The Biological Sulphate Removal Process

by

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DECLARATION

I the undersigned hereby declare that the work contained in this thesis is my own original work and that I have not in its entirety or in part submitted it at any university for a degree.

Signature:

Date:

SUMMARY

South Africa is one of the world's major coal producers, resulting in the second highest foreign exchange earner for South Africa. However, the mining industry contributes negatively to (ground) water pollution, due to the formation of acid mine drainage (AMD). AMD originates from the bacterial oxidation *(Thiobacillus ferrooxidans)* of pyrite (FeS) and contains high levels of sulphate and metals. Sulphate rich waters can be treated applying the biological sulphate removal technology.

This study concentrated on biologically removing sulphate from synthetic feed- and mine water, using the single-stage completely-mixed reactor system. The advantage of using this reactor system is that except for removing sulphate from about 2000 to less than 200 mg/ℓ, it can also partly biologically remove the formed sulphides. It was established that both ethanol and sugar can be used, as the carbon and energy source, however ethanol is more cost effective than sugar. Ethanol dosage and Hydraulic Retention Time (HRT) studies were undertaken to investigate at what concentration, the highest sulphate and sulphide removal rates were achieved. It was found that the highest sulphate reduction rates were obtained when using 1 mℓ ethanol/ℓ feed and that the removal rates were dependent on the HRT: the lower the HRT, the higher the sulphate reduction rate. The highest sulphide oxidation rate was achieved at the HRT of 6 h. It was, furthermore shown that the single stage completely-mixed reactor system could successfully be used to remove sulphate from Schoongezicht mine effluent, not only removing the sulphate, but also most of the metals, thereby increasing the mine effluent pH from 2.5 to 7.

The conclusion of this study was that a completely-mixed reactor system, as described in this thesis, can successfully be applied to treating acid mine drainage using ethanol (1 mℓ etanol/ℓ feed water) as the carbon and energy source at a hydraulic retention time as low as 4 hours. This technology has great potential for pilot- and full-scale treatment of sulphate rich effluents such as acid mine drainage.

OPSOMMING

Suid Afrika is een van die vernaamste steenkool produseerders in die wêreld, terwyl die uitvoer van steenkool die land se tweede hoogste verdiener is van buitelandse valuta. Ongelukkig dra hierdie industrie ook by tot die besoedeling van (grond) water, veral vanweë die vorming van suur myn afloop. Bakteriese oksidasie (deur *Thiobacillus ferrooxidans*) van piried (FeS) is hoofsaaklik verandwoordelik vir die vorming van suur myn afloop bevattende hoë konsentrasies van sulfaat en metale. Die toepassing van biologiese sulfaatverwyderingsprosesse vir die behandeling van sulfaatryke waters is vroeër gedemonstreer.

Die doel van hierdie studie was om 'n enkel-stadium reaktor met volledige vermenging te evalueer en te optimiseer om toegepas te word vir die biologiese verwydering van sulfaat vanuit sinteties bereide, sowel as mynwater. Hierdie reaktor is in staat om sulfaat te verwyder vanaf vlakke van ~ 2000 tot minder as 200 mg/ℓ. 'n Verdere voordeel gepaard met die gebruik van hierdie reaktor is dat die sulfied wat gevorm word tydens sulfaat-reduksie, gedeeltelik verwyder word deur die oksidasie daarvan na S^o. Die resultate wat behaal is in hierdie studie het aangedui dat beide etanol en suiker gebruik kan word as die koolstof en energiebron, terwyl etanol meer koste-effektief aangewend kon word. In teenstelling was metanol nie 'n geskikte koolstofbron vir sulfaatverwydering nie. Eksperimente is daarvolgens uitgevoer om toestande van optimum etanoldosering en hidroliese retensietyd (HRT) vir maksimum sulfaat- en sulfiedverwydering te bepaal. Die hoogste reduksie tempo's was verkry met 'n toediening van 1 mℓ etanol/ℓ invloei, en die effektiwiteit van verwydering was afhanklik van HRT. Hoe laer die HRT, hoe hoër die tempo van sulfaatverwydering. Die beste sulfaatverwyderingstempo was behaal teen 'n HRT van 6 uur. Die resultate het verder aangetoon dat die enkel-stadium reaktor met volledige vermenging in staat was om sulfaat effektief te verwyder, en die pH te verhoog vanaf na 2.5 tot 7, in mynuitvloeisels van 'n plaaslike steenkoolmyn.

Die gevolgtrekking uit hierdie werk is dat 'n volledig-gemengde reaktorstelsel, soos beskryf in die huidige studie, geskik is vir die suksesvolle behandeling van suur mynafloopwater met die gebruik van etanol (1 mℓ/ℓ toevoerwater) as koolstof- en energiebron by 'n hidrouliese retensietyd tot so laag as 4 uur. Dié tegnologie het groot toepassingspotensiaal vir volskaalse behandeling van sulfaatryke afloopwaters soos bv. suur mynafloop.

PREFACE

As with so many events in one's daily life, the work for this thesis was totally dependent on a sequence of events, all related to two contracts. The first one involved biological effluent treatment high in nitrate and the second one dealing with an effluent, which both had high ammonia and a high sulphate concentration. While finishing off the first contract, I got involved executing the second one, using the same reactor systems. This treatment protocol deviated from the traditional way of treating high sulphate concentrations and proved to be a novel and successful manner to gain the objectives. In the following years, research funding was invested to further study the biological sulphate and partly removal of the produced sulphides, which led to several international and national conference and published papers. From there it seemed a "small" effort to rework the results of this research work to make it the subject of my Masters thesis. The definition of the word small has to be re-defined, now that the thesis is finished!

FUNDING

The funding for executing the work as described in this thesis was provided by Anglo Coal, NRF (THRIP) and the CSIR (STEP).

The STEP funding provided for the initial research in the subject of biological sulphate removal. During the past 6-7 years, a close working relationship has been established between Dr. Angus Christi, Mr. Peter Gunther (Anglo Coal) and Dr. Jannie Maree (CSIR). Challenging problems relating to mine effluents have been solved due to the close collaboration between Anglo Coal and the CSIR. The funding of Anglo Coal has been instrumental for the further development of the technologies as investigated at the CSIR.

My involvement started in 1998-'99, when we could successfully remove high concentrations of sulphate from the "Schoonie" (Schoongezicht Mine) water, applying the CSIR technology for biological sulphate removal, using the single stage completely-mixed reactor system. The treatment of Schoonie water resulted in the operation and later in the commissioning of the CSIR-o-sure plant at Navigation Mine, Witbank (Plate 1).

THRIP funding started, once Anglo Coal was recognised as the CSIR-business partner. Part of THRIP funding was used to pay for my student fees, thus enabling me to enrol at the University of Stellenbosch.

ACKNOWLEDGEMENTS

The seed to use the conference papers as a base for this MSc thesis was sown and watered by my mentor Dr. Jannie Maree. Once it had taken root, the process became exiting and enjoyable. My thanks go to Jannie for his trust in my

iii

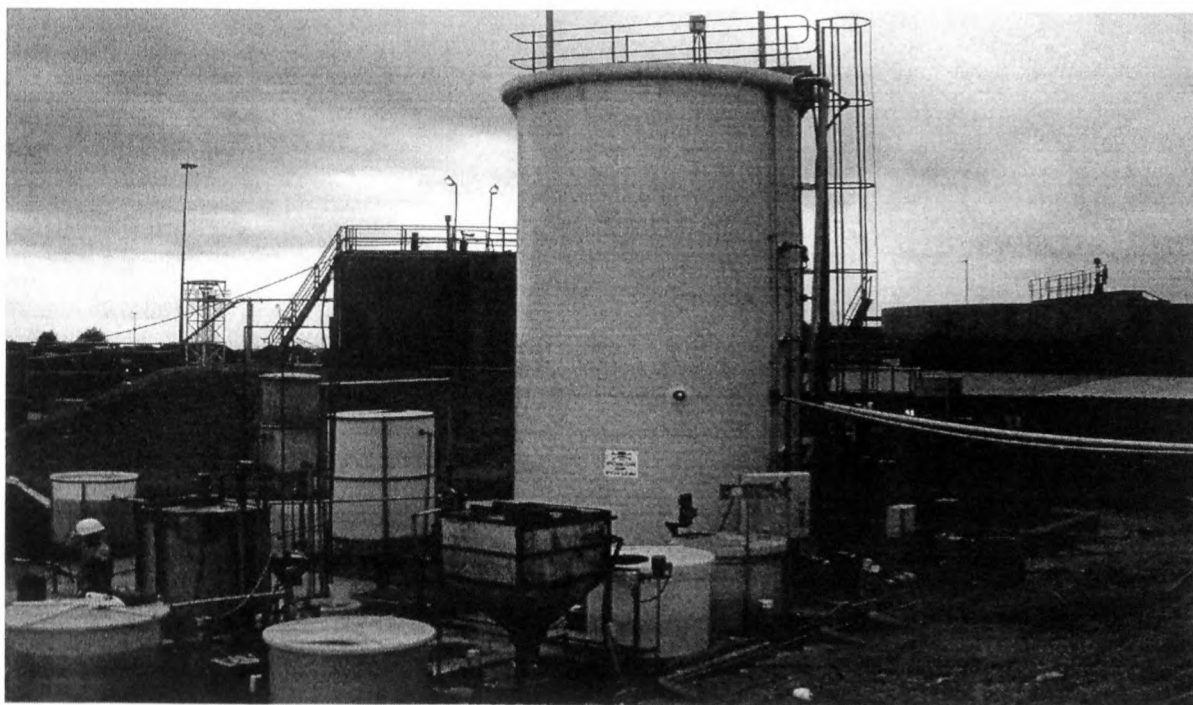capabilities and to Johan de Beer, my Programme Manager for his encouragement.

In addition, a big thank you to my promoter, Gideon Wolfaardt, who guided me through this process. Both Gideon and I worked in the "old" Watertek, at which time (late eighties), neither he nor me could have thought that we would ever enter the relationship of promoter and student.

I also want to thank my colleagues Yet Singmin, Sivuyelli Mnquanqeni, Ellenore Steyn, Daniel Khalo and Amos Tefu and especially my weekend helper, Linda Meyer, for their technical and maintenance assistance. They all in their own way did a great job. Thesis layout assistance was capably provided by Olga Webb.

Last but not least thanks to my family: to my daughters Martine and Annelies, who left the nest in time for me to concentrate on my studies and mostly to my husband Jan Meint, who showed loyalty, interest and patience and offered not only help where needed but also space in his "sacred" study!

DEDICATION

To my deceased parents who would have been very proud had they lived to experience this moment.

Plate 1: The CSIRosure Plant (Anglo Coal)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

ABBREVIATIONS

AB   : Acetogenic Bacteria
AMD   : Acid Mine Drainage
EGSB   : Expanded Granular Sludge Bed Reactor
HRT   : Hydraulic Retention Time
HSRB   : Hydrogen Utilizing Sulphate Reducing Bacteria
HMB   : Hydrogenotrophic Methanogens
MB   : Methanogenic Bacteria
MPB   : Methane Producing Bacteria
SOB   : Sulphide Oxidizing Bacteria
SRB   : Sulphate Reducing Bacteria
UASB   : Upflow Anaerobic Sludge Blanket
VFA   : Volatile Fatty Acids

THE BIOLOGICAL SULPHATE REMOVAL PROCESS

1.   INTRODUCTION

The economy in South Africa derives a significant proportion of its income from gold and coal-mining industries, as South Africa is one of the world's major coal producers and the third largest exporter in the world. Coal is the second highest foreign exchange earner for South Africa, with revenue from coal exports increasing from R6.5 billion in 1995 to R8.0 billion in 1996. Due to its scope and extent, the mining industry contributes negatively to the pollution of the water environment by producing salts to the surface and groundwater. These salts originate mainly from sulphuric acid when pyrite (FeS_2), which is associated with coal deposits, is exposed to oxygen and water during coal mining activities. This form of pollution is referred to as acid mine drainage and is considered a serious form of pollution as it occurs over the total area which has been mined with seepage into ground- and surface water. The problem can often be observed many years after the closure of the mine.

Due to the limited annual rainfall in South Africa, the country is considered a semi arid country. For that reason, water has been identified as the country's most limiting natural resource. Due to both the rapidly growing population and to the upliftment of the South African population in the rural areas, the total water demand for agriculture, housing, industrialisation and mining has increased rapidly. From this perspective, it is evident that all water sources have to be valued as an important commodity and thus that industrial effluents should be treated and reused. Annually an excess of 200 Mℓ/day of mining effluent is discharged in the water bodies of the Gauteng area, which resembles a sulphate load of at least 73000 t/annum and in Mpumalanga this contribution is estimated at 12000 t/annum (Maree, 1988).

The sulphur cycle (Fig. 1) is, like the carbon and nitrogen cycles, an essential process in nature. However, due to human activities, the cycle can be easily disturbed, both on a local and on global scale (Kuenen and Robertson, 1992). One of the major environmental pollutants in the sulphur cycle is the formation of SO_2 and other sulphur compounds by

the burning of fossil fuels, due to global industrialisation. The sulphur cycle consists of several steps: including an oxidative and a reductive component, which in a natural ecosystem should be in balance. On the reductive side, sulphate and sulphur function as an electron acceptor in the metabolic pathways, used by a wide range of anaerobic bacteria. On the oxidative side of the cycle, reduced sulphur compounds serve as electron donors for anaerobic phototrophic bacteria, which gain their energy from (sun)light or provide growth energy for the colourless sulphur bacteria. From an industrial management perspective, the best way to manipulate the sulphur cycle is to stop it at sulphur, which being insoluble, can be easily recovered.

Acid mine water can be treated *chemically* with lime and limestone neutralization technologies, however the residual sulphate in the form of gypsum ($CaSO_4.2H_2O$) is dependent on the solubility of gypsum, which is measured at about 1500 mg/ℓ as sulphate (SO_4). For removal of sulphate below this concentration, the *biological* sulphate reduction technology can be applied. In order to achieve biological sulphate reduction, anaerobic conditions, favoured by the sulphate reducing bacteria (SRB), and the presence of suitable carbon and energy sources, have to be adhered to. Successful sulphate reduction is typically associated with a pH increase due to the production of sulphide and alkalinity. Therefore, the sulphate reduction technology is particularly beneficial to industries experiencing acid mine drainage problems, as it results in removal of sulphate, in an increase in the pH of the treated water and often in metal removal. The latter occurs as a result of the formation of sulphides, followed by metal precipitation to form metal-sulphides.

In the presence of sulphate, the SRB utilize organic products as the carbon and energy source, providing electrons, while sulphate is used as the terminal electron acceptor with hydrogen sulphide (H_2S), CO_2, H_2O or HCO_3^- and in some cases acetate as the endproducts. In addition, some SRB species are able to grow with H_2 as electron donor, sulphate as electron acceptor and CO_2 as the sole carbon source. When sugars and other
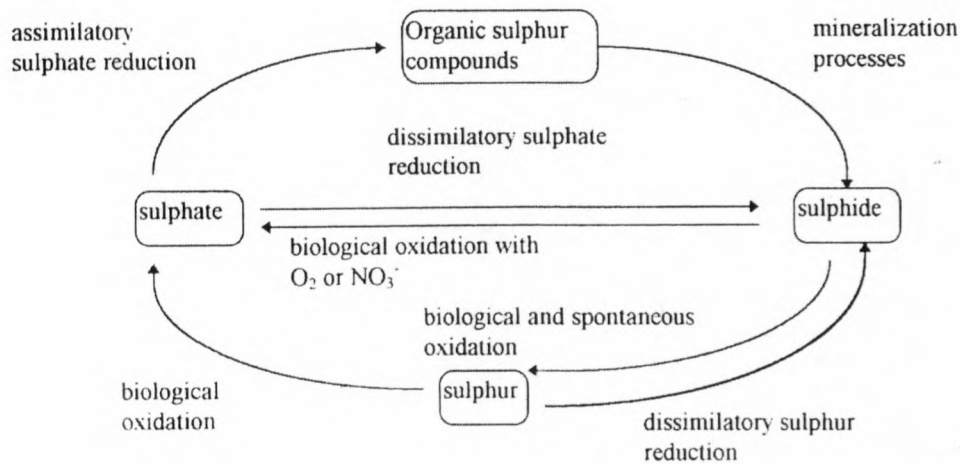
Figure 1:   **The Biological Sulphur Cycle.**

monomers are anaerobically fermented, intermediate products, such as the volatile fatty acids (VFA's) (e.g., butyrate and propionate) and ethanol are formed. In a well functioning bioreactor, these products will be subjected to acetogenesis, performed by the acetogenic bacteria (AB), to produce acetate. In a strict anaerobic, non-sulphate containing bioreactor, the acetate will then be utilized by the methanogenic archeae (MA) to produce CH_4 and CO_2. In an anaerobic reactor, many complex reactions take place in order to mineralize the organic matter. Carbohydrates, proteins, nucleic acids and lipids are first hydrolized to mono- and oligomers and then fermented to acetate, hydrogen and formate, which can be used by the MB (Oude Elferink, 1998). These bacteria can utilize hydrogen as the electron donor, using CO_2 as the electron acceptor, forming methane, an important energy source in the anaerobic degradation of organic matter.

In an anaerobic reactor a fierce competition exists between the MB and SRB as they both compete for the same substrate. The AB degrade organic matter, such as glucose and volatile fatty acids into acetate and hydrogen, which then form the substrate for the MB and the SRB. However, in anaerobic bio-reactors where both organic material and sulphate are present, the SRB play an important role in the degradation of the organic substrate and when there is an oversupply of sulphate, hydrogen is mainly consumed by

the SRB (Oude Elferink, 1998). In reactors with immobilized biomass the activity of hydrogenotrophic MB is completely suppressed within a few weeks when sulphate is added (Visser *et al.*, 1993).

In order to obtain biological sulphate reduction a carbon and energy source has to be provided, such as lactic acid (Middleton and Lawrence, 1977), wood dust and sewage sludge (Butlin *et al.*, 1949, 1960; Knivett, 1960; Sadana and Morey, 1962; Tuttle *et al.*, 1969; Conradie and Grütz, 1973). Although good sulphate removal was obtained using all these carbon sources, a long retention time of 5-10 days was required. Maree and Strydom, (1985) treated mine water with pulp mill effluent and sewage as energy sources. The disadvantage of using raw materials as the carbon and energy source is that a high COD load is added to a relative clean effluent, of which the excess has to be removed aerobically in a later stage, which will add to the operational costs. Furthermore, competition can arise for the intermediate products of the degradation of organic material, as AB, MB, MA and SRB compete for hydrogen, methanol and short chained fatty acids. More recently, good results for sulphate removal have been obtained using ethanol (De Smul *et al.*, 1997) and sucrose (Maree *et al.*, 1986, Greben *et al.*, 2000) and also methanol, both at thermophilic (Weijma *et al*, 1999) and at ambient temperatures (Tsukamoto and Miller, 1999).

Due to the development of improved reactor configurations, anaerobic, as opposed to the traditionally aerobic, treatment of wastewater were implemented as a feasible option. As the biological sulphate removal also occurs under anaerobic conditions, similar reactor configurations as for the anaerobical COD removal, can be used for biologically removing high sulphate concentrations. A biological sulphate reduction process was developed at the CSIR, Pretoria, South Africa (Maree and Strydom, 1985; Maree *et al.*, 1986). This three-stage process (anaerobic - aerobic – anaerobic), used for treating mining effluents employs up-flow packed bed reactors (or sludge blanket reactors) for anaerobic treatment, and an activated sludge system for aerobic treatment. Once the biological sulphate reduction process had been proven, many researchers concentrated their efforts on the most efficient reactor type. Among the most used reactor

configurations are the Upflow Anaerobic Sludge Bed Reactor (Lettinga, *et al.*, 1980), the Fluidized Bed reactor (Iza, 1991) and the Anaerobic Filter (Young and McCarty, 1969). These reactors are based on sludge immobilization and sludge retention, so that high biomass concentrations in the reactors can be maintained and therefore high organic loading rates can be applied. The advantage of sludge immobilization and the formation of a biofilm is that wash out of only the small particles of the biomass will occur. To avoid sludge loss due to wash out from the anaerobic reactor, the addition of a clarifier with a sludge return cycle to the reactor could be considered. However, due to the surface area of the clarifier, which is in contact with the environment, it can be assumed that a fair amount of air is introduced into traditionally a strict anaerobic reactor. A reactor system based on this principle was introduced by Maree *et al.,* (1997) as the single-stage, completely-mixed reactor configuration, which can remove sulphate and sulphide simultaneously, due to air diffusion into the reactor system.

The production of sulphides (in the gaseous form H_2S and in the dissociated forms HS^- and S^{2-}) during the sulphate removal process is considered a major problem, as sulphides are harmful to the environment. The produced sulphides are toxic to most bacteria at relative low concentrations and are fatally toxic to humans at gaseous concentrations of $800 - 1000$ ppm (Speece, 1996). Because of its toxicity, it is forbidden in most industrialised countries to drain sulphide-containing effluents into sewer pipes or surface waters (Janssen, 1996). In order to remove sulphide from waste streams, a number of physical and/or chemical processes are in place, such as air stripping, chemical precipitation and oxidation. Generally, these processes require large investments and operational costs, such as high temperatures or special chemicals. Therefore, investigations towards a (micro) biological approach have gained considerable interest. In principle, two different biotechnological processes can be used for the removal of the produced hydrogen sulphide. Cork (1985) suggested that photosynthetic bacteria, the green sulphur bacteria (*Chlorobium limicola*), use light energy to produce organic energy, and reducing H_2S to elemental sulphur ($S°$). Buisman (1989) showed that sulphide can be oxidized under oxygen limitating conditions by a group of colourless sulphur bacteria (Kuenen and Beudekker, 1982) to elemental sulphur.

Since South Africa is an arid country and since large areas of South Africa are polluted with underground acid mine drainage, it has become not only a priority, but a necessity to treat the mine effluents. Acid mine drainage (AMD), containing high concentrations of sulphate can be pre-treated with lime-stone precipitation, followed by the biological sulphate removal technology.

This study was undertaken with the aim to prove that both biological sulphate and biological sulphide removal could be achieved, in a single-stage reactor system, which is an improvement over other multi-stage systems. To obtain good sulphate and sulphide removal rates, several conditions and parameters were investigated, such as the reactor configurations, the utilisation of different carbon and energy sources and the implementation of decreasing hydraulic retention times.

2.   OBJECTIVES

The goal of this study was to evaluate a number of key parameters to optimise biological sulphate reduction and sulphide oxidation reactions, utilized in the treatment of industrial/mine effluents. The specific objectives were:

1.   To evaluate three reactor systems using artificial feed water resembling acid mine drainage:

   - A single stage completely-mixed reactor system

   - A single stage packed bed reactor system, with and without a clarifier

   - A single stage fluidized bed reactor system, with and without a clarifier

2.   To evaluate three different carbon and energy sources

   - Methanol, 1 mℓ methanol/ℓ feed

   - Ethanol, 0.25, 0.5, 1.0 and 2.0 mℓ ethanol/ℓ feed.

   - Sugar, 1.5 g sugar/ℓ feed

3.   To determine the optimum HRT for the maximum sulphate and sulphide removal rates, operating the different reactor systems and using the mentioned carbon sources. The range of HRT evaluated was from 50 to 4 h. In addition to artificial feed water, acid mine water, obtained from a colliery in the Mpumalangha area, was used to test the biological sulphate and sulphide removal technology.

3.   LITERATURE REVIEW

3.1   ORIGIN OF AMD

As discussed in the introduction, AMD is the result of mining activities, due to the exposure of pyrite to oxygen and water. Bacterial oxidation of sulphide minerals is the major factor in the formation of acid mine drainage, a common environmental problem in coal mining regions. AMD occurs because of the attack by *Thiobacillus ferrooxidans* on pyrite. When pyrite is first exposed during mining operations, it is slowly oxidised according to reaction (1)

$$FeS_2 + 3 \tfrac{1}{2} O_2 + H_2O \rightarrow Fe^{2+} + 2 SO_4^{2-} + 2 H^+ \qquad (1)$$
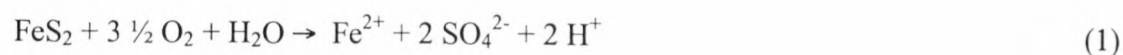
This reaction leads to acidic conditions, favoured by the bacterium *Thiobacillus ferrooxidans*, which causes the biological oxidation of ferrous (Fe^{2+}) to ferric ions (Fe^{3+}), which can react with more pyrite according to reaction (2):

$$FeS_2 + 14 Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2 SO_4^{2-} + 16H^+ \qquad (2)$$
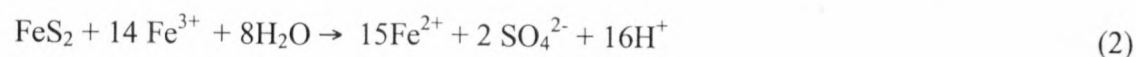
When more Fe^{2+} ions are formed, the bacterial oxidation to Fe^{3+} continues, thus initiating a cycle referred to as the *propagation cycle*. The breakdown of pyrite leads ultimately to the formation of Fe^{2+} and SO_4^{2-} ions, resulting in acid water, with a pH as low as pH 2. Under undisturbed conditions, the coal is not exposed to air, water or bacteria. However, when the coal seam is exposed, these elements can attack the pyrite, resulting in acid water. Furthermore, pyrite, occurring in the coal discard heaps can be oxidized and transformed by *Thiobacillus ferrooxidans,* with similar effect as discussed for the mine water effluents. The run-off of these coal discards often causes contamination of ground waters (Brock, 1997).

3.2   SULPHATE REDUCTION FROM AMD

3.2.1   Physical and Chemical Technologies

Due to the salination properties of AMD and the associated scaling and biocorrosion problems, as well as increased environmental awareness among the general population, methods are being investigated to remove the high sulphate concentration of AMD. Both physical (reverse osmosis, electrodialysis and ion exchange) and chemical (precipitation with barium salts and lime and limestone precipitation) methods have been tested and applied.

3.2.2   Biological

3.2.2.1 Passive treatment

In addition to physical and chemical removal of sulphate from AMD, the biological sulphate reduction/removal technology can be applied. A distinction is made between passive and active biological sulphate removal technologies. The passive treatment technology requires little maintenance and can find its application in rural mining areas, however, it can only treat relative small volumes of mining effluents (Pulles, 2000). The principle of the technology consists of a lined pit to which carbon material in the form of hay or straw has been added and through which the sulphate rich water is fed by means of gravity. In the bottom of the pit, anaerobic conditions prevail so that the SRB can utilize the straw or hay as the carbon and energy source and thus reduce the sulphate to produce sulphides. In the case of AMD, which usually contains a fair amount of metals, part of the produced sulphides will precipitate with the metals to form MeS that accumulate in the bottom of the pit. The other, gaseous part will rise to the surface of the pit and escape into the atmosphere.

Another passive way of treating sulphate rich wastewater can be achieved by wetland technology, as the plant-microbe associations in wetlands can serve both as the reactor

9

and as source of carbon for the sulphate reduction and water quality improvement (Batchelor *et al.*, 1998). Although wetlands have been used for mine water treatment, particularly with regard to metal removal, and appear attractive as sulphate reducing systems, they suffer from at least two potential limitations:

- Wetlands tend to cycle sulphur as there is nothing that effectively removes the produced sulphides unless the wetland receives a high metal input
- Wetlands are likely to be carbon limited.

These limitations can be overcome by supplementing the wetlands with additional carbon sources, when high flows are entering the wetland, however this will add to the passive treatment costs.

3.2.2.2 Active treatment

In contrast to the passive systems, the emphasis of this study will be on the active biological sulphate reduction technology. A major advantage of this technology is that much higher quantities of effluents can be treated. As indicated, sulphate-rich effluents can be treated biologically when SRB and organic matter are present. In the presence of sulphate, but also of sulphite (SO_3^{2-}) and thiosulphate ($S_2O_3^{2-}$), SRB are able to use several intermediate products of the anaerobic mineralization process. Besides the direct methanogenic substrates, such as hydrogen, formate, acetate, methanol and pyruvate (Bock *et al.*, 1994), they can also use propionate, butyrate, higher and branched fatty acids, lactate, ethanol and higher alcohols, fumarate, succinate, malate and aromatic compounds (Colleran *et al.*1995). In sulphidogenic breakdown of VFA, two oxidation patterns can be distinguished. Some SRB are able to completely oxidize VFA to CO_2 and sulphide as end-products, whereas other SRB can only carry out an incomplete oxidation of VFA with acetate and sulphide as end-products.

The carbon sources listed in Table 1 can be used by the SRB (Maree, 1988).

Table 1: **Organic substrates, mostly used for biological sulphate removal.**

(Table adapted from Middleton and Lawrence, 1977)

Acetate	Ethanol	Glycerol	Pyruvate
Alanine	Formate	Lactate	Succinate
Butyrate	Fructose	Malate	Sucrose
Citrate	Glucose	Propionate	Tartrate

3.3   MICROORGANISMS IN THE ANAEROBIC BIOREACTOR

3.3.1   Sulphate Reducing Bacteria

Ten genera of dissimilatory Sulphate Reducing Bacteria are currently recognised and are placed in two broad physiological subgroups (Brock *et al.*, 1997). The genera in group I, *Desulfovibrio, Desulfomonas, Desulfotomaculum,* and *Desulfobulbus* utilise lactate, pyruvate, ethanol, or certain fatty acids as carbon and energy source, reducing sulphate to hydrogen sulphide. The genera in group II, *Desulfobacter, Desulfococcus, Desulfosarcina, and Desulfonema*, specialise in the oxidation of fatty acids, particularly acetate, reducing sulphate to sulphide. The sulphate reducing bacteria are all obligate anaerobes and strict anaerobic techniques must be used for their cultivation. SRB are widespread in aquatic and terrestrial environments that become anaerobic due to active decomposition processes. The most known genus is *Desulfovibrio* which is common in aquatic habitats or water-logged soils containing abundant organic material and sufficient levels of sulphate. *Desulfotomaculum* consist of endo-spore forming rods primarily found in soil. *Desulfomonas* can be isolated from the intestine of mammals.

Certain SRB, among which the *Desulfosarcina, Desulfococcus* and certain species of *Desulfovibrio* are unique in their ability to grow chemolithotrophically with H_2 as electron donor, sulphate as electron acceptor and CO_2 as sole carbon source (Autotrophical growth).

11

3.3.2 Acetogenic Bacteria

Acetate is an important intermediate degradation product in an anaerobic reactor and can be produced both by AB and homoacetogenic bacteria. Homoacetogenic bacteria are obligate anaerobes that utilize CO_2 as a terminal electron acceptor, producing acetate as the sole product of anaerobic respiration. Electrons for the reduction of CO_2 to acetate can be derived from H_2, a variety of C_1 compounds, sugars, organic acids, alcohols, amino acids and certain nitrogen bases. Many homoacetogens can also reduce NO_3^- and $S_2O_3^{2-}$. However, CO_2 reduction is probably the major reduction of ecological significance (Brock et al., 1997).

3.3.3 Methanogenic Bacteria

The MB are CO_2 reducing bacteria, belonging to a major group of Archaea. They utilize H_2 as the electron donor, according to the following reaction (3):

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{3}$$

Methane formation occurs only under strictly anoxic conditions, therefore methanogenesis is restricted to anoxic habitats (Brock et al., 1997)

When growing on H_2 and CO_2, the MB are autotrophical, with CO_2 serving as both carbon source and electron acceptor. In addition to CO_2, some alcohols, formate, methanol and several methylamines and methylmercaptan can be converted to methane by certain MB species. The three classes of methanogenic substrates known, are listed below:

1   The CO_2 substrates, CO_2, CO and formate ($HCOO^-$)

2   Methyl groups (CH_3OH) (through reduction)

3   Acetate: $CH_3 COO^-$ (reaction 4):

$$CH_3 COO^- + 2H_2O \rightarrow CH_4 + HCO_3^- \tag{4}$$

3.4 COMPETITION FOR SUBSTRATE IN THE ANAEROBIC REACTOR

When considering the affinity of the SRB, the AB and the MB for substrates such as acetate, CO_2 and H_2, it is evident that these groups of bacteria may out-compete each other for their preferred substrate. In the sulphate reducing stage, a complete reduction of sulphate to sulphide is desired. Channelling of reducing equivalents towards the SRB is enhanced by the ability of the SRB to effectively compete with other anaerobic bacteria for the available organic substrate and the sensitivity of other bacteria for sulphide (Lens *et al.*, 1998).

The anaerobic process can become very complex in the presence of sulphate, because sulphate reducers will compete with MB for compounds such as formate and hydrogen, and with AB for compounds such as propionate and butyrate (Colleran *et al*, 1995). Until recently, only limited investigations have been conducted on the likely outcome of the competition between SRB and MB. Once the factors, influencing the outcome of this competition are known and applied, they can avoid the risk of process failure. Moreover, practical engineering manipulations could force the bacteria to either go the sulphidogenic or the methanogenic route.

O'Flagerty *et al.*, (1998) studied the population structure of biomass from a full-scale anaerobic reactor after 5 years of operation, with the purpose to obtain an improved understanding of long-term competition between SRB and other anaerobic microorganisms, such as the MB, the AB and other (synthropic) bacteria. The results showed that the SRB carried out an incomplete oxidation of propionate to acetate. It was observed that the SRB and synthropic bacteria competed for butyrate and ethanol. However, in the case of hydrogen, the SRB out-competed the MB, which confirmed the results of other studies, which demonstrated that H_2 and CO_2 are primarily used by the SRB, provided that sufficient sulphate is available (Visser, 1995). It is thought that the SRB keep the hydrogen concentration below the threshold level for the MB (Lovley, 1985). Oude Elferink *et al.*, (1994) showed that the hydrogen utilizing sulphate reducing bacteria (HSRB) gain more energy from the consumption of molecular hydrogen, have a

13

higher substrate affinity, growth rate and cell yield than the hydrogen utilizing methanogenic bacteria (HMB). These authors also suggested that in the presence of sulphate, compounds, such as alcohols, lactate, propionate and butyrate, may be oxidized directly by the SRB without the intermediate formation of hydrogen. They presented the following conclusions from their investigation:

1.   SRB will compete with MB for hydrogen, formate and acetate.
2.   In general, SRB have better growth kinetic properties than MB.
3.   Reactor conditions, such as pH, temperature, sulphate and sulphide concentrations, can influence the microbiological processes in the bioreactor and can determine whether these processes will proceed via the sulphidogenic or the methanogenic pathway.

O'Flagerty et al., (1998) further showed that acetogenic bacteria also played a role in the utilization of H_2 and CO_2 in their study of the anaerobic reactor, converting these substrates to acetate as shown in 3.3.2. Furthermore, it was shown that even after 5 years of reactor operation, the SRB failed to out-compete the acetate utilizing MB.

In general, the findings of Harada et al., (1994) (1998) were confirmed by those of. O'Flagerty et al., They showed that when the sulphate concentration in the bio-reactor increased from 30 to 100 to 600 mg SO_4/ℓ, the SRB utilized almost 5, 30 and 40-75% of the COD present. It was observed that propionate accumulated significantly when no or low levels of sulphate were present. Therefore, it can be deduced that SRB strongly contribute to the degradation of propionate to acetate. The study of Harada et al., (1994) confirmed furthermore that the activity of the HMB decreased with increasing sulphate concentrations. It can be assumed that the SRB contribute to the degradation of propionate to acetate using hydrogen. It was also shown that the SRB were poor competitors of MPB for acetate. Only during long-term operation, the SRB started to out-compete the MPB for acetate.

Omil et al., (1997) also studied the competition between acetate utilizing MB and SRB,

14

operating two UASB reactors, at a reactor pH of 8. The UASB reactors treated a VFA mixture of acetate, propionate and butyrate (5:3:2, on COD basis) and only acetate, respectively, at different COD: Sulphate ratios. It was found that in excess of sulphate (COD: Sulphate ratio lower than 0.67), the SRB became predominant in relation to the MB, when the reactors were operated from 250 to 400 days on acetate as the electron donor. They also described that a high reactor pH of 8, a short solid retention time (<150 days) and the presence of substantial SRB population in the inocculum may considerably reduce the time required for acetate utilizing SRB to out-compete MB.

Speece (1996) listed the most important factors known to influence the competition between the MB and SRB:

- Substrate (COD) concentration in feed
- sulphate concentration in feed
- maximum specific utilization rate (Kmax)
- half velocity constant (Ks)
- thermodynamics/free energy of the reaction
- nutrient availability
- adhesion properties
- proximity of cells (biofilms versus dispersed cells)
- temperature
- substrate type
- long term shifts.

Visser's studies (1995) indicated that the reactor pH should be added to the list of parameters, as the results of his investigation showed that the SRB favoured a reactor pH of 8.

3.5 CARBON AND ENERGY SOURCES

3.5.1 General

Since the 1970's the application of anaerobic wastewater treatment has increased dramatically. The advantage of anaerobic treatment over aerobic treatment is the low energy input and the low sludge yield. The main advantage is that the end product of the anaerobic degradation of organic matter is the production of methane gas (CH_4), a potential energy source. However, as already indicated, when sulphate forms part of the organic waste, the SRB will use the available organic matter as their carbon and energy source to reduce sulphate with hydrogen sulphide, partly as gas and partly dissolved in the treated water, as the end product. Due to this reason, many operators of anaerobic treatment plants consider sulphate rich effluents troublesome, as during anaerobic treatment of these wastewaters, the reactor will turn sulphidogenic rather than methanogenic.

When treating AMD or other sulphate containing industrial effluents, which contains no or insufficient electron donor and carbon source for a complete sulphate reduction, addition of an appropriate electron donor is required. The selection of the electron donor depends on the costs of the added electron donor per unit reduced sulphate and on the potential pollution of the additive in the waste stream. Probably the cheapest carbon and energy source to be used in the biological sulphate reduction technology is sewage and possible other types of industrial waste liquors. McKinney and Conway (1957) discussed sulphate as a possible terminal electron acceptor for the anaerobic biological waste treatment and Pipes (1960) developed a process with potential practical application using activated sludge. Domka *et al.,* (1977) surveyed a variety of municipal wastes, such as sewage, dairy waste and sugar plants as the carbon and energy source for the biological sulphate reduction (Postgate, 1984). Although sewage is a relative cheap product, the question in South Africa is whether enough sewage is available in the areas where AMD is produced. Butlin (1960) earlier calculated that a large sewage works, processing about 6 000 tonnes of sludge per day, could reduce about 60 000 tonnes of sulphate per day.

One of the main disadvantages of using a raw product as the carbon and energy source is the high concentration of residual COD in the treated water.

More recently, Rose, (2000) applied the use of primary sewage sludge as the carbon and energy source for the biological treatment of sulphate in AMD, operating the so-called Rhodes Biosure process. It is based on the hydrolysis of complex carbon sources in a novel Falling Sludge Bed Reactor, providing an easily accessible feed for SRB activity. In another instance, the question arose how much sewage sludge is needed to treat 500 Mℓ/day MD, flowing to the already severely impacted Vaal River system. Maree (1988) listed the volumes needed of three relatively cheap carbon and energy sources and their equivalent COD values required to reduce 1 g of sulphate (Table 2).

Table 2:   **Carbon source requirements for sulphate reduction.**

Carbon Source	COD Value (mg O$_2$)/ℓ	Required Volume (mℓ)
Molasses	1 000 000	1.2
Spent liquor	290 000	9.3
Raw sewage sludge	50 000	95.6

The study of Coetser *et al.,* (2000) evaluated several raw, and more refined, carbon sources for potential use in passive treatment systems to treat AMD. They found that Kikuyu grass cuttings, silage and hay, together with propionic-, butyric- and lactic acid were the preferred carbon sources to give the most effective sulphate reduction, while acetic acid, pyruvate and ethanol did not result in effective sulphate reduction. Further studies, are, at present undertaken by Pulles (2000) and Rose (2000) to investigate the role, which the microorganisms play in the degradation of plant- and other ligneous material to the preferred carbon and energy source(s) for the SRB in passive and active treatment systems (personal communication).

Maree *et al.,* (1986) utilized molasses, which contained 40% sucrose, 6% fructose and 5% glucose as the carbon and energy source. The SRB, however, could not use the sugars directly, but only after fermentation to pyruvate and lactate. The lab-scale study consisted

of two or three stages. Sulphate was reduced to sulphide in the primary anaerobic stage in the presence of excess molasses. Remaining, poorly degradable, soluble organic matter was removed in the second stage, which was either an aerobic or an anaerobic stage. Part of the molasses consisted of non-biodegradable matter, which required an additional third stage to the reactor system to remove the remaining difficult to degrade organic material from the final effluent. From this research work, it became evident that the added electron donor should cause little, if any, remaining pollution, as linking a third reactor for additional treatment will add to installation and operational costs.

The studies focussing on the competition for substrate between SRB and MB (3.4) demonstrated that SRB have the advantage over the MB, when H_2 is used as the energy source. Therefore, an alternative option for the energy source could be provided in the form of hydrogen gas. In order to add the required carbon source, the use of synthesis gas was considered. Du Preez *et al.,* (1992) successfully used synthesis gas, consisting of a mixture of H_2, CO and CO_2 in the biological sulphate reduction technology. Synthesis gas (also called producer gas) can be generated from any material containing carbon and hydrogen. It is in general easily available, as several industries dispose of it as a waste product. The gas originates from industrial sources, such as steam and methane, through the oxidation of fuel oil and also through coal gasification. The resultant mixture, containing on average 30% hydrogen, 7% carbon dioxide, 60% carbon monoxide and 3% nitrogen gas, is a suitable energy and carbon source for SRB because of the following reasons:

1. No organic compounds are added to the reactor

2. As it is a by-product from coal burners it is easily available

3. Low- grade coal, containing sulphur products, used to produce the gas can safely be utilized since the resulting sulphur products can be treated with the sulphate containing effluents.

The studies of Du Preez *et al.,* (1992), operated both continuously as well as in batch, showed that good sulphate reduction was achieved. They found that both H_2 and CO gases were utilized, according to the following reactions (5 and 6):

$$4H_2 + SO_4^{2-} \rightarrow 4H_2S + 2\,OH^- + 2H_2O \tag{5}$$

$$CO + 4H_2O \rightarrow H_2 + CO_2 \ \text{(called "water-shift" reaction)} \tag{6}$$
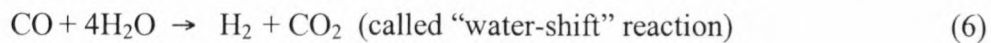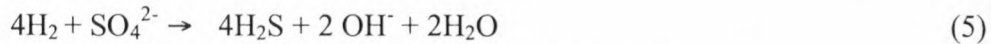
This water-shift reaction was described by Karpilova *et al.,* (1983) for *Desulfovibrio desulfuricans* and *Desulfovibrio baculatus,* and by Yagi (1958) and Yagi and Tamyia (1962) for *Desulfovibrio desulfuricans.* Non-SRB, growing on CO, can also carry out the same shift reaction as demonstrated by Kluyver and Schnellen (1947), using *Methanosarcina barkerii.* Levy *et al.,* (1981) described the production of CO_2 from CO by mixed culture anaerobes, while Du Preez *et al.,* (1992) reported on the oxidation of CO to CO_2 by microorganisms living symbiotically with SRB. The watershift reaction can also be carried out by the photosynthetic bacterium *Rhodospirrilium rubrum* by converting CO to CO_2 and H_2, as reported by Klasson *et al.,* (1990). The main advantage of this water-shift reaction is that the potentially harmful CO gas is converted to H_2 and CO_2, thereby providing additional H_2, the preferred electron donor for sulphate reduction (Van Houten, 1996). An alternative to the use of producer gas, consisting of CO, H_2 and CO_2, is the use of a mixture of H_2 and CO_2 (80%: 20%), resulting in a volumetric sulphate reduction rate of 30 g $SO_4/(\ell.d)$ (Van Houten, 1996). This sulphate reduction rate was achieved within 10 days of operation at 30 °C using a gas-lift reactor, which provided good mass transfer rates, with pumice as carrier material for the SRB. On its own H_2 gas is too expensive to be used as the energy source, but in the combination with CO and CO_2, it is an elegant and economic alternative.

An overview of various carbon sources used and the corresponding sulphate reduction rates are presented in Table 3.

Table 3: **Summary of reported carbon sources used for biological sulphate reduction with the sulphate reduction rates obtained (adapted Olthoff *et al.*, 1985a).**

Reference	Specific re-duction rate g SO$_4$/ (g VSS.d)	Reduction rate g SO$_4$/ (ℓ.d)	Temp. °C	Carbon source
du Preez *et al.*, (1992)	0.13	1.6	25	30% H$_2$, 59% CO; packed bed reactor
Burgess and Wood (1961)	-	4.5	35	Primary sewage sludge
Maree and Strydom (1987)	0.11	6.4	27	Molasses; packed bed reactor
Maree and Hill (1989)	0.20	0.8	27	Molasses; completely-mixed reactor
Van Houten (1996)		30	30	H$_2$ (80%) CO$_2$ (20%)
Greben and Maree (2000)	2.32	8.4	22	Ethanol, Completely-mixed reactor
Greben *et al.*, (2000a)	1.06	12.4	22	Sugar, Completely-mixed reactor
Middletton and Lawrence (1977)	0.03	-	-	Acetic acid
Obarsky et al. (1978)	-	0.2	35	Rubber waste effluent
Oleszkiewicz and Hilton (1986)	-	10.2	35	Cheese whey with gas stripping
Pipes (1960)	0.11	1.2	35	Waste activated sludge
Raboline (1971)	-	2.8	-	Primary sewage sludge
Sadana and Morey (1962)	0.08	2.4	35	Primary sewage sludge

3.5.2 Methanol

3.5.2.1 Sources of methanol.

The occurrence of methanol in nature is partly due to the fungal biodegradation of methoxylated aromatics and pectin. The methoxylated aromatics, such as vanillic acid and ferulic acid, are components of the ecologically significant lignin polymers (Florencia, 1994).

Sources of methanol as one of the organic constituents in industrial wastewater include:

- the production of polyester fibres
- the manufacturing of olive oil
- the production of potato starch

Furthermore, methanol is generated in condensation processes in industry, such as coal-gasification plants and in kraft pulping mills where it is the main organic pollutant.

3.5.2.2 Anaerobic methylothrophic microorganisms

Under anaerobic conditions, methanol can be utilized by several groups of microorganisms. In the presence of electron acceptors such as nitrate or sulphate, methanol can be converted to CO_2 by nitrate and sulphate reducing bacteria. In the presence of CO_2, acetogens can produce acetate and butyrate from methanol and the methanogens reduce methanol into methane without requiring any external electron acceptor (Florencia, 1994). The reduction of nitrate or sulphate produces more free energy than the formation of methane or acetate from methanol. The biological reactions, which represent the anaerobic degradation of methanol, are presented in Table 4. Heijthuijsen and Hansen (1986) concluded from their investigation, that in synthropic association with a hydrogenotroph, such as a HSRB, most of the methanol present can be completely oxidized to H_2/CO_2 via acetogens.

Table 4: Selected biological reactions involved in the anaerobic degradation of methanol.

Reactions
Methanogens
1. $4\,CH_3OH \rightarrow 3\,CH_4 + HCO_3^- + H^+ + H_2O$
2. $CH_3OH + H_2 \rightarrow CH_4 + H_2O$
Acetogens
3. $4\,CH_3OH + 2\,HCO_3^- \rightarrow 3\,CH_3COO^- + H^+ + 4\,H_2O$
4. $CH_3OH + 2\,H_2O \rightarrow 3\,H_2 + H^+ + HCO_3^-$
5. $10\,CH_3OH + 2\,HCO_3^- \rightarrow 3\,CH_3CH_2CH_2COO^- + H^+ + 10\,H_2O$
6. $4\,CH_3OH \rightarrow 3\,CH_3COO^- + 2\,H^+ + 4\,H_2$
Sulphate reducers
7. $4\,CH_3OH + 3\,SO_4^{2-} \rightarrow 3\,HS^- + 4\,HCO_3^- + H^+ + 4\,H_2O$
8. $CH_3OH + 3\,HSO_3^- \rightarrow HS^- + HCO_3^- + H^+ + H_2O$

3.5.2.3 Sources of methanol degrading/utilizing microorganisms

The occurrence of the anaerobic methylothrophic microorganisms has been observed in a variety of environments, e.g. in aquatic fresh water and marine sediments, sewage digester sludge, human faeces and in the rumen of cattle. Although they can be found at a very broad range of temperatures and pH values, the best growth rate for most of them occurs at a near neutral pH and at mesophilic temperatures. The temperature and pH range for optimal growth for the sulphate and nitrate reducers, utilizing methanol as the electron provider is given in Table 5 (Florencio, 1994).

3.5.2.4 Sulphate reducing bacteria and methanol

Braun and Stolp (1985) and Nanninga and Gottschall (1986) were among the first authors to report the use of methanol as electron donor for sulphate removal. Nanninga and Gottschall (1986) were able to isolate *Desulfovibrio carbinolicum* from an anaerobic wastewater treatment plant, which was able to reduce sulphate with methanol as the energy source. Braun and Stolp (1985) showed that a SRB isolated from sewage sludge was capable of degrading methanol after growth on pyruvate, malate and fumarate. They observed that ^{14}C-methanol was completely oxidized to CO_2, but no ^{14}C was incorporated in the cell material. They therefore proposed that methanol was not used as a carbon source. Davidova and Stams (1996) researched the degradation of methanol in anaerobic sludge at temperatures over 60 °C. They found that a consortium of bacteria, obtained from anaerobic granular sludge could degrade methanol at 65 °C via sulphate reduction and acetogenesis. Sulphate reduction was the dominating process (S^{2-} /acetate= 2.5). About 30% of the methanol was converted to acetate by acetogenic bacteria, while the SRB degraded the remainder of the produced acetate to H_2 and CO_2 in syntrophy with hydrogen-consuming SRB, according to the following reactions (7-13):

$$4CH_3OH + 3SO_4^{2-} \rightarrow 4\ HCO_3^- + 3HS^- + 4H_2O + H^+ \tag{7}$$

$$4CH_3OH + 2HCO_3^- \rightarrow 3CH_3COO^- + 4H_2O + H^+ \tag{8}$$

$$3CH_3COO^- + 3SO_4^{2-} \rightarrow 6\ HCO_3^{3-} + 3HS^- \tag{9}$$

$$4CH_3OH + 8H_2O \rightarrow 4\ HCO_3^- + 4H^+ + 12H_2 \tag{10}$$

$$12H_2 + 3SO_4^{2} - + 3H^+ \rightarrow 3HS^- + 12H_2O \tag{11}$$

$$CH_3OH + 8HCO_3^- \rightarrow 12CHOO^- + 4H^+ + 4H_2O \tag{12}$$

$$12CHOO + 3SO_4^{2-} + 3H^+ \rightarrow 3HS^- + 12\ HCO_3^- \tag{13}$$
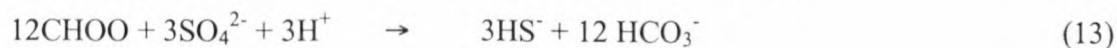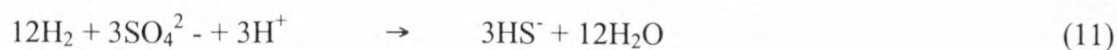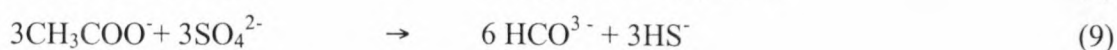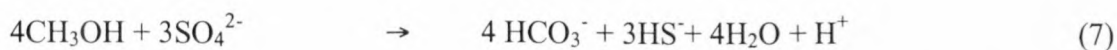
The authors concluded that the isolated sulphate reducer was unable to grow with methanol as such, but used hydrogen and formate, which are the degradation products of methanol. SRB are more efficient in hydrogen utilization than methanogenic bacteria. Hard *et al.* (1997) looked for a cheap carbon source, as they wanted to remediate acid mine water using facultatively methylotropic SRB. They managed to isolate six strains of

23

SRB, on agar plates with methanol as sole carbon and energy source, originally taken from mud from a wastewater pond and from drainage pipes of a disused sugar factory. All strains were isolated on methanol, but were also able to utilize lactate, pyruvate, acetate and a number of other carbon and energy sources without additional carbon sources. Very few mesophilic SRB growing on methanol as sole carbon and energy source have been described. Other strains reported to grow with methanol have special requirements, such as an additional carbon source (Braun and Stolp, 1985, Nanninga and Gottschal, 1986, 1987) or a high temperature (Davidova and Stams, 1996 and Weijma *et al.*, 1999).

Table 5: **Selected anaerobic microorganisms capable of growth on methanol.**

Microorganisms	pH range	Temperature (°C)
Sulphate and nitrate reducers		
Desulfovibrio carbinolics	5.3 - 8.7	37 - 38
D. alcoholovarans	7.0	35 - 37
Desulfobacterium catecholicum	6.9 - 7.1	28
D. anilini	6.9 - 7.5	35
Desulfotomaculum orientis	6.8 - 7.1	35 - 37
D. kuznetsovii	7.0 - 7.2	60 - 65
D. strain T90A	6.5 - 7.5	42 - 78
Hyphomicrobium spp.	5.0 - 8.5	25 - 35
Paracoccus denitrificans	nr	30

n.r. not reported

The work of Weijma *et al.*, (1999) and Weijma (2000) showed that they achieved a volumetric sulphate reduction of 15 g $SO_4^{2-}/(\ell.d.)$ when methanol was used as the carbon and energy source. They operated two Expanded Granular Sludge Bed (EGSB) reactors at 65 °C and at a reactor pH of 7.5, while the HRT was 14 and 3.5 h respectively. They

24

observed that methanol degradation to methane occurred via the intermediates H_2/CO_2 and formate, as was also indicated by the study of Davidova and Stams (1996).

Tsukamoto and Miller (1999) proved sulphate reduction could initially be obtained using a combination of lactate and methanol as the substrate, followed by only methanol. They could reduce sulphate concentration from 900 mg/ℓ in the feed down to 454 mg/ℓ in the effluent.

3.5.3 Ethanol

Ethanol is the product of yeast fermentation, as yeasts have the ability to carry out the two opposing modes of metabolism: fermentation and respiration. In the presence of oxygen, yeasts grow efficiently on sugar substrates, producing yeasts cells and CO_2. In the absence of oxygen, yeasts switch to an anaerobic metabolism, resulting in a reduced cell yield, but in significant amounts of alcohol. The yeast *Saccharomyces cerevisiae* is mainly used for alcohol production. The yeasts are initially responsible for the alcohol production, but in order to obtain a purer and more concentrated alcohol, it has to undergo one or more distillation procedures. (Brock *et al.,* 1997). For industrial purposes, ethanol is produced by distillation of crude oil e.g. at Sasol, the South African Synthetic Oil Industry or by fermentation of raw sugars at the Sugar Refineries e.g Illovo, Durban, South Africa.

Ethanol in the presence of AB and SRB represents a substrate that can be oxidized to acetate, which then can be oxidized by the acetate utilizing SRB, such as *Desulfuromonas acetoxidans* and *Desulfobacter postgatei*. These microorganisms are often unable to metabolise lactate and pyruvate, however can oxidize ethanol completely to CO_2. The reactions (14-18) involved are:

$$2C_2H_5OH + H_2O \quad \rightarrow \quad 2CH_3COO^- + H^+ + 2H_2 \tag{14}$$
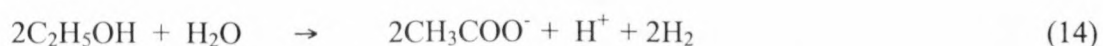
The produced hydrogen can be used as the energy source by the SRB in the presence of sulphate (15):

$$4H_2 + SO_4^{2-} + H^+ \rightarrow \quad HS^- + 4H_2O \quad\quad\quad (15)$$

$$2C_2H_5OH + SO_4^{2-} \rightarrow \quad 2CH_3COO^- + HS^- + H^+ + 2H_2O \quad\quad\quad (16)$$

$$\underline{2CH_3COO^- + 2SO_4^{2-} \rightarrow \quad 4HCO_3^- + 2HS^-} \quad\quad\quad (17)$$

$$2C_2H_5OH + 3SO_4^{2-} \rightarrow \quad 3HS^- + 3HCO_3^- + 3H_2O + CO_2 \quad\quad\quad (18)$$
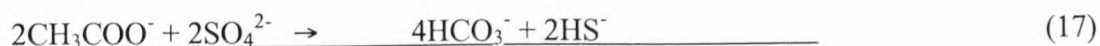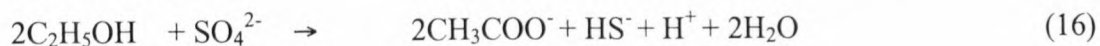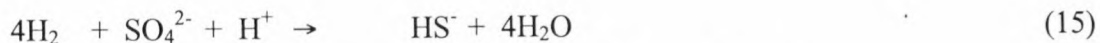
The first ethanol fed flue gas desulphuration plant was constructed in The Netherlands in 1994 (Hoogovens Technical Services E and E 1994, IJmuiden, The Netherlands) and recently the first bio-desulphuration unit for the treatment of contaminated groundwater of a zinc factory has become operational (Buisman et el., 1996).

Ethanol has been identified as an intermediate during the degradation of organic matter in most anoxic ecosystems investigated by many researchers (Kaspar and Wuhrmann, 1978; Lovley et al., 1982; Schink et al., 1985). Szewzyk and Pfennig (1990) concentrated in their study on the competition for ethanol by the SRB and other fermenting bacteria. The results of the competition experiments, in continuous culture, showed that SRB are able to successfully compete with fermenting bacteria under low substrate concentrations. This confirms the important role of the SRB in the anaerobic degradation process. The results of the study of Szewzyk and Pfennig showed that SRB are not only terminal degraders, comparable to the MB, but that they also compete successfully with the fermenting bacteria in the process of organic degradation.

That ethanol can be used as the preferred carbon and energy source was shown in the investigation of De Smul et al., (1997). They indicated that good sulphate reduction (80-85%) was obtained when the reactor pH was controlled above pH of 7.8. They also found that in their reactors, the oxidation of ethanol proceeded mainly via acetate, but due to the fact that the reactor pH was higher than 7.8, the acetotrophic SRB out-competed the methanogens, confirming the findings of Visser (1995).

3.5.4 Sugar

Many microbial reactions occur in the bioreactor when sugar is the carbon and energy source, according to the anaerobic glucose fermentation, called glycolysis. The reactions involved are energy yielding as 2 molecules of ATP are gained when one molecule of glucose is converted into 2 molecules of pyruvate, of which the fermentation products can be:

2 molecules ethanol and 2 molecules CO_2

2 molecules lactate and 2 molecules hydrogen

1 molecule lactate + 1 molecule acetate + 1 molecule formate + 3 molecules hydrogen

1 molecule lactate+ 1 molecule acetate + 1 molecule H_2 + 1 molecule CO_2 + 2 molecules hydrogen

Another group of obligate anaerobic bacteria, which can utilize CO_2 as the terminal electron acceptor producing acetate, are the homoacetic bacteria. Electrons for the reduction of CO_2 to acetate can come from H_2, sugars, organic acids, alcohols and amino acids. The reactions taking place in these circumstances are as follows (19-21):

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \tag{19}$$
$$2\,CO_2 + 4\,H_2 \rightarrow CH_3COOH + 2H_2O \tag{20}$$
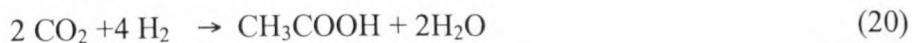
Homoacetogens ferment glucose via the glycolytic pathway converting glucose to two molecules of pyruvate, from which 2 molecules of acetate are formed:

$$2\ \text{pyruvate} \rightarrow 2\,CH_3COOH + 2\,CO_2 + 2H_2 \tag{21}$$
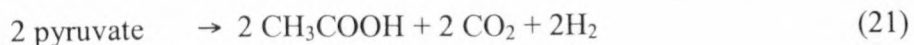
The third acetate comes from the reduction of 2 molecules of CO_2 generated in the mentioned reaction, using four electrons generated from the glycolosis and the four produced during the oxidation of two pyruvates to two acetates. Starting from pyruvate the overall reaction can be written as (22):

27

$$2 \text{ pyruvate} + 2H_2 \rightarrow 3 \text{ CH}_3\text{COOH.} \tag{22}$$
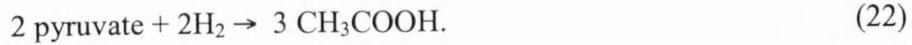
The acetate so produced can thus be utilized by the SRB to reduce sulphate. When sulphate is reduced in the presence of acetate by the SRB, it can be expresses according to the following reaction (23):

$$CH_3COOH + SO_4^{2-} \rightarrow HS^- + 2HCO_3^- + H^+ \tag{23}$$

Homoacetogenesis can be expressed in the next reaction, using the produced $2HCO_3^-$ and H^+ (24)

$$4H_2 + H^+ + 2HCO_3^- \rightarrow CH_3COO^- + 4H_2O \tag{24}$$

As can be observed from the above mentioned reactions, many complex interactions can take place in the anaerobic bioreactors, and a great number of factors, such as pH, temperature, presence of enough substrate, nutrients and many others, will determine in which way the microorganisms will carry out the fermentation of the organic matter.

3.5.5. Economy of electron donors

Van Houten (1996) undertook a cost comparison between the use of ethanol versus producer gas as the energy sources for the biological sulphate reduction technology, to be used in an European country, such as The Netherlands. He suggested that for small-scale applications, ethanol would probably be more cost effective to use than producer gas, however, when observing large biotreatment systems, he indicated that the use of synthesis (producer) gas becomes distinctly cheaper.

Maree (1988) did a similar study for South Africa. He found that when 1500 mg/ℓ sulphate had to be removed totally, the cost would amount to:

28

Energy source	Cost (R/m^3)
Sugar	1.89
Ethanol	1.43
Methanol	0.93,

This indicated that methanol is cheaper than ethanol, which in turn is cheaper to use than sugar.

3.6   REACTOR TYPES

Wastewaters containing high COD and/or sulphate concentrations can be treated anaerobically as opposed to aerobically due to the development of new reactor types. These reactor types are based on sludge immobilisation and sludge retention. In these systems, the solids retention time is uncoupled from liquid retention time. As a re-sult, high biomass concentrations are maintained in the reactor and thus high loading rates can be applied. However, the main problems encountered in the anaerobic treatment of industrial effluents, is washout of the biomass. Full-scale fluidised bed reactors in-stalled in the 1980's could never operate optimally as a result of an unsolvable biomass inventory problem. This was blamed on excessive organic loading rates, which caused the biomass to detach from the carrier medium (Colleran, *et al.,* 1994). Growth of bio-mass is much related to the energy dissipation in this aqueous environment. Quiescent aqueous conditions foster the growth of superior settling biomass. This is a big advantage for anaerobic treatment as opposed to aerobic, for which considerable energy must be dis-sipated to enable oxygen transfer to activated sludge (Speece, 1996). Speece (1996) listed the favourable conditions for high concentration anaerobic biomass immobilization:

- Fixed surfaces or carrier agents to facilitate development of biofilms
- Appropriate design to accommodate deterioration of bacterial settling charac-teristics
- Quiescent conditions in the inlet zone as well as upper sections of the reactor to maximize development of large high-density biomass aggregates.

29

The anaerobic reactors discussed in this study are:

- The upflow packed bed reactor, which relies entirely upon biofilm attachment to the packing material
- Fluidised bed configuration, which forms biofilms on the high settling velocity particles
- The completely-mixed reactor system, to which a clarifier needs to be added to avoid sludge loss.

Both the upflow packed bed and the fluidised bed reactors operate on the principle of bio-mass immobilization and the formation of biofilms. There is a profound communal synergism existing within dense biomass, which may be exploited by utilizing biofilms and granules in anaerobic processes. The peculiar aggregation of anaerobic microorganisms into biofilms and granules optimises the cooperation between the partner organisms by reducing the diffusion distance for the transfer of the metabolites. From this follows, that the anaerobic aggregate is a highly structured and layered consortium, which can stabilize the metabolic arrangement to create optimal environmental conditions for all its members (Speece, 1996).

Wolfaardt et al., (1994) described that microbial activities, which result in macro-scale environmental changes and which can be measured in physical and chemical terms, occur at micro-scale. Many processes occurring in the environment are not possible with single species populations but require consortial activities (Geesey and Costerton, 1986). Such activities typically are interactions between two or more populations in a given community, which enable organisms to maximize their metabolic capabilities and to maintain community integrity and stability.

Consequently, many of the biological processes relevant to industry have been viewed as a black box. A better understanding of the mechanisms which microbial communities apply in nature to proliferate under hostile environments, such as biofilm and floc formation, can, through microbial activity manipulations, result in process optimisation.

30

Anaerobic digesters and reactors represent an area in which metabolic cooperation between bacteria has been extensively studied. Anaerobic digestion of waste, such as industrial and municipal effluent, is efficient only when microbial aggregates in the form of biofilms, sludge granules and flocs are present (Wolfaardt *et al.,* 1994). The term microbial aggregate is chosen to mean those associations of microorganisms that are largely microbial biomass plus varying amounts of extra cellular polymeric materials produced by microbes themselves.

3.6.1 Packed Bed Reactor

This type of reactor offers benefits by providing an inlet region for large dense biomass aggregates to develop, which are not prone to washout, and provides a surface, which facilitates biofilm accumulation. The packing material can vary considerably, from material such as pumice, small silica particles to plastic or ceramic rings. The first prototype anaerobic upflow packed bed reactor was constructed in the United States, of which the packing material consisted of 8 cm diameter rocks. It was found that this media did not work well due to insufficient void volume. Due to the use of faulty packing material, the value of a packed bed reactor in its early days was not fully recognized. However, the potential of upflow packed bed reactors for the anaerobic treatment of wastewater has been demonstrated with the development of improved packing material, notably when materials that provide a high surface-to-volume ratio are used (Speece, 1996). With respect to flow, they can be completely filled (anaerobic filter) or intermittently dosed (trickling filter) (Metcalf and Eddy, 1991).

3.6.2 Fluidized Bed Reactor

The fluidised-bed reactor is similar to the packed-bed reactor in many respects, but the packing medium is expanded by the upward movement of fluid (air or water) through the bed. The porosity of the packing material can be varied by controlling the flow rate of the fluid (Metcalf and Eddy, 1991). The most important criterion for optimum functioning of the fluidized bed reactor lays in the choice of carrier material. Speece (1996) listed the

31

most desirable characteristics of the fluidization material:

- Withstands physical abrasion
- Provides maximum cumulative pore surface and volume area for the bacteria to adhere to
- Minimizes required fluidization velocity
- Enhances non-limiting diffusion/mass transfer
- Provides an irregular surface to protect biomass from abrasion.

Types of immobilization carriers include sand, coal, activated carbon, polyurethane foam, fired clay and porous glass beads. In most cases, the use of activated coal is the preferred choice, due to its exterior roughness and absorptive properties. Speece (1996) emphasises the need for media/biomass separation equipment, directly following a fluidised bed reactor, to avoid total biomass loss. This is especially important to prevent biomass wash out in the case of process failure.

3.6.3   Complete-Mixed Reactor

Traditionally the completely-mixed reactor system was used for the aerobic treatment of wastewaters, rather than for anaerobic treatment. In order to avoid washout of the biomass, a clarifier or settler has to be added to the system, thus increasing the capital costs. The advantage of the completely-mixed reactor system is the potential continuous contact between substrate and biomass, when the particles, entering the tank are immediately dispersed throughout the tank, in proportion to their statistical population. Complete mixing can be accomplished in round or square tanks if the content of the tank is uniformly and continuously redistributed (Metcalf and Eddy, 1991). The disadvantage of the completely-mixed reactor system is the occurrence of poorly settling biomass (Speece, 1996). When this biomass collected in the settler, will not settle, it results in wash out of the particles, thus fouling the effluent with suspended solids. This phenomenon also occurs in the case of anaerobic sludge. However in general, flocs with good settling properties are formed. It has been observed (Metcalf and Eddy, 1991) that

cell aggregates can also form in completely-mixed systems, usually when inorganic material is present to form the core of such cell aggregates.

3.7   SULPHIDE

3.7.1. Toxicity

The product of biological sulphate reduction is sulphide and as already indicated in the Introduction, sulphide is toxic for many anaerobic bacteria. Sulphide accumulation can result in a severe inhibition of the purification process or might even cause total process failure. Most studies on sulphide toxicity have focussed on the inhibition of MB (McCartney and Oleszkiewicz, 1991, Lens *et al*, 1998, Oude Elferink, 1998), but the maximum sulphide concentration that can be tolerated by other microorganisms in the bioreactor without loss of reactor efficacy, should also be known. Therefore, the inhibition of sulphide on AB and SRB activity, has been studied (Table 6). Originally, Schlegel (1981) suggested that the inhibitory effect of sulphide is caused by undissociated H_2S because only neutral molecules can permeate the cell membrane. However, the exact H_2S inhibition mechanisms have not been explained yet. Many studies have been dedicated to sulphide toxicity on sulphate reduction. The results of these studies have been described by several researchers and have been listed in Table 6 (adopted from Lens *et al.,* 1998). In general, these studies demonstrated that, under mesophilic conditions, both granular and suspended sludge are more tolerant to H_2S inhibition at a higher pH of around 8. This finding can be ascribed to the fact that H_2S occurs as HS^- at a pH >6.9. It was also shown that the sludge was more sensitive to the concentration of H_2S than to the concentration of TS.

Table 6: Un-ionized sulphide (H$_2$S) and Total Sulphide (TS) concentration causing a 50% inhibition of methanogenesis, sulphate reduction or the degradation of specific substrates.

Sludge type	Substrate	T (°C)	pH	H$_2$S (mg/ℓ)	TS (mg/ℓ)	Reference
Sulphate reduction						
Desulfovibrio desulfuricans	Lactate	35	7.0	250	500	Okabe *et al.*,(1992)
Sludge susp	Lactate/ Acetate	35	7.2-7.6	NR	83	McCartney and Oleszkiewicz (1991)
Sludge susp	Lactate	35	7.0	>300	NR	McCartney and Oleszkiewicz (1993)
			8.0	185	2244	
Sludge granules	Acetate	30	7.0 -7.4	171	615	Visser *et al.*, (1996)
			8.1-8.3	57	1125	

* sludge susp = sludge suspension   ** NR= Not reported

3.7.2   Biological Sulphide Oxidation

Due to their toxic effect on the SRB, it is desirable that the sulphides, produced during sulphate removal are removed from the bioreactor. Partial biological sulphide oxidation (Buisman, 1989, Janssen, 1996) to elemental sulphur (S°) is a relatively cheap option, especially when partial sulphide oxidation can be achieved in the same reactor in which the sulphate reduction is accomplished (Maree *et al.*, 1997). Colourless sulphur bacteria (*Thiobacillus* spp) oxidize sulphide to S° or to sulphate in the presence of air (O$_2$). The electrons of sulphide are used to convert oxygen into H$_2$O, while CO$_2$ is the main carbon source. Under oxygen limited conditions, that is, dissolved oxygen concentrations below 0.1 mg/ℓ, S° is the major end product of sulphide oxidation, while sulphate is formed under sulphide-limiting conditions (Janssen, 1996). S° formation requires four times less oxygen compared with complete oxidation to sulphate (according to reactions 25 and 26)

and consequently, a lower energy consumption for aeration.

$$2HS^- + O_2 \rightarrow 2\,S^\circ + 2\,OH^- \qquad\qquad (25)$$

$$2HS^- + 4O_2 \rightarrow 2\,SO_4^{2-} + 2\,H^+ \qquad\qquad (26)$$

Janssen (1996) observed that the S° particles excreted by the *Thiobacillus* are very small (submicron range) and sometimes need a flocculant, such as a polymere, to be precipitated. In reactors with long solid retention times, the sulphur particles form aggregates, on which the thiobacilli have been noticed to immobilize. This concept of immobilizing thiobacilli on carrier material such as S° particles, can be applied in reactors operating under autotrophic conditions.

3.8   THE INFLUENCE OF AIR ON THE SRB

Dilling and Cypionka (1990) described the aerobic respiration in SRB. They found that cultures of *Desulfovibrio desulfuricans* (strain CSN) reduced 5 mM O_2 with H_2 as electron donor. Aerobic respiration was not coupled with growth, but resulted in ATP formation. Besides H_2, organic electron donors, such as formate, lactate, ethanol and pyruvate, as well as inorganic sulphur compounds, e.g. H_2S, thiosulphate, sulfite, were used for aerobic respiration. Sulphite and thiosulphate were oxidized completely to sulphate. The capability of aerobic respiration was also detected *in Desulfovibrio vulgaris, D. sulfodismutans, Desulfobacterium autrophicum, Desulfobulbus propionicus* and *Desulfococcus multivorans.* However, although these groups of bacteria can respire oxygen, no bacteria capable of dissimilatory sulphate reduction in the presence of O_2 have been identified so far (Marschall *et al.,* 1993). Marschall *et al.,* (1993) found in their study that O_2 can be a true electron acceptor for SRB, but at the same time, it exerts toxic effects, even at low O_2 concentration. They also found that SRB isolated from periodically oxic environments (activated sludge, top layer of marine sediment) were not better adapted to oxic conditions than their laboratory strains. Due to the successful competition for electrons, it was found that the presence of oxygen prevents reduction of sulphur compounds. The authors described that after increased electron supply at very

low O_2 concentrations a little sulphide production could be observed, concomitant with aerobic respiration. They concluded with the statement: "The isolation of oxygen-tolerant SRB that can reduce sulphate instead of O_2 in the presence of both of these electron acceptors remains a challenge for future research".

From these studies, it can be concluded that a small amount of air entering the reactor may not harm the SRB, however, when they start using the O_2 for their respiration, less sulphate will be reduced and at the same time less O_2 is available for the oxidation of the produced sulphide.

3.9   BIOLOGICAL SULPHUR (S°) FORMATION

When sulphate is biologically reduced, sulphides are produced, which can be biologically oxidized to elemental sulphur, thus closing the biological sulphur cycle. When biologically produced elemental sulphur is not exposed to air, it is inert and can be disposed off in the environment. However, elemental sulphur can be recovered and used for industrial purposes, e.g. for the production of sulphuric acid. Maree (1988) remarked that South Africa had an annual sulphuric acid production in 1987 of 4410 000 tonnes. The sources for sulphur at that time were:

- Imported sulphur   29.6%
- Pyrite   35.0%
- SO_2 contained in smelter off-gases   11.2%
- Sulphur recovery from coal mining   22.7%
- Sulphur recovery from gypsum   1.6%

Sulphuric acid production from these five raw materials was estimated to be 3145 000 tonnes, representing 71.3% utilization of the production capacity (Maree, 1988).

Five major industries in South Africa have been identified to be dependent on sulphuric acid, being:

1. The gold and uranium mines
2. The base metal mines
3. Cement manufacturers
4. The paper industry
5. The fertilizer industry.

In 1987, it was necessary to supplement the local sulphur production with the importation of 36000 tonnes (as S). Sulphur was imported from Canada, which, due to the low Rand/Canadian Dollar exchange rate, implies that this importation will results in a direct money outflow from South Africa. It is thus envisaged that when the biological sulphate reduction technology is applied to the large volumes of AMD produced per annum, an additional benefit can be found in the production of significant supplies of biological sulphur. Maree (1988) estimated that 50 000 tonnes of sulphur can be recovered from mining effluents per annum.

In principle, sedimentation of the sulphur fraction proceeds very slowly, but in the presence of a suitable polyelectrolyte, the rate at which the sulphur particles settle can be increased (Buisman *et al.*, 1993). However, the use of polyelectrolytes should be minimized in order to avoid high operational costs and to enable the reuse of the high purity biological sulphur obtained. In order to convert the biologically formed sulphur into a feasible product, an economically and technically feasible sulphur separation method should be developed.

37

4 EXPERIMENTAL

4.1 EVALUATION AND SELECTION OF REACTOR TYPE

4.1.1 Introduction

Various anaerobic reactor configurations have been used for biological sulphate removal. The preferred configurations for anaerobic treatment of wastewater are mostly the Upflow Anaerobic Sludge Bed (UASB) Reactor (Lettinga, *et al.*, 1980), the Fluidized Bed (FB) reactor (Iza, 1991) and the Anaerobic Filter (Young and McCarty, 1969). In an anaerobic reactor the AB degrade sucrose, glucose and volatile fatty acids into acetate and hydrogen, which then form the substrate for the MB and the SRB. As indicated (Introduction and 3.4), the SRB play an important role in the degradation of the organic substrate in anaerobic bioreactors where both organic material and sulphate are present. When an excess of sulphate is present, hydrogen is mainly consumed by HSRB (Oude Elferink, 1998). In reactors with immobilized biomass the activity of HMB is completely suppressed within a few weeks when sulphate is added (Visser *et al.*, 1993). In a single stage anaerobic reactor, which contains both high organic and sulphate concentrations the SRB degrade both the sulphate and the organic matter, forming sulphide and bi-carbonate.

The aims of this study were to determine the effects of the following parameters on the sulphate reduction- and sulphide oxidation rates:

- The reactor type, (single-stage fluidized, packed bed and the completely-mixed reactor configuration).
- The presence or absence of a clarifier
- Decrease in the hydraulic residence time (from 50 to 5 h), when operating the:
  1) single-stage fluidized,
  2) packed bed and
  3) completely-mixed reactor configuration.
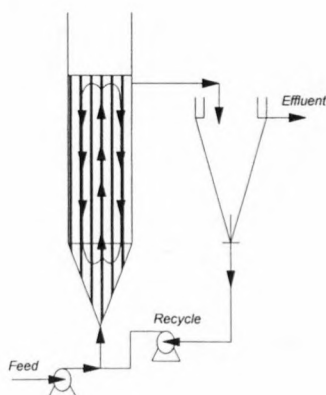
Figure 2:   Single-stage, fluidized-bed reactor with clarifier configuration.

Single - stage, fluidized-bed reactor with clarifier configuration

These units consisted of fluidized-bed reactors (volume 18 ℓ each) with a clarifier (15 ℓ). Although a fluidized bed reactor is not usually fitted out with a clarifier, in this instance the clarifier was added, in order to investigate its function to obtain partial sulphide oxidation. The reactors were operated without support medium for six months (Fig. 2). The reactors received the feed and the recycle discharge of the clarifier, while the overflow of the clarifier was discharged as waste.

Single - stage, packed bed reactor with a clarifier.

An immobilization support medium (plastic rings with rough surfaces) was placed in the fluidized-bed reactor to modify it into a packed bed reactor (Fig.3)

Single - stage, packed bed reactor without a clarifier.

This design consisted of a column reactor (volume of 14.5 ℓ) (Fig. 4) packed with plastic rings with rough surfaces as immobilizing material. Sludge was recycled from the bottom of the reactor to a level, two thirds up the full length of the reactor. The feed inlet pipe was at the bottom, while an air inlet pipe was fitted at the top to feed a small amount of air to the reactor system.

Single - stage completely-mixed reactor

The completely-mixed reactor system comprised of a reactor (volume15 ℓ) and a clarifier (volume15 ℓ)(Fig. 5). Both reactor and clarifier were open to the atmosphere so that air could diffuse into the reactor system. The operational temperature was maintained at 20-22 °C.
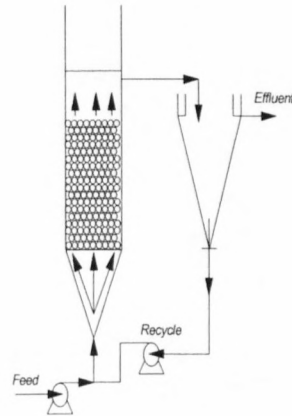
Figure 3: **Single-stage, packed bed reactor with a clarifier**.
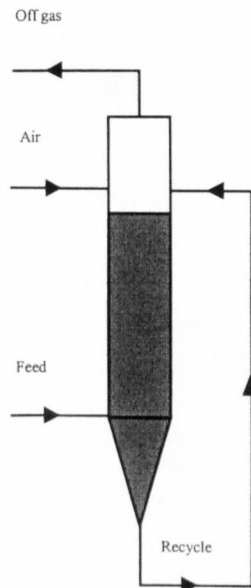
Figure 4: Single-stage, packed bed reactor system without a clarifier.
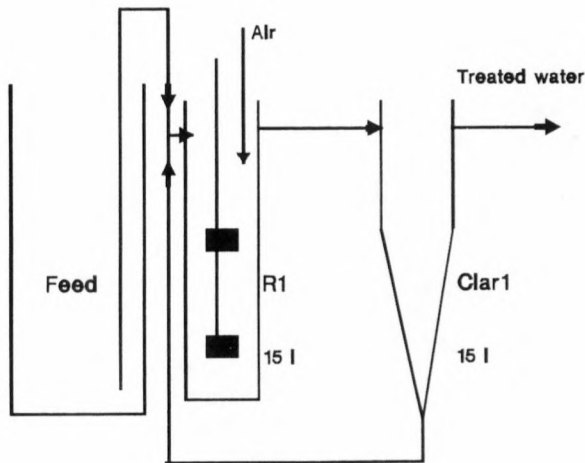
Figure 5: Completely-mixed reactor.

4.1.2.2 Biomass

All reactors were inoculated with pre-conditioned, anaerobic sludge, originally obtained from the municipal sewage treatment plant at Daspoort, Pretoria. Once the sludge had been used for sulphate reduction, it was kept in the cold room after use and re-used to seed the reactors, when new experiments were started.

4.1.2.3 Feedstock

All reactors received synthetic feed, of which the sulphate (in the form of $CaSO_4$) and the COD concentrations were 1500 mg/ℓ each, which resulted in a COD/SO_4 ratio of 1. The feed was supplemented with both macro nutrients: 75 mg/ℓ Ammonia-N and 15 mg/ℓ Phosphate-P and micro nutrients: 100 µg/ℓ Fe, 210 µg/ℓ Co, 0.28 µg/ℓ Mn, 0.44 µg/ℓ V, 0.25 µg/ℓ Ni, 0.48 µg/ℓ Zn, 0.40 µg/ℓ Mo, 0.18 µg/ℓ B, 0.37 µg/ℓ Cu.

4.1.2.4 Carbon and energy source

Both sucrose (1.5 g/ℓ feedstock) and technical grade ethanol (1 mℓ/ℓ feedstock) (Crest Industries, Johannesburg) were used as carbon and energy sources, depending on the

experiment. Although the reservoir containing the feedstock was kept at 4 °C, the sucrose in the feed fermented to acids, resulting in a feed pH of 4 <pH<6. At the start of the experiment, a pH controller was added to maintain the reactor pH at 7.5, using sodium bicarbonate.

4.1.2.5 Analytical

Manual determinations of sulphate, sulphide, alkalinity, COD, pH and redox potential were carried out according to analytical procedures as described in Standard Methods (APHA, 1985). With the exception of the feed COD and sulphide, all analyses were carried out on filtered samples. The acidity determination of the feed was done by titrating the feedwater with 0.1 N NaOH to a pH of 9.0. The COD samples were pre-treated with a few drops of H_2SO_4 and N_2 gas to correct for the COD value caused by the sulphide concentration.

4.1.2.6 Hydraulic retention time (HRT)

The feed rates of the reactors were varied to obtain different HRT's, ranging between 5 and 50 h.

The experimental conditions are given in conjunction with the tables of results.

4.1.3 Results and Discussion

4.1.3.1 Reactor configuration

Table 7 shows the volumetric sulphate reduction and the specific sulphate reduction rates in the completely-mixed, the fluidized bed and the packed bed reactor configurations, when using artificial feedstock with ethanol as carbon and energy source. The feed rates were 100, 90 and 98 ℓ/d resulting in a HRT of 3.6, 4.8 and 4.4 h, respectively. From the results in Table 7 it can be observed that the sulphate reduction rate in the completely-mixed system was 4.8 g SO_4/(ℓ.d), in the packed bed system it was 4.9 g SO_4/(ℓ.d) and in the fluidized bed system, the rate was 3.3 g SO_4/(ℓ.d). These results indicated that in both

the completely-mixed and the packed bed reactor systems the conditions were favourable so that good sulphate reduction could be obtained and that the sulphate reduction in the fluidized bed system was less efficient, due to operational circumstances.

Table 7: **Effect of reactor type on the sulphate and the specific sulphate reduction rates.**

Reactor System		Completely-mixed	Fluidized Bed	Packed Bed
Feed	units	Values		
Feed rate	ℓ/d	100	90	98
HRT	h	3.6	4.8	4.4
Rates				
Vol. SO_4 red. rate	g SO_4/(ℓ.d)	4.8	3.3	4.9
Spec. SO_4 red. rate	g SO_4/(gVSS.d)	2.8	0.24	n.a.
Ratios				
COD/SO_4		0.78	1.16	0.77
Sulphide/SO_4		0.19	0.18	0.11

The specific sulphate reduction rate in the completely-mixed reactor system was the highest at 2.8 g SO_4/(gVSS.d). This finding can be ascribed to the biomass, which became sulphate reducing specific as it was exposed to a high sulphate load in the completely-mixed reactor for at least 6 months. Furthermore, in this reactor type (continuous mixing) the substrate and biomass were in constant contact, which resulted in a better sulphate removal rate. The specific sulphate reduction rate in the packed bed is not given, because the biomass in a packed bed is not in suspension and thus the VSS is non-measurable.

4.1.3.2 Hydraulic Retention Time (HRT)

Fluidized bed reactor with clarifier

Table 8 shows the results of the volumetric sulphate and specific sulphate reduction rates using artificial feed with sucrose as the carbon and energy source for a fluidized bed reactor at different feed rates. It can be noted that when the HRT was decreased from 51

to 15.8 h, the volumetric sulphate reduction rate increased from 0.6 to 1.5 g $SO_4/(\ell.d)$. Thus, a decreased HRT resulted in an increased volumetric sulphate reduction rate. The specific sulphate reduction rate increased from 0.07 to 0.14 when the HRT was decreased. Similar results were obtained in a replicate study, where the volumetric sulphate reduction rate increased from 0.3 to 1.4 when the HRT decreased from 51 to 15.8 h

The packed bed reactor

Similar results as for the fluidized bed reactor were obtained for the packed bed reactor: When the HRT was decreased from 15.8 to 5.6 h, thus increasing the loading rate, the volumetric sulphate reduction rate increased from 1.4 to 3.9 g $SO_4/(\ell/d)$. Thus, lowering the HRT in a packed bed reactor had a favorable effect on the volumetric sulphate reduction rate.

Table 8: **The effect of HRT operating a fluidized bed reactor with clarifier.**

Parameter			
Feed rate	ℓ/d	10.8	35
HRT	h	51	15.8
Period	d		
Carbon Source		sugar	sugar
Rates:			
Vol. SO_4 reduction	g SO_4 (ℓ/d)	0.6	1.5
Spec. SO_4 reduction	g $SO_4/$ (g.VSS.d)	0.1	0.14
Ratios:			
COD/SO_4		0.67	0.6
S/SO_4		0.05	0.091

Completely - mixed Reactor system

The results in Table 9 show that the volumetric sulphate reduction rate increased from 2.5 to 6.6 g SO$_4$/(ℓ.d) when the HRT decreased from 12 to 4.8 h and the specific sulphate reduction rate increased from 1.2 to 2.6 g SO$_4$/(g VSS.d). Similar to the fluidized- and packed bed reactors, the sulphate reduction rate increased when the HRT decreased. In general, this suggests an inverted relationship between sulphate removal efficiency and HRT. This relationship can be ascribed to the fact that when the HRT decreases, the feedrate increases, resulting in a higher sulphate load entering the reactor. When the SRB are well adapted to sulphate removal and when enough COD is available, the SRB become more efficient in sulphate removal, due to the higher sulphate load. These factors together resulted in an increased sulphate removal rate.

Table 9: **The sulphate reduction rates in a completely-mixed reactor with clarifier, using artificial feed and ethanol as energy and carbon source.**

Parameter	Unit		Values	
Feed rate	ℓ/d	30	50	75
HRT	h	12	7.2	4.8
Carbon Source		Ethanol	Ethanol	Ethanol
Rates:				
Vol. SO$_4$ reduction	g SO$_4$ /(ℓ.d)	2.5	4.5	6.6
Spec. SO$_4$ reduction	g SO$_4$/ (gVSS.d)	1	2.4	2.6
Ratio's				
COD/SO$_4$		0.77	0.61	0.61
S/SO$_4$		0.13	0.17	0.14

The influence of the decrease in HRT on the COD/Sulphate ratio and the competition between methanogenic and sulphate reducing bacteria

Applying the fluidized bed reactor at two different HRT's (51 and 15.8 h) resulted in COD_{used}/sulphate$_{removed}$ ratios of 0.67 and 0.60, respectively (Table 8). These values correspond well with the theoretical COD/sulphate ratio of 0.67, the stoichiometrical amount (mol) of COD (sugar) needed to remove 6 mol of sulphate (reaction 27). When the sulphate is reduced to sulphur, the COD/sulphate ratio amounts to 0.50 (reaction 28).

$$C_{12}H_{22}O_{11} + 6SO_4^{2-} \rightarrow 6CO_2 + 6HS^- + 6HCO_3^- + 5H_2O \qquad (27)$$

$$C_{12}H_{22}O_{11} + 8H_2SO_4 \rightarrow 8S + 12H_2CO_3 + 7H_2O \qquad (28)$$
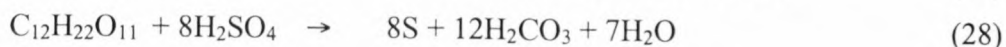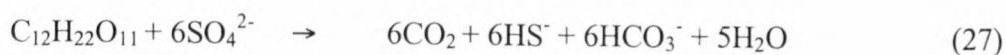
The experimental COD_{used}/sulphate$_{removed}$ ratios in the packed bed reactor were 0.57 and 0.96 at HRT of 15.8 and 6.1 h, respectively. When the HRT was decreased from 12 to 7.2 h in the completely-mixed system, the experimental COD_{used}/sulphate$_{removed}$ ratio decreased from 0.77 to 0.61(Table 9), where after it remained stable, even though the HRT was decreased to 4.8 h.

The experimental COD_{used}/sulphate$_{removed}$ ratios results as obtained in the completely-mixed reactor confirmed the theory that when the sulphate load to a reactor increases, the amount of COD utilized decreases, due to the fact that mainly the SRB utilize the available COD. This finding indicated that at low feed rates the MB degraded the COD as effectively as the SRB. Visser (1995) reported that at low sulphate concentrations the MB compete with the SRB for acetate, but at higher sulphate concentrations the SRB become dominant and mainly use the available hydrogen. Ng *et al.,* (1999) reported that MB do not favor a fast feeding rate, thus not a low HRT. The results in Table 7 showed that the experimental COD_{used}/sulphate$_{removed}$ ratio in the three reactor systems varied from 0.78 to 1.16 to 0.77, using the completely-mixed, the fluidized bed and the packed bed reactor configuration, respectively. These results indicate that the COD utilization in the completely-mixed and packed bed reactors was more efficient and thus economical than in the fluidized bed reactor.

4.1.3.3 The effect of air on the sulphide oxidation rate

The theoretical Sulphide/Sulphate ratio during sulphate reduction is 0.33 (32/96) according to reaction 18 (3.5.3). When the experimental sulphide/sulphate ratio is lower than 0.33, it can be assumed that part of the formed sulphide escaped as gas and that it was partly oxidized to elemental sulphur (reaction 25, shown in 3.7.1)

Fluidized bed with a clarifier

The results in Table 8 show that the experimental $sulphide_{produced}/sulphate_{removed}$ ratios varied from 0.05 to 0.09 in the fluidized bed reactor with clarifier, when feeding artificial medium at a HRT of 51 h and 15.8 h, respectively. When the HRT was decreased to 6.1 h, the experimental $sulphide_{produced}/sulphate_{removed}$ ratio increased to 0.18. As the theoretical sulphide /sulphate ratio is 0.33, it is proposed that a part of the sulphide produced was oxidized to elemental sulphur in the fluidized bed reactor, which due to the formation of yellow/white particles could be observed in the reactor.

The sulphide/sulphate ratios in a duplicate reactor operated under the same condition as described above varied from 0.11 to 0.15 at HRTs of 51 to 15.8, respectively. These values were not as low as in the other fluidized reactor, but low enough to propose that sulphide oxidation took place. The oxic zones in a reactor system, with a clarifier in place is at the top of the clarifier. It is assumed that air can enter the reactor system due to the re-circulation stream from clarifier to the reactor.

Fluidized bed and a packed bed reactor without a clarifier

The results of sulphide reduction when feeding artificial feed with ethanol as carbon and energy source, at two (almost) similar HRT values, are given in Table 10.

It can be observed from Table 10 that the experimental sulphide/sulphate ratios were the same (0.24) in both fluidized and packed bed reactor systems *without* clarifiers. Although

these values were lower than the theoretical ratio of 0.33, it was not as low as obtained in reactors *with* clarifiers, as seen in Table 8, when the sulphide/sulphate ratio was as low as 0.05 and 0.09 at a similar HRT. It was concluded that O_2 provided through air diffusion from the clarifier surface had a favourable effect on the sulphide oxidation rate. This oxidation process was observed in the form of a thick yellow-white layer on the surface of the clarifier (Plate 2). To confirm that the biological oxidation indeed was the most active at the top of the clarifier, the sulphide concentration was measured at the top, the center and the bottom of the clarifier of the reactors. The results are given in Table 11.

The results in Table 11 show that in the three reactor systems, the sulphide concentration was the lowest at the top of the clarifier, indicating that sulphide oxidation occurred at the top of the clarifier, which is the most oxic zone in the reactors. As the sulphide oxidizers are mainly distributed at the oxic zones of the reactor, it can be advised to add additional oxygen for the sulphide to sulphur oxidation, to the most oxic zone of the reactor.

Table 10: **The sulphide / sulphate ratio using fluidized and packed bed reactor systems without clarifiers.**

Parameter	Unit	Fluidized bed reactor	Packed bed reactor
Feed rate	ℓ/d	50	55
HRT	h	7	6.3
Rates:			
SO_4 red.rate	$g/(\ell.d)$	1.6	3.2
COD util.rate	$g/(\ell.d)$	2.3	4.4
Ratios:			
COD/SO_4		1.45	1.36
S/SO_4		0.24	0.24

The fact that not all sulphide produced was removed by biological oxidation may be ascribed to

1 The molar $(O_2/S^{2-})_{consumption}$ ratio.

Janssen (1996) indicated that under conditions of oxygen limitation, which is at molar $O_2/S^{2-}_{consumption}$ ratio between 0.5 and 1.0, the system produces mainly thiosulphate and sulphur whereas at molar $(O_2/S^{2-})_{consumption} > 1.0$, sulphate is the primary oxidation product. A maximal sulphur formation is obtained at a molar $(O_2/S^{2-})_{consumption}$ between 0.6 and 1.0 and not at the stoichiometric value of a molar $(O_2/S^{2-})_{consumption} = 0.5$, because of the formation of thiosulphate. This means that it is not possible to completely convert all produced sulphide into elemental sulphur, but that some sulphate formation will also occur, either directly due to an excess of oxygen or indirectly due to the formation of thiosulphate under oxygen-limiting conditions.

2 The rate of the reaction:

$$2HS^- + \tfrac{1}{2}O_2 \rightarrow S + H_2O$$
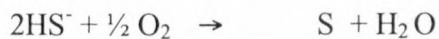
may be slower than the sulphate reducing reactions, such as:

$$2C_2H_5OH + 3SO_4^{2-} \rightarrow 3HS^- + 3HCO_3^- + 3H_2O + CO_2$$

This means that the sulphide oxidizing bacteria cannot oxidize the formed sulphides fast enough and thus a certain sulphide concentration will remain in the reactor. According to Janssen (1996), not all sulphide is converted into sulphur due to a limitation in biological activity especially in highly loaded bio-reactors. It must then be taken into account that chemical oxidation of sulphide becomes relatively more important, resulting in the formation of thiosulphate, according to the following equation.

$$2HS^- + 2O_2 \rightarrow H_2O + S_2O_3^{2-}$$

51

No analyses for thiosulphate were carried out to confirm this hypothesis.

4.1.4 Summary

Three reactor configurations, both with and without a clarifier have been evaluated for the anaerobic treatment of sulphate-rich wastewater. The results of this evaluation indicated that the highest sulphate reduction rate was obtained in the packed bed reactor at 4.9 g $SO_4/(\ell.d)$, feeding synthetic feed with ethanol as carbon and energy source at a feed rate of 98 ℓ/d. When feeding the same feedstock at 100 ℓ/d, the sulphate reduction rate obtained in a completely-mixed reactor configuration was 4.8 g $SO_4/(\ell.d)$, while it was 3.3 g $SO_4/(\ell.d)$ in a fluidized bed reactor configuration at a feed rate of 90 ℓ/d. The use of a completely-mixed reactor system for the reduction of sulphate rich effluents, operating at ambient temperature (22-22 °C) is non conventional and a novel approach to treating sulphate rich effluents. As stated in the introduction to this investigation, the preferred configuration for anaerobic treatment of wastewater is the UASB reactor. The advantage of the UASB reactor system is the development of granular sludge, while the operating temperature is 30-35 °C, since the SRB belong to the group of mesophilic bacteria.

Sulphate was reduced via sulphide to sulphur resulting in a low sulphide $_{produced}$/ sulphate $_{removed}$ ratio. The best sulphide oxidation occurred in the reactor systems with a clarifier in place as more than one third of the formed sulphide was oxidized to elemental sulphur. At low feed rates, thus at high HRT's better sulphide oxidation was obtained, as shown in the in the sulphide$_{produced}$/sulphate$_{removed}$ ratios.

4.2   EFFECT OF CARBON SOURCE ON SULPHATE REMOVAL

4.2.1   Introduction

Over the past 10 to 15 years the emphasis for sulphate removal from wastewater streams has moved away from the traditional chemical treatment to biological treatment. It has been proven that sulphate can be removed biologically (Maree and Strydom 1985, Maree *et al.,* 1986), provided that a suitable carbon source is available, such as lactic acid (Middleton and Lawrence, 1977). Omil *et al.,* (1997) described the use of acetate and other volatile fatty acids (VFA) but found that the competition between sulphate reducing (SRB's) and methanogenic bacteria (MB) was in favour of methane, rather than, sulphide production. Visser (1995), however, showed that the reactor pH of higher than 7.5, shifted the competition in favour of the SRB. Swezyk and Pfennig (1990) indicated that ethanol can be used as carbon and energy source, but also described competition between and SRB and MB. O'Flaherty *et al.* (1998) found that ethanol and short chain VFA, such as propionate and butyrate were degraded faster by SRB when enough sulphate was present in the reactor. A potentially cheaper chemical to be used is methanol (Braun and Stolp, 1985), although they described that methanol can only be used as an electron donor and that an additional carbon source is needed. Tsukamoto and Miller (1999), however, showed that methanol could be used as carbon source for microbiological treatment of acid mine drainage, while Weijma *et al.,* (1999) obtained good sulphate removal rates using methanol as the electron donor under thermophilic conditions. Van Houten (1996) proved that 30 g $SO_4/(\ell.d)$ could be removed in an upflow anaerobic sludge blanket reactor, using a combination of CO_2/H_2 gases as carbon and energy source, respectively.

The aims of this study were to determine the volumetric and specific sulphate reduction, and the sulphide removal rates, when using sugar, methanol and ethanol as carbon and energy sources in a single stage completely-mixed reactor system at decreasing HRT's.

ethanol as the carbon and energy sources (Table 12). When using methanol the HRT was constant at 24 h. When sugar was used, the feed pH was around 4, due to the bacterial degradation of sugar, even when the feed was stored at 4 °C. In order to maintain the reactor pH at 7.5, a pH controller was installed adding a $NaHCO_3$ solution to the reactor. However, once the sulphate reduction had started, sufficient alkalinity was produced and the reactor pH could be maintained at values between 7 and 8.

4.2.2.3 Analytical

The analytical procedures were carried out as described in section 4.1.2.5.

Table 12: **The experimental periods, determined by the increased feedrates in the reactors with sugar and ethanol as the carbon and energy sources.**

Period	Number of days Sugar-reactor	Number of days Ethanol-reactor	HRT (h)
1	8	6	24
2	15	6	12
3	3	7	7.2
4	5	7	4.8
5	7	7	3.6

4.2.3 Results and Discussion

The suitability of sugar, methanol and ethanol as carbon and energy sources for biological sulphate reduction in a complete-mix reactor is shown in Table 13.

Table 13: Experimental conditions, chemical composition of feed and treated water, reaction rates and stoichiometric ratio's between various parameters when comparing sucrose methanol and ethanol as carbon and energy source, feeding synthetic feed.

Parameter	Unit	Carbon and energy source										
		Sucrose reactor					MeOH reactor	EtOH reactor				
HRT (h)		24	12	7.2	4.8	3.6	24	24	12	7.2	4.8	3.6
Feed:												
Sulphate	mg/ℓ	1683	1691	1550	1725	1600	1630	1550	1372	1600	140	1320
COD	mg/ℓ	1500	1381	2365	188	1350	2061	1630	1326	1550	1523	1434
Alkalinity	mg/ℓ						-	-	79		89	85
Acidity	mg/ℓ	100	100	200			-	- .			-	-
pH		4.2	4.3	3.9	4.1	4	7.3	7	7.1	6.71	7.1	7
Treated:												
Sulphate	mg/ℓ	1090	850	47	83	35	1402	648	316	257	75	598
COD	mg/ℓ	922	640	552	109	615	563	729	526	726	714	869
Alkalinity	mg/ℓ	1026	728	610	920	752	140	777	543	543	641	441
Sulphide (S)	mg/ℓ	85	85	183	98	194	29	218	156	234	190	141
VSS	g/ℓ	7.9	11.3	11.7	12.5	13.2	5.2	5.3	2.6	1.9	2.5	1.7
pH		7.2	7.3	7.6	6.9	7.3	7.6	7.7	7.7	7.5	7.7	7.3
Rates:												
Vol. SO_4 reduction	g/(ℓ .d)	0.6	1.7	5	8.2	10.4	0.2	2.5	2.5	4.5	6.6	4.8
COD utilization	g/(ℓ .d)	0.7	1.7	6	4	4.9	1.5	2	1.9	2.7	4.0	3.8
Specific SO_4 reduction	g SO_4/ (g VSS.d)	0.1	0.2	0.4	0.7	0.8	0.04	1.2	1.0	2.4	2.6	2.8
Specific COD utilization	g O_2/ (g VSS.d)	0.1	0.2	0.5	0.3	0.4	0.3	0.7	0.7	1.4	1.7	2.2
Ratios:	Theor. value											
COD/SO_4	0.67	0.86	1.00	1.20	0.49	0.48	6.60	0.80	0.77	0.61	0.61	0.78
S^{2-}/SO_4	0.33	0.14	0.10	0.12	0.10	0.12	0.13	0.10	0.13	0.17	0.14	0.19

4.2.3.1 Sugar

The volumetric and specific sulphate reduction rates (maximum) were determined to be 10.4 g SO_4/(ℓ.d) and 0.8 g SO_4/(g VSS.d), respectively. The volumetric sulphate reduction rate increased from 0.6 to 10.4 g SO_4/(ℓ.d) and the specific sulphate reduction rate increased from 0.1 to 0.8 g SO_4/(g VSS.d) when the HRT decreased from 24 h to 3.6 h. The increase in the volumetric sulphate reduction rate can be ascribed to the gradual VSS increase from 7.9 to 13.2 g/ℓ, while the increase in the specific sulphate reduction rate was likely due to adaptation of the biomass.

Stoichiometric relationship between theoretical and actual ratios for COD_{used}/ sulphate$_{removed}$, sulphide$_{produced}$/sulphate$_{removed}$ and alkalinity$_{produced}$/sulphate$_{removed}$.

COD_{used}/sulphate$_{removed}$ ratio

The theoretical value for the COD_{used}/sulphate$_{removed}$-ratio (mg O_2/mg SO_4) is 0.67 (reaction 27) and 0.50 (reaction 28) when sulphate was reduced to sulphide and sulphur respectively.

The experimentally determined values were found to be greater than 0.67 (0.86, 1.00 and 1.20) for retention times higher than 5 h (24 h, 12 h, and 7.2 h respectively) and smaller than 0.67 (0.49 and 0.48) for retention times lower than 5 h (4.8 h and 3.6 respectively). The higher COD_{used}/sulphate$_{removed}$-ratios measured at longer retention times indicated that a portion of the organic carbon was not utilised for sulphate reduction but possibly fermented to methane by the methanogens. Visser's results (1995) indicated that the SRB are more competitive at longer rather than at shorter retention times, whereas in this study the results showed that at shorter residence times lower COD_{used}/sulphate$_{removed}$-ratios were measured, thus that the faster growing SRB out-competed the slower growing MB for the available carbon in solution. The better COD utilisation at shorter residence times can possibly be ascribed to the utilisation of H_2 (reactions 29 and 30), as hydrogen is consumed by SRB when excess sulphate is present (Visser *et al.,* 1993). The low

$COD_{removed}$/sulphate$_{removed}$ values of 0.49 and 0.48 at HRT of 4.8 and 3.6 h can be contributed to the fact that parts of the sulphate was immediately reduced to sulphur (reaction 28).

When sugar is the carbon and energy source, the SRB can utilize the sugar and produce hydrogen, (29) utilized by the SRB, according to reaction (30)

$$C_{12}H_{22}O_{11} + 5\ H_2O + 4SO_4^{2-} \rightarrow 4CO_2 + 8H_2 + 4HS^- + 8HCO_3^- + 4H^+ \quad (29)$$

$$8H_2 + 2SO_4^{2-} + 2H^+ \rightarrow 2HS^- + 8H_2O \quad (30)$$
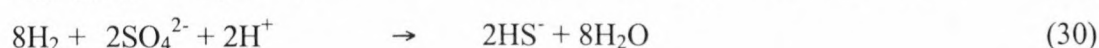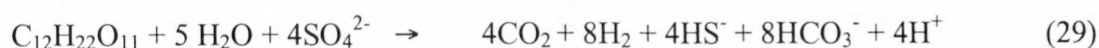
Sulphide$_{produced}$/sulphate$_{removed}$ ratio

The theoretical value for sulphide$_{produced}$/sulphate$_{removed}$ ratio is 0.33 and 0.00 when sulphate is reduced to sulphide and sulphur respectively. The experimental sulphide$_{produced}$/sulphate$_{removed}$ varied between 0.10 and 0.14. The fact that the actual values were between the theoretical values for sulphide and sulphur as end-products indicated that only a portion of the sulphate is reduced to elemental S° via sulphide, while the balance of the reduced sulphate remained in the sulphide form. The low sulphide concentrations could partially be contributed to sulphide escaping in the gas form and partially due to the sulphide oxidation to sulphur. This result was confirmed by the formation of a yellow-white layer (sulphur) on top of the clarifier during the experiment (Plate 2). The sulphide oxidation results obtained in this study confirmed those for the study on reactor evaluation.

4.2.3.2 Methanol

Methanol was not a suitable carbon source to sustain sulphate reduction. Using methanol the sulphate reduction rate was only 0.2 g SO_4/(ℓ.d). When no sulphate reduction was obtained at the HRT of 24 h, no further experiments were carried out at decreased HRT's. The actual COD_{used}/sulphate$_{removed}$ value of 6.6 (Table 13) was much higher than the theoretical value of 0.67 when sulphide is the end-product or 0.5 when sulphur is the end-

product. This indicated that other microorganisms were competing for the same COD in the anaerobic reactor. Oremland and Polcin (1982) showed that the MB out-compete SRB for methanol. Visser (1995) stated that, the released electrons (in terms of COD), during the anaerobic degradation of organic matter in the presence of sulphate, are used by SRB and MB. When hydrogen is the available substrate the SRB will out-compete the MB. When only methanol is present, the methanol will be used for methanogenesis and not for sulphate reduction. This finding differs from the results from Weijma et al., (1999) who described sulphate reduction and methanogenesis with methanol as the carbon and energy source, however their experiment was conducted at 65 °C, whereas this investigation was carried out at room temperature. The different results can be ascribed to the difference in affinity for methanol as substrate between mesophilic and thermophilic SRB. Methanol utilization is carried out by specific genus of the thermophilic eubacterial SRB (*Desulfomaculum*).

The actual sulphide/sulphate ratio of 0.13, is greater than 0 (sulphide $_{produced}$/sulphate$_{removed}$-ratio when sulphur is the end-product). However, it was less than 0.33 (sulphide$_{produced}$/sulphate$_{removed}$-ratio when sulphide is the end-product). This finding indicated that apart from sulphide escaping as a gas, in addition both sulphide and sulphur were formed as end products.

4.2.3.3 Ethanol

Ethanol, like sugar, is a suitable carbon and energy source for biological sulphate reduction in a complete-mix reactor (Table 13).

High reaction rate

The volumetric and specific sulphate reduction rates in this study were determined to be 6.6 g SO_4/(ℓ.d) and 2.8 g SO_4/(gVSS.d) respectively. The volumetric sulphate reduction rate increased from 2.5 to 6.6 g SO_4/(ℓ.d) when the HRT decreased from 24 to 4.8 h and the specific sulphate reduction rate from 1.2 to 2.6 g SO_4/(g VSS.d). At the HRT of 3.6 h,

the volumetric reduction rate decreased to 4.8 g $SO_4/(\ell.d)$, although the biomass (VSS) increased to 2.5 g/ℓ, to decrease to 1.7 g/ℓ at HRT of 3.6 h. With ethanol the specific sulphate reduction rate was higher (2.8 g SO_4/(g VSS.d) than in the case of sugar (0.8 g SO_4/(g VSS.d). This is due to the lower VSS-concentration in the case of ethanol (1.7 to 2.6 g/ℓ) than in the case of sugar (7.9 to 13.2 g/ℓ). It was observed that when sugar is used as the carbon and energy source, the biomass increased in mass as can be seen from the VSS values (Table 13). When ethanol is used, the biomass decreased in the reactor. This finding seems to indicate that in the case of sugar enough COD is present for cell division and growth, as can be seen from the increase in the VSS values. When ethanol is used, the VSS decreased. This finding may indicate that in the case of ethanol, the ethanol is only used for energy to reduce the sulphate and not enough is left for cell growth. From this result, it can be concluded that when ethanol is used as the carbon and energy source, a small amount of sugar should be added to the reactor, for maintaining the mass of the sludge.

Stoichiometric relationship between theoretical and actual ratios for $COD_{removed}$/ $sulphate_{removed}$, $sulphide_{produced}$/$sulphate_{removed}$ and $alkalinity_{produced}$/$sulphate_{removed}$

As in the case of sugar, the COD_{used}/ $sulphate_{removed}$ ratio's at the different HRT were close to the theoretical value of 0.67, showing that most COD was used by the SRB for the sulphate reduction and that only a small amount was utilized by the MB. At the lower HRT values (of 7.2 and 4.8 h), the $COD_{removed}$/ $sulphate_{removed}$ ratio was lower than the theoretical value, which was also observed at the lower HRT, when sugar was used. The exact explanation for these results is not clear. It is possible that it is due to an analytical error (although the results were very consistent) or alternatively, it can possibly be ascribed to the fact that part of the sulphate was reduced to sulphur in one step. Further tests to verify this assumption will have to be carried out. The theoretical values for $ethanol_{removed}$/$sulphate_{removed}$ are 0.32 and 0.24 when sulphate is reduced to sulphide and sulphur respectively.

General

The specific sulphate reduction rates, using sugar (0.8 g SO_4/(g VSS.d)) and ethanol (2.8 g SO_4/(g VSS.d)) are higher than the value of 0.03-0.13 g SO_4/(g VSS.d) obtained from other studies as shown in Table 3, in the Introduction (Olthof *et al*., 1985). This can be ascribed to the production of hydrogen, when both sugar and ethanol are degraded, resulting in a high reaction rates. In addition, the relative low sulphide concentration in solution in the single-stage process contributed to the obtained results, as high sulphide concentrations are toxic to SRB.

4.2.4 Summary

The aim of this study was to determine the volumetric and specific sulphate reduction rates using sugar, ethanol and methanol as a carbon and energy sources. The results indicated that sugar and ethanol were found to be suitable carbon and energy sources resulting in a volumetric and a specific sulphate reduction rate of 10.4 and 4.8 g SO_4 g/(ℓ.d) and 0.8 and 2.8 g SO_4/(gVSS.d), respectively, at a HRT of 3.6 h. Methanol was found to be an unsuitable carbon source for sulphate removal at room temperature. The experimental sulphide/sulphate ratio was consistently lower than the theoretical value, which indicated that part of the formed sulphide was oxidized to sulphur as was observed in the previous study (section 4.1).

61

4.3.   SYSTEM OPTIMIZATION

4.3.1   Introduction

As was shown in 4.1 and 4.2, good sulphate removal was obtained using the different reactor systems, with sugar and/or ethanol as the carbon and energy source. It was also discussed that the disadvantage of using waste material such as sewage sludge usually results in the non-degradable, residual Chemical Oxygen Demand (COD), whereas the disadvantage of carbon sources, such as sugar and ethanol is the relative high running costs. However, if effective sulphate removal can be achieved with smaller volumes of ethanol, the use of ethanol can be considered a feasible option. Weijma *et al.*, (1999) successfully used methanol, which is cheaper to use than either sugar or ethanol, however methanol can only be used at 65 °C. The results of studies 4.1 and 4.2 also showed that when the feedrate was increased, thus lowering the HRT, higher sulphate removal rates were obtained.

During biological sulphate reduction sulphides are produced, which can partly escape in gaseous form and which are partly dissolved in the treated effluent. As they are harmful to the environment, it is desirable to remove these sulphides. Buisman (1989) and Janssen (1996) showed that HS^- produced during biological sulphate reduction, can be oxidised to elemental sulphur, in a two stage anaerobic/aerobic process, provided that the oxygen level is kept low. The findings of both study 4.1 and 4.2 indicated that the produced sulphides were partly oxidized to sulphur in the completely-mixed reactor system.

The aim of this study was to determine the effect of decreasing the ethanol dosage (2, 1, 0,5 and 0.25 mℓ ethanol/ℓ feed) and a decrease of the HRT from 12 to 6 to 4 h on the sulphate reduction and the sulphide oxidation rates and on the $COD_{used}/sulphate_{removed}$ ratio in a completely-mixed reactor system.

4.3.2   Materials and Methods

4.3.2.1 Reactor configuration, biomass, feedstock and the carbon and energy source

Three single stage completely-mixed reactor configurations were used (see sections 4.1.1 and 4.2.1). The biomass and the feedstock were the same as discussed in sections 4.1.1 and 4.2.1. Ethanol (Crest Industries, Johannesburg) was used as the carbon and energy source. The feed COD was 345, 651, 1467 and 2800 mg/ℓ, respectively, when 0.25, 0.5, 1.0 and 2.0 mℓ ethanol/ℓ feed was added. The operating temperature was kept at room temperature (20 – 22 °C).

4.3.2.2 Experimental

Three reactor systems were operated under identical conditions during a stabilization period, which was period 1. During period 2, the ethanol dosage varied from 0.5, to 1 to 2 mℓ ethanol per ℓ feed at a constant HRT of 12 h. In period 3, the HRT was decreased from 12 to 6 to 4 h, while the ethanol concentration remained constant at 1 mℓ/ℓ feed. In period 4, the ethanol dosage was decreased, as in period 2, however, now the HRT was maintained at 6 hours. During period 5 the ethanol dosages were decreased from 1 to 0.5 to 0.25, while the HRT was decreased to 4 hours (Table 14).

4.3.2.3 Analytical

The analytical procedures were carried out as discussed under 4.1.2.5

Table 14:   **The experimental conditions during periods 1-5 for reactors CM 1,2 and 3.**

Period (days)	Ethanol dosage (mℓ/ℓ)	HRT (h)
1 (30)	0.8	12
2 (30)	0.5, 1 and 2 respectively	12
3 (30)	1	12, 6 , 4
4 (30)	0.5, 1 and 2 respectively	6
5 (30)	0.25, 0.5 and 1 respectively	4

4.3.3 Results and Discussion

4.3.3.1 Sulphate reduction rates, sulphide oxidation rate and S^{2-}/SO_4 ratio

The results of the sulphate reduction and sulphide oxidation rates and the S^{2-}/SO_4 ratios during experimental period 2-5 are given in Table 15.

Ethanol concentrations at higher HRT (12h)

When the reactors were operated under the same conditions (period 1), the sulphate reduction rates in all three reactors were similar, varying from 1,9 to 2,0 g $SO_4/(\ell.d)$ (results not shown).

The results, which are average results from the duration of the experimental periods, in Table 15 show that when the ethanol dosage was increased from 0.5 to 1.0 mℓ/ℓ feed, the volumetric sulphate reduction rate increased from 1.6 to as high as 3.2 g $SO_4/(\ell.d)$, which was a proportional increase. When, however the ethanol dosage was doubled again, the sulphate reduction rate did not increase as before. This indicated that 2 mℓ/ℓ feed is excessive and will unnecessarily add to the running costs. However when 2 mℓ/ℓ feed was added, it proved possible to obtain total sulphate removal as, towards the end of the experimental period, the residual sulphate concentration in the reactor was <10 mg/ℓ (data not shown).

The specific sulphate reduction rate was the highest at 3.2 and 2.0 g $SO_4/(g\ VSS.d)$, when 1 mℓ/ℓ feed was added. The specific sulphate reduction rate was the lowest at 0.7 g $SO_4/(g\ VSS.d)$ when 0.5 mℓ ethanol/ℓ feed was added and it was 1.4 g $SO_4/(g\ VSS.d)$, when 2 mℓ ethanol/ℓ feed was added. This finding showed that 1 mℓ ethanol/ℓ feed is sufficient to obtain good sulphate reduction. It was observed that the residual amount of COD in the reactors increased dramatically when the ethanol concentration was increased from 0.5 to 1 to 2 mℓ/ℓ feed.

Table 15: The volumetric and specific sulphate reduction rates, the sulphide oxidation rate and the S^{2-}/SO_4^- ratio as functions of the HRT and the ethanol dosage.

HRT (h)	12				6				4			
EtOH mℓ/ℓ feed	0.5	1.0	1.0	2.0	0.25	0.5	1.0	1.0	0.25	0.5	1.0	1.0
Vol. SO$_4$. red rate: g SO$_4$/(ℓ.d)	1.6	2.3	3.2	3.7	1.9	2.7	4.5	5.1	1.6	4.1	6.1	7.7
Spec. SO$_4$. red rate: g SO$_4$/ (g VSS).d	1.7	0.7	3.2	2	1.4	1.3	1.3	2.3	0.8	1.4	1.7	2.3
S^{2-}/SO_4 ratio	0.16	.06	.14	.08	.07	.13	.16	.16	.20	.17	.2	.18
S^{2-} ox. rate g O$_4$/(ℓ.d)	0.3	0.9	2.4	3.0	1.2	1.4	2.3	2.4	0.6	1.9	2.4	4

Ethanol concentrations at lower HRT (6 and 4h)

The results, in Table 15 show that, when 0.25 mℓ ethanol/ℓ feed was added to the reactors at both the HRT of 6 and 4 h, the volumetric sulphate reduction rates were lower (1.9 and 1.6 g SO$_4$/(ℓ.d)), than the sulphate reduction rates (of 2.7 and 4.0) and (4.5 and 6.1) g SO$_4$/(ℓ.d) at 0.5 and 1.0 mℓ ethanol/ℓ feed, respectively. These results indicated a linear relationship between the ethanol concentration and the sulphate reduction rate.

When the ethanol dosage was doubled from 0.25 to 0.5 mℓ ethanol/ℓ feed, the volumetric sulphate reduction rate did not increase two-fold at the HRT of 6 h, but at the HRT of 4 h, it increased more than double. When the ethanol dosage increased from 0.5 to 1.0 mℓ ethanol/ℓ feed, the volumetric reduction rates increased, however they did not double. The same finding can be noted from the specific sulphate reduction rates, although the rates increased, they did not increase with the same percentage as the ethanol dosage. The overall results indicated that the ethanol dosage of 0.25 mℓ/ℓ feed at a HRT of 4 h was too

low for the high sulphate load entering the reactor and that adding 0.5 mℓ/ℓ feed at both HRT of 4 and 6 h, resulted in adequate sulphate reduction, but that the highest sulphate reduction rates were obtained when 1 mℓ/ℓ feed was added.

Decrease in HRT at a constant ethanol dosage (1 mℓ ethanol/ℓ feed)

The results of a decrease in the HRT while the ethanol concentration was kept constant at 1 mℓ ethanol/ℓ feed are depicted in Table 15. These results show that when the HRT was decreased from 12 to 4 h, the volumetric sulphate reduction rate increased from 2.3 to 7.7 g SO_4/(ℓ.d). This result indicated that at lower HRT the biomass became increasingly sulphate reducing specific, because methanogenic bacteria do not favor a fast feed rate (Ng *et al.,* 1999*)*. Moreover, due to the lower HRT, the sulphate load in the reactor increased. It thus seems likely that at high sulphate loads in the reactor, the SRB out-competed the MB, which was also demonstrated by others (Omil *et al.,* 1998, Colleran *et al.,* 1995).

The highest specific sulphate reduction measured was 3.31 g SO_4/g (VSS.d), which was higher than the specific rate of 2.8, which was achieved at a HRT of 3.6 h, when feeding synthetic feed with 1 mℓ ethanol/ℓ feed as the carbon and energy source (Table13).

The overall results (Table 15) of the 4 different experimental periods showed that

- **at HRT of 12 h**: the highest sulphate reduction rate of 3.7 g SO_4/(ℓ.d) was obtained when the ethanol dosage was 2.0 mℓ ethanol/ℓ feed

- **at HRT of 6 h**: the highest sulphate reduction rate of 5.1 g SO_4/(ℓ.d). was obtained when the ethanol dosage was 1.0 mℓ ethanol/ℓ feed

- **at HRT of 4 h**: the highest sulphate reduction rate of 7.7 g SO_4/(ℓ.d). was obtained when the ethanol dosage was 1.0 mℓ ethanol/ℓ feed

These results indicate that an increase in the ethanol dosage and a decrease in the HRT resulted in optimal sulphate reduction rates. The higher the ethanol concentration, the better sulphate removal, but at the same time a higher residual COD concentration in the

treated water. Not only will a higher ethanol dosage increase the running costs, it will also necessitate a secondary aerobic treatment for COD removal. Any additional reactor system will, however, add to the operational costs. From this study, it was learned that when 1.0 mℓ ethanol/ℓ feed is added as the carbon and energy source and the HRT is kept at 4-6 h, optimal sulphate removal is achieved.

The relationship between the ethanol dosage, the HRT and the sulphate reduction rates is given in Figures 6 and 7.

4.3.3.2 Sulphide removal

Sulphide produced/ sulphate removed ratio

The experimental sulphide/sulphate ratios, shown in Table 15, (0.06, 0.08 and 0.07), (0.13, 0.16 and 0.16) and (0.2, 0.17 and 0.2) indicate that part of the produced sulphide was removed, due partly to H_2S gas escaping, but mainly due to sulphur oxidation. The ratios were the lowest at the highest HRT (12 h) and were the highest at the lowest HRT (4 h). These results confirmed the results from the previous study (section 4.1) that at lower HRT not enough air entered the reactor to oxidize the produced sulphides. When observing the S^{2-}/SO_4 ratios in Table 15, this theory was confirmed as at the lowest HRT, the highest Sulphide produced/sulphate removed ratio was obtained and at the highest HRT, the lowest ratio. In all instances, the experimental ratios were lower than the theoretical value of 0.33.

It can be concluded from these results that under the specific reaction conditions that the volumetric reaction rate of sulphide oxidation to sulphur is slower than the volumetric sulphate reduction rate, resulting in un-oxidised sulphide in the effluent. The lower rate of sulphide oxidation than sulphate reduction at lower HRT (Fig. 7) may possibly be explained by oxygen limitation. The available air in the reactor is due to air diffusion

4.3.4 Summary

The results of this study showed that an increase in the ethanol dosage and/or a decrease in the HRT, while maintaining the feed sulphate concentration at approximately1500 mg/ℓ, had a positive effect on the volumetric and specific sulphate reduction rates, when operating at room temperature. When ethanol was dosed at 0.5, 1 and 2 mℓ/ℓ feed, the sulphate reduction rate increased from 1.6 to 3.2 to 3.7 g SO_4/(ℓ.d). When the HRT decreased from 12 to 4 h, the sulphate reduction rate increased from 2.3 to 7.7 g SO_4/(ℓ.d). These results indicated that the sulphate reduction rate increased with increased ethanol dosages and with decreased HRT and thus that the sulphate reduction rate is dependent on the ethanol concentration and the HRT.

The experimental S^{2-}/SO_4 ratios were the lowest (0.06, 0.14, 0.06 and 0.07) at the highest HRT (12 h), followed by 0.13, 0.16, 0.16 and 0.16 at the HRT of 6 h and the highest at 0.20, 0.17, 0.20 and 0.18 at the lowest HRT (4 h). In all instances, the experimental ratios were lower than the theoretical S^{2-}/SO_4 ratio of 0.33. This finding can be ascribed to partial H_2S gas escaping and to the oxidation of the produced sulphides to sulphur. The sulphide oxidation rate followed the pattern of the sulphate reduction rate, showing that the sulphide oxidation rate is a function of the sulphate reduction rate and the amount of oxygen present in the reactor.

4.4 **APPLICATION OF THE SULPHATE / SULPHIDE REMOVAL TECHNOLOGY IN THE MINE INDUSTRY**

4.4.1 Introduction

Mining effluents are often acidic, containing high concentrations of sulphates and metals. Traditionally this type of wastewater was treated chemically by the addition of lime, to increase the pH and to precipitate the heavy metals as metal hydroxides. However, this method can only be applied to a sulphate concentration of about 1500-2000 mg/ℓ, the solubility of $CaSO_4.2H_2O$ in water. The residual sulphate concentration can be removed, applying the biological sulphate removal technology (Maree and Strydom, 1985; Maree *et al.*, 1986). Over the past 15 years, this treatment mode has gained increased popularity, due to the development of better reactor configurations and the formation of granular sludge (Lettinga *et al.*, 1980, Hulshoff Pol, 1989). As shown (4.2), sulphate can be removed biologically as sulphide or sulphur, if a suitable carbon and energy source is available, such as ethanol or sugar. The disadvantage of using either sugar or ethanol as the carbon and energy source is the additional costs and COD loading to organic free water. Du Preez *et al.,* (1992) showed that sulphate can be reduced biologically when either hydrogen and/or producer gas were used as the energy and carbon and energy source, respectively. To avoid incurring high additional costs, the idea of an integrated treatment system was conceived; in which initially the high sulphate load is treated with limestone till the sulphate concentration is reduced to approximately 1500 mg/ℓ, the solubility of gypsum ($CaSO_4.2H_2O$). The remaining sulphate concentration can then be treated biologically, with the advantage that less carbon and energy source is needed than in the case of a sulphate concentration of e.g. 2500 mg/ℓ. Other researchers, e.g. Rose (2000), Pulles (2000) concentrate their investigations on the use of primary sewage sludge or lignin rich material, with the disadvantage that high residual COD concentrations will occur in the treated water. Moreover, not enough sewage sludge is available in the regions where the acid mine water has to be treated.

When acid mine water is treated biologically, the produced sulphides, which are dissolved in the treated effluent, can be removed through metalsulphide precipitation (Greben *et al.*, 2000b). Metal sulphides have low solubilities and can be discarded with the sludge wasting process. Depending on the effluent pH, part of the formed sulphides will escape in gaseous form, and a part will be oxidised to elemental sulphur provided that the oxygen level is kept low.

The aims of this investigation were to determine: the stability of the completely-mixed reactor system, the volumetric and specific sulphate reduction rates, the stoichiometric relationship between various parameters and the efficiency of metal removal from acid mine water.

4.4.2 Materials and Methods

4.4.2.1 Reactor configuration, biomass, feedstock and the carbon and energy source

Two completely-mixed reactor systems as described in 4.1 were used in this investigation. The same biomass as discussed in 4.1.1 and 4.2.1 was used, while ethanol (Crest Industries, Johannesburg) was used as the carbon and energy source (1 mℓ ethanol/ℓ feed). Initially the first reactor received synthetic feed, of which the sulphate (in the form of $CaSO_4$) and the COD concentration was 1500 mg/ℓ. The feed water for the second reactor comprised of undiluted mine water (composition see Table 18). Both reactors were inoculated with biomass conditioned to sulphate removal. Both the reactor and the clarifier were open to the atmosphere and the operating temperature was kept at room temperature (20 – 22 °C).

4.4.2.2 Analytical

The analytical procedures were carried out as discussed under 4.1.2.5

4.4.2.3 Experimental

The experiment for the first reactor was divided in four experimental periods, which were determined by the feed composition and by the HRT (Table 16). During the first period, a synthetic feed was used, of which the effluent was collected. During the second, third and fourth period, this effluent was mixed with acid mine water, obtained from a coal mine, and the mixture served as feed water for the reactors. The experimental period for the second reactor, receiving undiluted mine water as feed stock, was defined by the HRT. Due to the fact that undiluted acidic mine water was fed to the reactor, the HRT was kept relatively high at 18 and 14.4 h (Table 17).

Table 16: **Experimental periods as determined by the feed composition and the HRT.**

Period in days	HRT	Feed composition
1-13	12	Synthetic feed
16-27	10	part a.m.w*: part reactor effl** = 1:1
60-70	5.3	part a.m.w: part reactor effl = 1 :1
84-95	4.3	part a.m.w: part reactor effl = 1 :1

*:a.m.w = acid mine water   **:reactor effl= treated synthetic feed water

4.4.3   Results and Discussion

4.4.3.1 Process stability

Figure 8 shows the sulphate concentration of the feed and treated water over a period of 65 days in the first reactor. It was observed that sulphate was removed from as high as 3000 mg/ℓ to less than 200 mg/ℓ (as SO_4). From days 10 to 40 the biomass adapted to the feed water and feed rate (as shown in Fig. 8), where after the reactor pattern became stable.

imperative to include a pH controller which will keep the reactor pH constant in the range of 7.5-8.5.

4.4.3.2 *The stoichiometric relationship between various parameters as shown in Table 17*

COD/sulphate

The experimental COD/SO_4 ratio varied between 0.55 and 0.84. The theoretical values are 0.67 and 0.50, when sulphate is reduced to sulphide and to sulphur, respectively, excluding COD requirement for cell production. The experimental values were in some instances as low as 0.55, for which no direct explanation is clear. It may indicate that a portion of the sulphate was converted to sulphur and not to sulphide. In the case of sulphur as end product, ascribed to the activity of the sulphide oxidizing bacteria (*Thiobacilles*, spp), the theoretical feed COD requirement amounts to only 75% of that when sulphide is the end product. COD is also needed for new biomass growth and other bacteria present in the reactor will use part of the available COD for their consumption. Ethanol as carbon source can be degraded to hydrogen, which will mainly be used by the SRB, and acetate, which forms the substrate for the MB. Visser (1995) indicated that fierce competition takes place for the same (carbon) substrate in an anaerobic reactor. The MB and the SRB degrade actetate via methanogenesis and sulphate reduction, respectively. His study revealed that the reactor pH exerts a strong effect on the competition of the SRB (pH optimum at 8-8.5) and the MB (pH optimum at 6.5-7.5), thus for the sulphidogenic reactor it can be advised to maintain the reactor pH close to 8.

Table 17: **The results as obtained from the experimental conditions as shown in Table 16.**

Determinand	Unit	Values						
		First reactor				Second reactor		Theoretical ratio
		Synthetic	A.m.w : Treated synthetic feed			Acid mine water		
Dilutions			50:50	50:50	50:50	100	100	
Feed rate	(ℓ/d)	30	36	67.6	83	20	25	
HRT (h)*		12	10	5.3	4.3	18	14.4	
Feed:								
Sulphate	mg/ℓ	1550	1950	1715	1912	2500	3150	
COD	mg/ℓ	1630	1319	1444	1316	1694	1627	
Alkalinity	mg/ℓ	200	600	600	600			
Acidity	mg/ℓ	-	300	300	300	600	600	
pH								
Treated:								
Sulphate	mg/ℓ	235	755	329	397	628	194	
COD	mg/ℓ	553	569	282	286	535	600	
Alkalinity	mg/ℓ	518	945	1111	781	573	712	
Sulphide (S)	mg/ℓ	181	201	273	213	170	281	
Calcium						305	301	
VSS	g/ℓ	5.3	2.2	3.7	3.4	3.4	3.6	
pH		7.73	8.01	7.79	7.96	8.15	7.87	
Rates:								
SO_4 reduction	g/(ℓ.d)	2.5	2.9	6.2	8.4	2.5	4.9	
Specific SO_4 reduction	g SO_4/ (g VSS.d)	0.47	1.29	1.67	2.47	0.74	1.36	
Ratios:								
COD/SO_4		0.82	0.63	0.84	0.68	0.8	0.55	0.67
S/SO_4		0.16	0.17	0.2	0.15	0.09	0.1	0.33

* HRT is based on reactor volume only and excludes clarifier volume.

Sulphide/sulphate

The experimental S^{2-}/SO_4 ratio varied between 0.1 and 0.2 (Table 17). The theoretical value is 0.33 (mass ratio) when sulphate is reduced to sulphide. The fact that the experimental values are less than the theoretical value of 0.33 supports the finding that a portion of the sulphate is converted to sulphur, as was seen in the previous studies. Another reason for the low sulphide/sulphate ratio is because of the metal sulphide precipitation. Due to the lower sulphide concentrations in the reactors, the process is more stable as high sulphide levels are toxic to the SRB, causing instability in the process (Oleszkiewicz and Hilton, 1986).

4.4.3.3 *Biological treatment of acid mine water*

Table 18 shows the chemical composition of the acid mine water, the diluted acid mine water with the treated synthetic feed water (50%: 50%) and the treated acid mine water.

From the results in Table 18, it can be observed that:

- 90% sulphate reduction was achieved (2 250 to 220 mg/ℓ), while COD (due to ethanol addition) was reduced from 2020 to 1115 mg/ℓ (as O_2). Thus, it can be concluded that ethanol is a suitable energy and carbon source to use for sulphate reduction in acid mine water.
- Alkalinity was generated to raise the pH from 3.2 to 8.3
- Water with a maximum alkalinity of 1160 mg/ℓ (as $CaCO_3$) was produced.
- The macro nutrients, phosphate and nitrate, added to the feed water were completely utilized
- Iron and copper were removed completely and aluminium, manganese and zinc to <4 mg/ℓ. Aluminium precipitated as $Al(OH)_3$ and the other metals as metal sulphides.
- A portion of the produced sulphides were oxidized to elemental S.

76

Table 18: **Chemical composition of acid mine water (AMW), the diluted acid mine water and of the treated acid mine water.**

Parameter	Chemical composition of:		
	A.m.w	A.m.w and treated feed	Treated diluted a.m.w.
Carbon Source	Ethanol	Ethanol	Ethanol
pH	3.2	7.8	8.3
Sulphate (mg/ℓ SO_4)	2250	1550	220
Sulphide (mg/ℓ S)	5	22	106
COD (mg/ℓ O_2)	28	2020	1115
Acidity (mg/ℓ $CaCO_3$)	600		
Alkalinity (mg/ℓ $CaCO_3$)		600	1160
Nitrate (mg/ℓ N)		0.73	0
Orthophosphate (mg/ℓ P)	2.8	7.5*	0.5
Calcium (mg/ℓ Ca)	305	269	246
Magnesium (mg/ℓ Mg)	178	157	141
Iron (mg/ℓ Fe)	139	0.06	0.04
Aluminum (mg/ℓ Al)	11.7	< 5	3.5
Manganese (mg/ℓ Mn)	8.4	3.18	1.25
Copper (mg/ℓ Cu)	0.35	0.01	0.01
Zinc (mg/ℓ Zn)	113	3.1	3.1

* Macro-nutrient added to the synthetic feed

4.4.4 Summary

The results from this study showed that the single stage completely-mixed reactor system could successfully be used to biologically reduce sulphate from the acid mine drainage diluted with the treated water from the artificial feed. It is thought that both sulphate and the produced sulphide were removed via sulphur using ethanol as carbon and energy source, treating both artificial feed water and acid mine water for a period of 95 days. Sulphate was reduced from approximately 2000 mg/ℓ to almost 400 mg/ℓ, while the sulphide concentration was between 200 and 300 mg/ℓ. The maximum volumetric sulphate reduction rate achieved was 8.4 g SO_4/(ℓ.d). The VSS value in the reactor varied between 3 and 4 g/ℓ, resulting in a specific sulphate removal rate varying from 0.47 to 2.47 g SO_4/(g VSS.d). The experimental COD/sulphate ratio was between 0.63 and 0.84, which was in accordance with the theoretical value of 0.50 and 0.67 (see 4.2.3). The experimental sulphide/sulphate ratio was less than the theoretical value of 0.33 due to escaping H_2S gas, the partial conversion of sulphate to sulphur and due to metal sulphide precipitation. When undiluted acid mine water was treated at HRT's of 18 and 14.4 h, the volumetric sulphate removal rate increased from 2.5 to 4.9 g SO_4/(ℓ.d), while the specific sulphate removal rate increased from 0.74 to 1.36 g SO_4/(g VSS.d). The overall results showed that iron and copper were completely removed and aluminium, manganese and zinc to less than 4 mg/ℓ. Consistent sulphate removal rates resulted in good alkalinity production, causing the pH of the acid feedwater, fed directly to the reactor system (without pre-treatment), to increase from 3.2 to 8.0.

5.   GENERAL CONCLUSIONS

In this study, a number of key parameters, for achieving the highest biological sulphate reduction and sulphide oxidation rates, when treating artificial and industrial/mine effluents, were evaluated. The objectives to evaluate three reactor systems, using different carbon and energy sources at various concentrations were achieved. Furthermore, the influence of the hydraulic retention time on the sulphate removal and sulphide oxidation rates were investigated. Lastly, the application of the non conventional completely-mixed reactor system, using improved process conditions, such as the optimum ethanol concentration and evaluating the HRT was tested on the treatment of diluted and undiluted mine effluent. The following conclusions were made:

1.   **High SO_4 removal rates can be obtained, using:**

- The single-stage completely-mixed reactor system
- Ethanol and/or sugar as the Carbon and Energy Source

In addition
- The sulphate reduction rate is a function of the HRT: -

   the lower the HRT, the higher the sulphate reduction rates.

- The sulphate reduction rate is a function of Ethanol dosage

2.   **Sulphide can be removed partially, due to**

   1. H_2S gas escapes
   2. Sulphide oxidation
   3. Metal sulphide (MeS) precipitation

In addition

- The sulphide oxidation rate is a function of the sulphate removal rate and the available O_2

3.   **Biological Sulphate Removal Technology can successfully be applied to treat Acid Mine Water.**

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