasing sulfuric acid concentration, the K_h of particulate hydrolysis decreased. One of several reasons were thought to be responsible for this, including the release of

toxic compounds due to solubilisation of the sludge with sulfuric acid. Previous studies showed that the addition of sulfuric acid lowered digestion efficiency (Young *et al.*, 1995), although the reason for this was not discussed. Although hydrolysis inhibition is quite unusual compared to methanogenesis, Speece (1996) reported this to be a possibility.

7.2.6 Kinetics Of Sulfidogenesis and Methanogenesis

The kinetic studies looking at the final stages of digestion for acetate degradation showed that for a volumetric sulfate concentration of up to 120 mg/l, a mixed microbial consortia of ASRB and AMPB were both responsible for acetate uptake. As the concentration increased to 220 mg/l, the K_s value decreased, showing that SRB were not competitive for acetate at high sulfate concentrations. In terms of propionate uptake SRB were shown to be the main oxidisers of this substrate, even though the affinity of AB for propionate was much higher than a mixed consortia containing active SRB. The reactor in which the microbial consortia were most competitive for acetate and propionate was the one receiving the lowest sulfate concentration of 1.47 g/l. For butyrate degradation, even after prolonged exposure to sulfate, butyrate-oxidising SRB were not able to out-compete AB. As for valerate uptake, there was also no significant difference in the uptake rates at any sulfate concentrations, indicating that SRB were not significant oxidisers of this VFA.

7.2.7 Compliance Of The Final Sludge Effluent

Putting the results of this research into a wider legislative perspective, despite the lower VS destruction levels in the sulfuric acid-fed reactors compared to the methanogenic reactor, all the reactors meet USEPA (1994) requirements for at least 38% reduction in VS of untreated sludges. This level is deemed necessary to reduce vector attraction to the biosolids. As long as the sludge meets the necessary pathogen reduction criteria, the sludge would be classified as "Exceptional Quality" that can be applied onto any type of land. Consequently, full-scale application of the hybrid H_2S Generation system to meet these requirements is feasible under the current US legislation which has been used as a guideline by many counties including New Zealand (NZWWA, 2002). In the case of EU legislation, no criteria are allocated for acceptable VS destruction levels, since the main thrust of the legislation is to ensure

pathogen and metal removal and adequate digestion to prevent vector attraction (European Commission Proposal, 2000).

7.3 ORIGINAL PROJECT OBJECTIVES & CONCLUSIONSS

1) To determine the extent of sulfate reduction required in an H_2S Generator in order for a sufficient amount of sulfur to be recycled through the three stages of the Sulfate Digestion process. Initially, sodium sulfate was used rather than sulfuric acid in order to start-up the H_2S Generator without compromising pH integrity as a result of acid addition. The effect of hybrid sulfate digestion on the effluent sludge quality compared to conventional anaerobic treatment was investigated:

- The amount of sulfate as sulfuric acid required to be added to a primary sludge of approximately 2.5% concentration to reduce the pH to 1.8 (to destroy pathogens and solubilise metals) is 5g/l. This is equivalent to a COD:SO_4 ratio of approximately 5:1;

- The use of sodium sulfate as a source of sulfate at a COD:SO_4 of 5.45:1 had no detrimental effects on the digestion efficiency of primary sludge compared to a completely methanogenic reactor.

2) To investigate the effect of using sulfuric acid as a source of sulfate in an H_2S Generator. This is the ultimate aim of the project since sulfuric acid must be used to solubilise heavy metals from the raw primary sludge. Lab-scale reactors were run at different COD:SO_4 ratios, and the sludge treatment efficiency observed to see whether there is a difference in digestion efficiency using sulfate as an acid rather than a salt;

- Sulfuric acid concentrations in the feed of the hybrid Sulfide Generators in the range of 1.47g/l to 4.33 g/l had increasingly detrimental effects on the digestion efficiency of the primary sludge due to the inhibition of the hydrolysis stage:

- $COD:SO_4$ ratios of up to 5:1 had no detrimental effects on the final stages of digestion - namely sulfidogenesis and methanogenesis - when sulfate was used in either a salt or acid form;

3) To build a profile of the microbial kinetics involved during sulfate digestion compared to conventional anaerobic methanogenic digestion, including areas such as hydrolysis, sulfidogenesis and methanogenesis using small-scale batch tests with seed sludge derived from large-scale reactors running at various $COD:SO_4$ ratios:

- At a volumetric sulfate concentration of up to 120 mg/l, a mixed microbial consortia of ASRB and AMPB are both responsible for acetate uptake. At higher concentrations, SRB are no longer competitive. SRB are also the main oxidisers of propionate at all sulfate concentrations, whereas butyrate-oxidising SRB are not able to out-compete AB. SRB are insignificant oxidisers of valerate at any sulfate concentration.

4) To design, build and set-up an H_2S Bioscrubber that can be connected to a H_2S Generator without compromising the anaerobic conditions in the latter reactor. The sulfide oxidation efficiency of the bioscrubber was investigated:

- An optimal recirculation arrangement between the air-lean and air-rich bioscrubber was derived to prevent aeration of the air-lean bioscrubber. It was shown that the novel design for the bioscrubbers has an average sulfide removal efficiency of 92% with an average inlet H_2S concentrations of 0.8%; however, the majority of the oxidised product was in the form of S^0 and not sulfate as a result of a lack of *Thiobacillus* growth.

7.4 SUGGESTIONS FOR FUTURE WORK

7.4.1 Microbial Analysis Of The Sludge In Each Reactor

Using FISH and rRNA Hybridisation techniques, a more accurate, quantitative insight into the type of bacteria involved in anaerobic digestion could be obtained, down to the species level. Of interest would be to look at the differences in the relative

numbers of various trophic groups at differing COD:SO_4 ratios when sulfuric acid is fed to a sulfidogenic reactor. This may give some answers regarding the effects of inhibition of the hydrolysis stage on the various groups involved in anaerobic digestion. Also, such information would help to contribute to a model for sulfidogenic anaerobic digestion as has been developed for conventional anaerobic digestion by the IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes (Batstone *et al.*, 2002).

7.4.2 Investigating The Reasons For Hydrolysis Inhibition

The research conducted here has shown the use of sulfuric acid as a source of sulfate in sulfidogenic reactors has negative effects on the hydrolysis stage of anaerobic digestion. An in-depth analysis of a primary sludge before and after acidification with sulfuric acid (metals, organic and inorganic compounds) to identify potential toxicants, and subsequently performing ATA's on a seed sludge, would help to develop or apply biomass that may be able to degrade this material.

7.4.3 Determining Differences In Yield Coefficients Between MPB's And SRB's

One method of determining whether the theory of a higher Yield coefficient for SRB being responsible for higher TS concentrations in the effluent of the sodium sulfate-fed reactor compared to the methanogenic reactor would be to follow the method suggested by Metcalf & Eddy (1991) to determine various kinetic coefficients from lab data. Several anaerobic CSTR reactors would be run with the same influent feed concentration but at different HRT's (at least 5 were recommended). The steady-state effluent substrate and VSS concentrations could then be used to determine Y (as well as other kinetic data) by plotting the combined results. The equations of the lines would then refer to the various kinetic data. Ten reactors in all would be required to run at various HRT's (5 Methanogenic and 5 Sulfidogenic) to obtain an adequate number of data points for kinetic analysis. A suggested COD:SO_4 of 5:1 should be maintained in the Hybrid H_2S Generators as this ratio has demonstrated that adequate sulfate is present to stimulate SRB growth. Continuous H_2S stripping would also be required to prevent sulfide toxicity. A synthetic feed should be used for easier and more accurate control of the COD mass balance.

The method of Metcalf and Eddy (1991) suggests linearising the data in the graphs by using the reciprocal of the axes, so that the slope of the line and intercept are used to determine the various kinetic data. By doing this, a great loss in accuracy of the results is incurred, as demonstrated by the linearised form of the Michaelis-Menten equation (Lineweaver-Burke plot) (Weber et al., 1996). It would be preferable to analyse the non-linear form of the plots with suitable analysis software e.g. with NLREG software (see Sherrod, 1996).

7.4.4 H_2S Bioscrubber Investigations

The preliminary investigations into sulfide oxidation in a novel bioscrubber configuration showed the potential of this design as a polishing stage for anaerobic reactors that required biogas-recirculation. However, optimal hydrodynamic conditions need to be investigated, and nutrient levels and threshold sulfide limits need to be explored. Respirometry tests could also be carried out on different types of packing in order to find the optimum types for these bacteria. As in the case of anaerobic digestion, FISH probing into the exact types of organisms involved in sulfide oxidation under various process conditions could help quantify and optimise the performance of the bioscrubbers.

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APPENDIX A

Protocol of Analysis for Gas Chromatography

A1. Protocol for the Perkin-Elmer 8700C gc-HWD

METHOD: GASES
SECTION 1 GC CONTROL

	1
OVEN TEMPEATURE (DEG C)	45
ISO TIME (MIN)	2.0
RAMP RATE (DEG C/MIN)	-
HWD 1 RANGE	HIGH
HWD 1 POL	A-B
HWD 1 ZERO	ON
FID SENS	LOW
FID 2 ZERO	OFF
INJ 1 TEMP	60
DET 1 TEMP	125
DET 2 TEMP	OFF
CARRIER 2	0.0 PSIG
EQUILIB TIME	2.0 MIN
TOTAL RUM TIME	2.0 MIN

SECTION 2 TIMED EVENTS

TIME	DET	EVENT		NOTES
-0.20		HOLD		(RUN button is pressed to start countdown)
0.00	1	ATTN	512	(Sample is injected into Port A with simultaneous pressing of RUN button)
0.02		RELAY 0	ON	
0.20		RELAY 0	OFF	
1.95		RELAY 1	ON	
1.98		RELAY 1	OFF	

SECTION 3 DATA HANDLING

DATE ACQUISITION 1&2		REPORT 1&2	
ENABLED	YES	REPORT TYPE	SEPARATE
		CALC TYPE	%

START TIME	0.00 MIN	CALIB FIT	ZERO
END TIME	2.00 MIN	AREA/HT CALC	AREA
WIDTH	5	OUTPUT	
SKIM SENS	0	PRINT TOL	0.0000
BASELINE CORR B-B		SCREEN	YES
		PRINTER	YES
AREA SENS	10000	NO PEAK ALARM	NO
BASELINE SENS	2500		
PEAK IDENTIFICATION 1&2		QUANTIFICATION 1&2	
UNRETD PEAK TIME	0.00 MIN	SCALING FACTOR	1.0000
AREA/HT REJECT	1.0000		
MULTIPLE REF PEAK	NO		
REF PK: TIME	0.00 MIN		
TIME TOL	0.10 MIN		

A2. Protocol for the Perkin-Elmer 8500C gc-FID: Manual Injection

METHOD: MANUAL VFA ANALYSIS

SECTION 1 GC CONTROL

	1	2
OVEN TEMPEATURE (DEG C)	90	130
ISO TIME (MIN)	2.3	1.8
RAMP RATE (DEG C/MIN)	20.0	
FID 2 SENS	HIGH	
DET ZERO	ON	
INITIAL DET	2	
DET 2 TEMP	300	
PRESSURE 2	3.0 PSIG	
EQUILIB TIME	0.0 MIN	
TOTAL RUM TIME	6.1 MIN	

SECTION 2 TIMED EVENTS

TIME	EVENT		NOTES
-1.10	HOLD		(RUN button is pressed to start countdown)
-1.00	PTV TEMP	100	
-0.50	RELAY 2	ON	
-0.40	PTV TEMP	340	
0.00			(Sample injected, count to 4, RUN button pressed and syringe

		detracted)
0.50	PTV TEMP	0
1.00	RELAY 2	OFF

SECTION 3 DATA HANDLING

DATE ACQUISITION		REPORT	
START TIME	0.00 MIN	CALC TYPE	%
END TIME	6.10 MIN	AREA/HT CALC	AREA
		PRINT TOL	0.0000
WIDTH	3	OUTPUT	
SKIM SENS	0	SCREEN	YES
BASELINE CORR B-B		PRINTER	YES
DET 1 AREA SENS	50		
DET 2 AREA SENS	7615		
DET 1 BASE SENS	4		
DET 2 BASE SENS	370		

PEAK IDENTIFICATION		QUANTIFICATION	
UNRETD PEAK TIME	0.00 MIN	SCALING FACTOR	1.0000
AREA/HT REJECT	1.0000		
REF PK: TIME	0.00 MIN		
TIME TOL	0.10 MIN		

A3. Protocol for the Perkin-Elmer 8500C GC-FID: Autosampler Method

In order for the g.c. to go into automatic mode, it is necessary for the screen to go blank, which happens after 15 minutes of no buttons on the g.c. being pressed. Therefore, the g.c is set with two consecutive 'SETS' of methods, the first as a 'dud' method which last 15 minutes, and the next one is the actual autosampler method. The 'dud' method is described below.

METHOD: DUD 15 MINUTES
SECTION 1 GC CONTROL

	1
OVEN TEMPEATURE (DEG C)	90
ISO TIME (MIN)	7.0
RAMP RATE (DEG C/MIN)	-
FID 2 SENS	HIGH
DET ZERO	ON
INITIAL DET	2
DET 2 TEMP	300

PRESSURE 2	3.0 PSIG
EQUILIB TIME	0.0 MIN
TOTAL RUM TIME	7.0 MIN

SECTION 2 TIMED EVENTS

TIME	EVENT	NOTES
-8.00	HOLD	

SECTION 3 DATA HANDLING

DATE ACQUISITION		REPORT	
START TIME	0.00 MIN	CALC TYPE	%
END TIME	7.00 MIN	AREA/HT CALC	AREA
		PRINT TOL	0.0000
WIDTH	3	OUTPUT	
SKIM SENS	0	SCREEN	NO
BASELINE CORR B-B		PRINTER	NO

DET 1 AREA SENS	50
DET 2 AREA SENS	7615
DET 1 BASE SENS	4
DET 2 BASE SENS	370

PEAK IDENTIFICATION			QUANTIFICATION	
UNRETD PEAK TIME		0.00 MIN	SCALING FACTOR	1.0000
AREA/HT REJECT		1.0000		
REF PK:	TIME	0.00 MIN		
	TIME TOL	0.10 MIN		

A4. AUTOSAMPLER PROGRAMMES

The autosampler method is identical to that described in A2. for manual injection, except for the following sections:

SECTION 2 TIMED EVENTS

TIME	EVENT		NOTES
-1.10	HOLD		(RUN button is pressed to start countdown)
-1.00	PTV TEMP	100	
-0.35	RELAY 2	ON	

-0.25	PTV TEMP	340
0.00		(Autoinjection of sample)
0.50	PTV TEMP	0
1.00	RELAY 2	OFF

SECTION 3 DATA HANDLING

OUTPUT TO SCREEN	NO
OUTPUT TO PRINTER	NO

AUTOSAMLER PROGRAMMES

PROG NO	FILL TIME (S)	INJECT TIME (S)	FLUSH TIME (S)	INJEC PER VIAL
0	10	0	10	1
1	15	4	30	2

SET 0

NAME	PRE AUTO		OPTIONS	
METHOD	DUD 15 MINUTES		CLEAN UP RUN	OFF
AS PROG	0		AUTO SENS	OFF
			PRINT METHOD	OFF
INITIAL VIAL	3		PRINT SET	OFF
NO OF SAMPLES	1			
FLUSH REQUIRED	0			

VIAL	TYPE	PROG	NAME (of sample vial)
3	SAMPLE	0	DUD SAMPLE

SET 3

NAME	VFA'S		OPTIONS	
METHOD	MAN. VFA ANAL.		CLEAN UP RUN	OFF
AS PROG	1		AUTO SENS	OFF
			PRINT METHOD	OFF
INITIAL VIAL	4		PRINT SET	OFF
NO OF SAMPLES	5			
FLUSH REQUIRED	2			

VIAL	TYPE	PROG	NAME (of sample vial)
4	SAMPLE	1	ACETATE
5	FLUSH	1	ACETATE
6	SAMPLE	1	PROPIONATE
7	FLUSH	1	PROPIONATE
8	SAMPLE	1	N-BUTYRATE
9	FLUSH	1	N-BUTYRATE
10	SAMPLE	1	ISO-BUTYRATE
11	FLUSH	1	ISO-BUTYRATE
12	SAMPLE	1	N-VALERATE
13	FLUSH	1	N-VALERATE

A5. Cool-Down Method Perkin-Elmer 8500C GC-FID

When not in use but still switched on, the gc-FID can be left in cool-down mode where the carrier gas supply can be turned off, and the oven, injector and detector temperatures lowered until the g.c is next required.

METHOD: COOLDOWN
SECTION 1 GC CONTROL

	1
OVEN TEMPEATURE (DEG C)	34
ISO TIME (MIN)	999.9
RAMP RATE (DEG C/MIN)	-
FID 2 SENS	LOW
DET ZERO	ON
INITIAL DET	2
DET 2 TEMP	125
PRESSURE 2	0.0 PSIG
EQUILIB TIME	0.0 MIN
TOTAL RUM TIME	999.9 MIN

SECTION 2 TIMED EVENTS

TIME		EVENT
0.01	PTV TEMP	0

SECTION 3 DATA HANDLING

DATE ACQUISITION		REPORT	
START TIME	0.00 MIN	CALC TYPE	%
END TIME	999.90 MIN	AREA/HT CALC	AREA
		PRINT TOL	0.0000
WIDTH	5	OUTPUT	
SKIM SENS	0	SCREEN	YES
BASELINE CORR B-B		PRINTER	NO

DET 1 AREA SENS	50
DET 2 AREA SENS	200
DET 1 BASE SENS	4
DET 2 BASE SENS	4

PEAK IDENTIFICATION QUANTIFICATION

UNRETD PEAK TIME	0.00 MIN	SCALING FACTOR	1.0000
AREA/HT REJECT	0.0000		

REF PK:	TIME	0.00 MIN
	TIME TOL	0.10 MIN

APPENDIX B

Chemical Composition of Biomedia

Table B1-Chemical composition of biomedia added to Nutrient Deficiency Test bottles using glucose as substrate.

	Volume Stock Solution	Amount to prepare 1L Biomedia	Compound	Conc. in stock solution	Final conc. in medium
A1	100 ml	10 ml	K_2HPO_4	65.3 g/l	653 mg/l
A2	100 ml	10 ml	$NaH_2PO_4.2H_2O$	19.5 g/l	195 mg/l
A3	100ml	1ml	Resazurin	0.5 g/l	0.5 mg/l
A4	-	443.9mg	NH_4HCO_3		443 mg/l
		3730 mg	$NaHCO_3$		3730 mg/l
A5	1000 ml	1ml	$Na_2S.9H_2O$	0.24 g/l	0.24 mg/l
A6	-	66 mg Vitamin Tablet	Vitamins +Trace Elements		
		(Holland & Barret ABC Plus)	Vitamin A		400 µg/l
			Vitamin D		2.5 µg/l
			Vitamin E		10 mg/l
			Vitamin C		30 mg/l
			Vitamin B-1		0.7 mg/l
			Vitamin B-2		0.8 mg/l
			Niacin		9 mg/l
			Vitamin B-6		1 mg/l
			Folic Acid		200 µg/l
			Vitamin B-12		0.5 µg/l
			Biotin		0.07 mg/l
			Pantothenic Acid		3 mg/l
			Vitamin K		15 µg/l
			Ca		81 mg/l
			P		75 mg
			Fe		7 mg/l
			Mg		50 mg/l
			Zn		7.5 mg/l
			I		75 µg/l
			Cu		1 mg/l

			Mn		1.25 mg/l
			K		20 mg/l
			Cl		18 mg/l
			B		35 µg/l
			Si		1 µg/l
			Cr		17.5 µg/l
			Se		17.5 µg/l
			Ni		2.5 µg/l
			Sn		5 µg/l
			V		5 µg/l

B1. Biomedia Preparation

One litre of biomedia is prepared by adding 10ml of solution A1 and A2, and 1ml of solution A3 to a conical flask containing 978ml de-ionised water. The flask is capped with a watch glass to prevent water loss and boiled for several minutes. It is left to cool down to room temperature while purging with a 30%CO_2:70%N_2 gas mixture. The solution is transferred to a 1L glass bottle continuously flushed with the same gas mixture. Compound A4 is added to the bottle. For the 'blank solution' containing no minerals or vitamins, 1ml of A5 is added to the mixture and then tightly sealed with a cap ready for use. For the mineral and vitamin-rich solution, compound A6 is also added to the bottle before being sealed.

APPENDIX C

Student's t-Test Table

Degrees of Freedom	p=0.05	p=0.025	p=0.01	p=0.005
1	12.71	25.45	63.66	127.32
2	4.30	6.20	9.92	14.09
3	3.18	4.17	5.84	7.45
4	2.78	3.50	4.60	5.60
5	2.57	3.16	4.03	4.77
6	2.45	2.97	3.71	4.32
7	2.36	2.84	3.50	4.03
8	2.31	2.75	3.36	3.83
9	2.26	2.68	3.25	3.69
10	2.23	2.63	3.17	3.58
11	2.20	2.59	3.11	3.50
12	2.18	2.56	3.05	3.43
13	2.16	2.53	3.01	3.37
14	2.14	2.51	2.98	3.33
15	2.13	2.49	2.95	3.29
16	2.12	2.47	2.92	3.25
17	2.11	2.46	2.90	3.22
18	2.10	2.44	2.88	3.20
19	2.09	2.43	2.86	3.17
20	2.09	2.42	2.84	3.15
21	2.08	2.41	2.83	3.14
22	2.07	2.41	2.82	3.12
23	2.07	2.40	2.81	3.10
24	2.06	2.39	2.80	3.09
25	2.06	2.38	2.79	3.08
26	2.06	2.38	2.78	3.07
27	2.05	2.37	2.77	3.06
28	2.05	2.37	2.76	3.05
29	2.04	2.36	2.76	3.04
30	2.04	2.36	2.75	3.03
40	2.02	2.33	2.70	2.97
60	2.00	2.30	2.66	2.92
120	1.98	2.27	2.62	2.86
infinity	1.96	2.24	2.58	2.81

Wilcoxon Signed-Rank z Table

z critical	Level of significance for a directional test
1.645	0.05
1.960	0.025
2.326	0.01
2.576	0.005
3.291	0.0005

APPENDIX D

Analysis of Kinetic Data Using NLREG Software

Since the Monod equation (e.q 2.21) does not have an asymptotic form, it would be impossible to derive an accurate μ_{max} value. It was therefore necessary to calculate the μ_{max} by using the asymptotic equation 1-D. Once the μ_{max} and K_s values were derived, they were used to calculate estimated μ values from the original experimental data for S using the regular Monod equation.

$$\mu = \mu_{max} + \frac{\alpha}{S} \qquad \text{(eq. 1-D)}$$

Where

α = constant

Taking the data in Table D1 as an example, μ_{max} and α were derived from the asymptotic equation 1-D to give a μ_{max} value of 59.5 and α of -73.1. Estimated μ values were calculated by using the derived μ_{max} and α-values and the original S-values to give the results of the third column in Table D1. In this case, the normal form of the Monod equation was used, i.e $\left(\mu = 59.5 + \frac{-73.1}{S}\right)$. A comparison of the actual and estimated μ is shown in Figure D1. K_s can now be calculated by rearranging equation 1-D to solve for S and putting the ½ μ_{max} value in place of μ as shown in equation 2-D. The result for K_s is 2.46.

$$K_s = S, \text{ when } \frac{\alpha}{\frac{1}{2}\mu_{max} - \mu_{max}} \qquad \text{(eq. 2-D)}$$

$$\therefore K_s = \frac{-73.16}{29.75 - 59.50} = 2.46$$

Table D1- Example data used to demonstrate method of μ_{max} and K_s estimation.

Experimental data for S (mg/l)	Experimental data for μ (d^{-1})	Estimated data for μ (d^{-1})
8.5	50	51
8.0	50	50
7.6	49	50
7.2	50	49
6.1	49	48
5.8	46	47
5.4	47	46
4.4	45	43
3.5	37	39
3.0	33	35
2.0	25	23
1.5	10	11

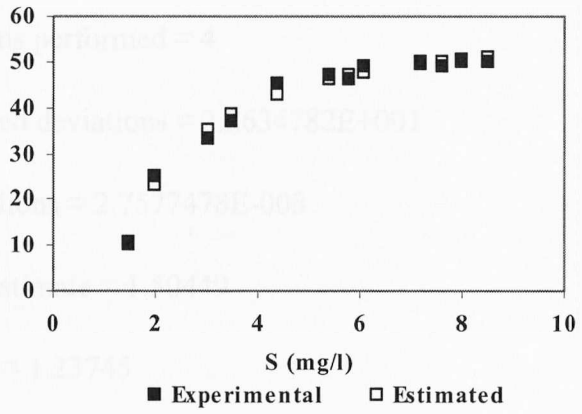


Figure D1- Difference between experimental data and that estimated by NLREG software.

Statistical analysis of the results: At the end of each analysis, the NLREG programme performs a series of statistical analyses on the data as listed below under "Analysis results calculated by NLREG software". The most important one to note is the highlighted "Proportion of Variance Explained" statistic which in this case is 98.7%. This statistic indicates how much better the entered equation predicts the dependent variable than just using the mean value of the dependent variable. If the entered equation perfectly predicts the observed data, the value of this statistic will be 1.00 (100%). If the function does no better a job of predicting the dependent variable than

using the mean, the value will be 0.00. Another important statistic to consider is the Standard Error (SE) of the μ_{max}. The SE is a measure of the amount of error in the prediction of y for an individual x.

Analysis results calculated by NLREG software:

Estimation of μ_{max}

Number of observations = 12

Maximum allowed number of iterations = 500

Convergence tolerance factor = 1.000000E-010

Stopped due to: Both parameter and relative function convergence.

Number of iterations performed = 4

Final sum of squared deviations = 2.2634782E+001

Final sum of deviations = 2.7577478E-008

Standard error of estimate = 1.50449

Average deviation = 1.23745

Maximum deviation for any observation = 2.13025

Proportion of variance explained (R^2) = 0.9870 (98.70%)

Adjusted coefficient of multiple determination (Ra^2) = 0.9857 (98.57%)

Durbin-Watson test for autocorrelation = 2.376

---- Descriptive Statistics for Variables ----

Variable	Minimum value	Maximum value	Mean value	Standard dev.
S	1.5	8.5	5.25	2.373528
μ	10	50	40.91667	12.59479

---- Calculated Parameter Values ----

Parameter	Initial guess	Final estimate	Standard error	t	Prob(t)
μ_{max}	1	59.4965196	0.8014428	74.24	0.00001
K_s	1	-73.1577805	2.652141	-27.58	0.00001

---- Analysis of Variance ----

Source	DF	Sum of Squares	Mean Square	F value	Prob(F)
Regression	1	1722.282	1722.282	760.90	0.00001
Error	10	22.63478	2.263478		
Total	11	1744.917			

APPENDIX E

Raw Data For Sulfate Reduction in Reactors A-C

Table E1- Raw data for sulphate reduction test in Reactors A-C.

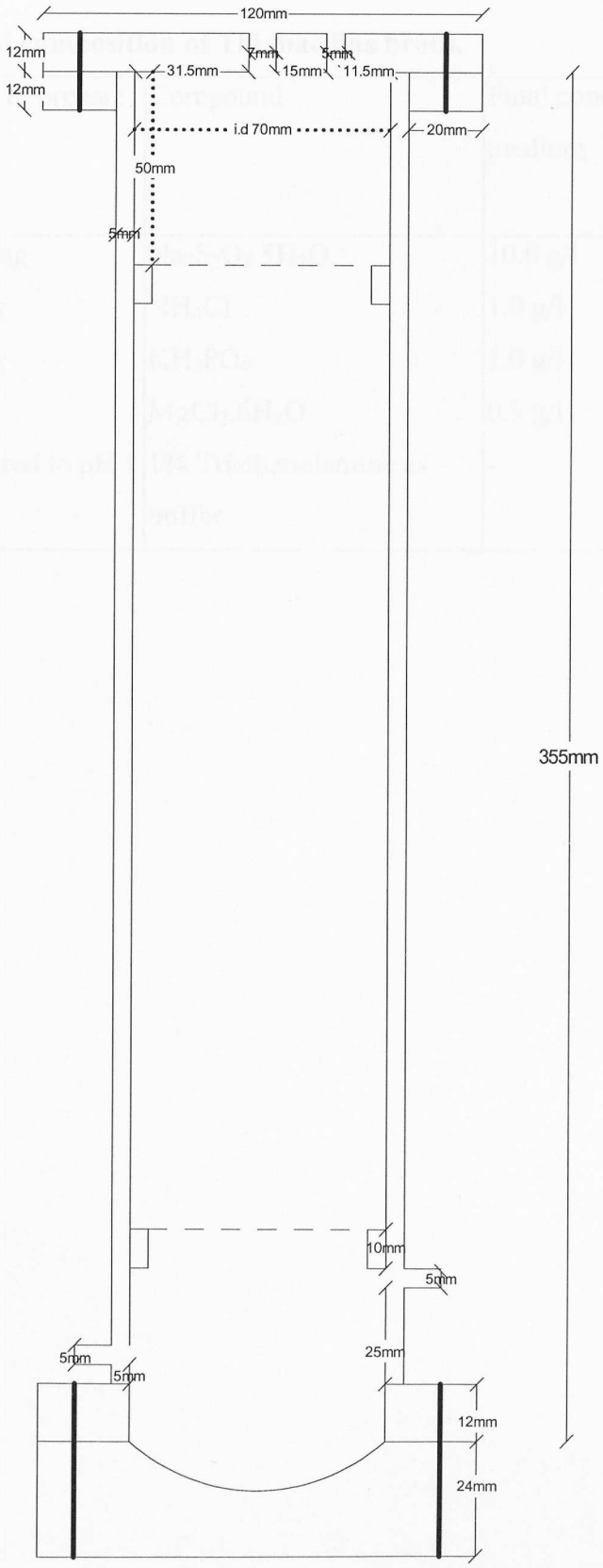
Trial	Reactor	VSS conc. (g/l)	Time (Hrs:Min)	Experimental data for S (g/l)	Experimental data for μ (d^{-1})	Estimated data for μ (d^{-1})
1	**A**	**0.4**	**10:45**	**0.075**	**0.052**	**0.174**
			11:35	0.074	0.029	0.171
			12:30	0.074	0.110	0.169
			13:30	0.072	0.042	0.162
			15:30	0.071	0.114	0.156
			16:34	0.069	0.238	0.146
			17:25	0.065	0.267	0.130
			18:15	0.062	0.104	0.109
			20:00	0.056		
2	**A**	**0.4**	**10:30**	**0.088**	**0.192**	**0.217**
			10:45	0.087	0.420	0.215
			11:00	0.085	0.031	0.209
			11:30	0.085	0.289	0.208
			12:00	0.082	0.211	0.199
			12:45	0.080	0.437	0.191
			13:00	0.078	0.065	0.185
			13:45	0.077	0.132	0.182
			14:40	0.075	0.159	0.174
			15:25	0.073	0.085	0.166
			16:40	0.071	0.362	0.158
			17:10	0.068	0.110	0.144
			18:22	0.066		
3	**A**	**0.4**	**10:00**	**0.097**	**0.302**	**0.241**
			10:20	0.095	0.150	0.236
			11:00	0.093	0.258	0.232
			11:45	0.090	0.342	0.224
			12:00	0.089	0.074	0.220
			13:00	0.088	0.230	0.216
			13:55	0.084	0.129	0.206
			14:30	0.083	0.242	0.202
			15:00	0.081	0.215	0.195
			16:00	0.077	0.222	0.182
			16:40	0.075	0.224	0.172
			17:45	0.071	0.107	0.155
			19:00	0.068		
1	**B**	**0.55**	**10:45**	**0.142**	**0.146**	**0.734**
			11:35	0.139	0.669	0.726
			12:30	0.125	0.654	0.686
			13:30	0.110	0.613	0.631
			15:30	0.082	0.240	0.475
			16:34	0.076	0.531	0.428
			17:25	0.066	0.509	0.324

			18:15	0.056	0.073	0.192
			20:00	0.053		
2	**B**	**0.55**	**10:30**	**0.133**	**0.526**	**0.710**
			10:45	0.130	0.762	0.701
			11:00	0.125	0.559	0.686
			11:30	0.119	0.830	0.666
			12:05	0.108	0.789	0.622
			12:45	0.096	0.902	0.564
			13:00	0.091	0.202	0.534
			13:45	0.087	0.845	0.512
			14:40	0.070	0.250	0.365
			15:25	0.065	0.238	0.318
			16:40	0.059	0.295	0.228
			17:10	0.055	0.039	0.175
			18:22	0.054		
3	**B**	**0.55**	**10:00**	**0.115**	**0.509**	**0.157**
			10:25	0.111	0.686	0.651
			11:00	0.101	0.752	0.634
			11:45	0.088	1.213	0.589
			12:00	0.081	0.376	0.516
			13:00	0.072	0.566	0.467
			13:55	0.061	0.147	0.393
			14:30	0.059	0.379	0.257
			15:00	0.054	0.034	0.229
			16:00	0.053	0.132	0.160
			16:40	0.051	0.040	0.147
			17:45	0.050	0.017	0.110
			19:00	0.231	1.587	0.091
			10:00	0.208	0.659	0.812
			19:00	0.050		
1	**C**	**0.62**	**10:40**	**0.142**	**0.130**	**0.734**
			11:10	0.139	0.593	0.726
			11:36	0.125	0.580	0.686
			12:08	0.110	0.544	0.631
			12:40	0.082	0.213	0.475
			13:45	0.076	0.471	0.428
			14:30	0.066	0.451	0.324
			15:25	0.056	0.065	0.192
			17:30	0.051		
2	**C**	**0.62**	**10:30**	**0.133**	**0.467**	**0.710**
			11:00	0.130	0.676	0.701
			11:35	0.125	0.496	0.686
			12:05	0.119	0.736	0.666
			13:00	0.108	0.700	0.622
			13:45	0.096	0.800	0.564
			14:25	0.091	0.179	0.534
			15:22	0.087	0.750	0.512
			16:35	0.070	0.222	0.365
			17:10	0.065	0.211	0.318
			17:55	0.059	0.262	0.228
			18:35	0.055	0.035	0.175
			19:10	0.035		
3	**C**	**0.62**	**9:30**	**0.241**	**1.500**	**1.330**
			10:15	0.212	1.098	1.296
			10:45	0.198	2.362	1.276

11:00	0.183	0.827	1.251
11:35	0.170	2.276	1.227
12:10	0.136	0.944	1.138
13:33	0.102	1.545	0.993
14:25	0.068	1.099	0.695
15:10	0.047	0.171	0.291
15:55	0.043	0.138	0.192
18:00	0.036		

APPENDIX F

Bioscrubber Measurements



NOT TO SCALE

APPENDIX G

Ingredients For Growing A *Thiobacillus* Culture

Table G1- Chemical composition of Thiobacillus broth.

Volume stock solution	Amount to prepare 1L	Compound	Final conc. in medium
1000 ml	10,000 mg	$Na_2S_2O_3.5H_2O$	10.0 g/l
	1000 mg	NH_4Cl	1.0 g/l
	1000 mg	KH_2PO_4	1.0 g/l
	500 mg	$MgCl_2.6H_2O$	0.5 g/l
	As required to pH 7	1% Triethanolamine as buffer	-