DETERMINATION OF THE SUITABILITY OF ALTERNATIVE CARBON SOURCES FOR SULPHATE REDUCTION IN THE PASSIVE TREATMENT OF MINE WATER

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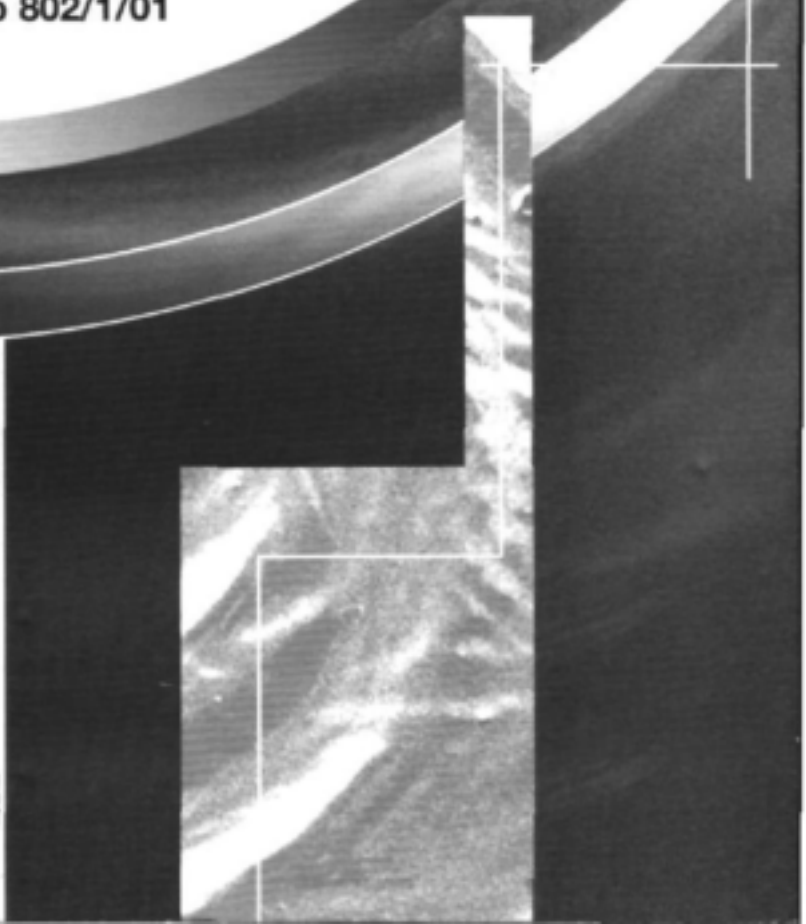

Water Research Commission

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by

S. DILL, T. E. CLOETE, L. COETSER and L. ZDYB

Report to the Water Research Commission
by
CSIR, Division of Water, Environment and Forestry Technology

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CSIR, Division of Water, Environment and Forestry Technology

in association with

University of Pretoria,
Department of Microbiology and Plant Pathology

Report to the

Water Research Commission

on

Determination of the suitability of alternative carbon sources for sulphate reduction in the passive treatment of mine water

by

S. DILL (Project Leader)

EXECUTIVE SUMMARY

Background

This project was undertaken by the CSIR, Pretoria, and the University of Pretoria, Department of Plant Physiology and Microbiology. The project was sponsored by the WRC and originated from another project on the passive treatment of mine water (WRC K5/700), which was also partially sponsored by the WRC.

By the time the motivation for the present project was submitted to the WRC, the passive mine water treatment project had been running for some 12 months. It was observed that the initial sulphate reduction achieved through the operation of the pilot scale plants, had declined significantly over the past weeks in most of the systems. Some of the questions asked in discussing the problem were related to the suitability of the carbon sources for the biological treatment of acid mine water, whilst others were related to the medium-to-long term suitability of such carbon sources in the treatment of acid mine water. Limited initial test work had been undertaken on the carbon sources included in the pilot plant systems and the need to better understand and test potential carbon sources for the use in passive mine water treatment systems was identified by the project team.

The primary objective of this project was to develop a quick test method for the assessment of potential carbon sources regarding their suitability for use in passive treatment systems. The objective was defined by developing a test procedure. The test procedure would compare the sulphate reduction under controlled experimental conditions in an anaerobic reactor using defined carbon sources such as lactic acid and others with undefined (complex) carbon sources.

Methodology

It was initially envisaged that up to 20 defined and undefined carbon sources could be tested in triplicate for their suitability to support sulphate reduction in passive treatment systems for mine water. The experiments would most likely require 20 to 30 days to obtain suitable data for the evaluation of the respective carbon sources. The main problem was that a larger number of anaerobic reactors would be required which could be sampled without changing the environmental conditions in the reactor. The envisaged volumes of matter to be incubated was 1,000 mℓ.

Intravenous feeding apparatus, in short 'drip bags', were identified as a suitable reactor for the experiments as they were quite readily available from hospitals in volumes of 1,000 and 5,000 mℓ.

These bags were made from durable plastic and were also gastight and flexible enough to expand to accommodate any gas produced during the testing of the carbon sources. Initial testing of the drip bags confirmed that the idea was feasible.

The initial work was followed up with experiments to evaluate defined and undefined carbon sources regarding their suitability to support sulphate reduction in the anaerobic reactors. Seven defined carbon sources were identified from a comprehensive literature review and used in further experimental work to generate baseline data regarding sulphate reduction with defined carbon sources. The removal of 90% of the sulphate over time was used as a benchmark for the evaluation of the experiments. The experiments confirmed that under the selected experimental conditions 90% of the sulphate in artificial acid mine drainage (initial sulphate concentration was approximately 2,500 mg/l) could be removed within seven days. Two carbon sources which achieved more than 90% sulphate reduction were butyric acid and propionic acid.

The other defined carbon sources used were found to be less effective, ranging from 73.6% sulphate removal for pyruvate to 87.7% sulphate removal for methanol. Some variation in the time period was also found for the same carbon source, probably due to a slight variation in the water chemistry between the different batches of mine water used in the experiments.

Experiments with undefined (complex) carbon sources confirmed that some of these carbon sources can remove sulphate from the artificial mine water quite effectively. Sulphate reduction in excess of 90% was achieved for kikuyu grass cuttings, silage, mushroom compost and hay. Fresh cow manure fell short of the benchmark by 9.0 % with 81.1 % sulphate removal. The other carbon sources ranged between 0% sulphate removal with composted cow manure, to 86.7% sulphate removal with fly ash.

Literature Review

The literature review showed that an increasing amount of work has been undertaken on bacterial sulphate reduction in acid mine water and industrial effluents since the mid 1960s. The majority of work cited in the literature is concerned with active systems for the removal of sulphate while the discussion of passive treatment systems for mine water treatment focuses largely on the removal of metals, particularly iron, rather then the removal of sulphate. No information pertaining to the assessment of carbon sources for the purpose of biological sulphate removal could be found in the literature and the work undertaken and documented in this report is therefore considered to be novel.

Availability of carbon sources

The review of the potential carbon sources available showed that a number of "renewable" carbon sources existed, such as grass and wood, whilst a number of waste products were also identified as potentially suitable carbon sources. The distribution of carbon sources needs to be considered in the context of the geographical distribution of the mines as transport of carbon sources beyond a radius of 100 km may not be cost-effective. It was confirmed that for a number of the carbon sources, that exist in the respective mining areas, alternative uses are established. Such uses may render the carbon sources a commercial commodity rather than a freely available waste product, Potentially , therefore, this could add to the capital cost of the passive treatment system as the cost of carbon could become one of the major cost items in the construction operation costs of the system.

Conceptual model

To document and better understand the complexity of the biocenosis in a passive treatment system for mine water, a conceptual model was developed of the main bacterial physiological groups potentially present in the sulphate reducing reactor of the mine water treatment system. The model described the fate of the complex carbon via the various pathways of the respective bacterial groups and provided an indication of preferable pathways, that would maximise the conversion of the complex carbon into suitable products for bacterial sulphate reduction. In addition, the model also indicated the competitive reactions that scavenge (remove) carbon which is potentially available for sulphate reduction. Such competing reactions lead to a net loss of the total carbon available for sulphate reduction in the treatment process, thereby reducing the overall efficiency of the treatment system. The environmental conditions in the sulphate reduction reactor therefore have to be controlled in such a manner as to minimise competing reactions and maximise the amount of carbon available for sulphate reduction.

Conclusions

The work undertaken during the project gave valuable insights into the suitability of certain defined and undefined carbon sources for the use of sulphate reduction in small-scale anaerobic reactors. The methodology developed was found to be reproducible and low in cost, and can easily be implemented in other laboratories to screen potentially suitable carbon sources for sulphate reduction. Not all the objectives of the project have been met satisfactorily and recommendations for further research work are given at the end of the report.

Recommendations for further work

The main issue requiring further work is the release of carbon from complex carbon sources over time. In particular, it is important to develop test procedures to simulate the 'ageing' of carbon in passive treatment reactors so that carbon release can be examined over medium-to-long term periods of 2 to 10 years, as well as a need for further work to more fully understand the sustainability of carbon release from potential carbon sources.

The specific outputs emanating from this project are as follows

Dill, S., T.E. Cloete, L. Coetser, & L. Zdyb. *Determination Of The Suitability Of Alternative Carbon Sources For Sulphate Reduction In The Passive Treatment Of Mine Water*. (This report). WRC TT

MSc thesis

Coetser, S.E. (1997). *Microbial sulphate reduction using defined carbon sources and artificial acid mine drainage*. University of Pretoria, Department of Microbiology and Plant Pathology.

MSc thesis (agric): Microbiology

Zbyd, L. (1999). "Microbial sulphate reduction as a method of passive treatment of acid mine drainage using undefined carbon sources", University of Pretoria, Department of Microbiology and Plant Pathology.

Poster and Oral Paper

Coetser, S.E., S. Dill & T.E. Cloete (2000). *Biological Sulphate Reduction in Artificial Acid Mine Drainage using Defined Carbon Sources*, BioY2K Conference in Grahamstown, South Africa.

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K5/802 DETERMINATION OF THE SUITABILITY OF ALTERNATIVE CARBON SOURCES FOR SULPHATE REDUCTION IN THE PASSIVE TREATMENT OF MINE WATER

The Steering Committee responsible for the project consisted of the following persons:

Mr HM du Plessis	Water Research Commission
Mr GN Steenveld	Water Research Commission
Mr W Pulles	Pulles, Howard and de Lange
Dr JP Maree	CSIR, Division of Water, Environment and Forestry Technology
Dr ME Aken	AMCOAL (Chamber of Mines)
Ms MC Eksteen	Department of Water Affairs and Forestry

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The project was conducted as a sister project of the Water Research Commission Project No. K5/700 entitled:

K5/700: PILOT SCALE DEVELOPMENT OF INTEGRATED PASSIVE WATER TREATMENT SYSTEMS FOR MINE EFFLUENT STREAMS.

Valuable contributions towards the project came from the Specialist Researchers and the Chairman of the Project Management Committee of this project. The authors therefore wish to record their sincere thanks to the following:

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1. THE PURPOSE OF THE PROJECT

1.1 Background and Motivation

In 1993 the Department of Water Affairs & Forestry (DWA&F) identified a need to investigate the potential application of passive treatment systems for mine waters in South Africa. The Chamber of Mines Research Organisation (COMRO) was invited to prepare a proposal in this regard. In compliance with this request, COMRO in association with Steffen Robertson & Kirsten (SRK) and the CSIR, submitted a proposal in 1994. However, on the grounds of the subsequent disbanding of COMRO and a concern expressed by the Water Research Commission (WRC) regarding the anticipated high value of the research on this topic, the WRC contracted the CSIR to conduct a literature review on the need for passive mine water treatment systems in South Africa. This investigation revealed the potential benefits of developing passive treatment for application in South Africa, but identified the lack of established local or international experience with systems designed to treat sulphate rather than metals. It also identified the acute lack of information with regard to the suitability and sustainability of carbon sources to drive the required sulphate reduction process. Further research in this regard was therefore strongly recommended.

In deliberations between Pulles, Howard & De Lange (PHD), the Chamber of Mines and other interested parties, it was felt that there was adequate information available to proceed with *in situ* pilot scale studies in mine water discharges rather than undertake pilot scale research consisting of prolonged laboratory studies. In 1995 the WRC, in association with the Chamber of Mines and other interested parties, appointed PHD to undertake a pilot scale project to investigate the use of passive treatment systems for the treatment of mine waters. The systems were duly designed based on the best available information, constructed and commissioned.

With the pilot scale research presently under way, it was recognised that a suitable and/or sustainable carbon source supply is essential for long-term and cost-effective passive biological sulphate reduction. The supply of carbon is a primary factor in both the design and capital costs, as well as in the costs of maintenance and operation of the system. The pilot scale research has also shown that a significant factor in the viability of a carbon source may be the residual organics and nutrients that are released during the degradation of the carbon source which, in turn, must be removed from the wastewater to meet acceptable final effluent discharge quality objectives. The removal of these residual components could affect significantly the design, capital, maintenance and operational aspects of the system.

It was also evident that preferred carbon sources, such as mushroom compost (often recommended internationally) or domestic sewage sludge, may not be readily available at a specific mine location. It

may be necessary, therefore, to find alternative carbon souces for which there is presently little information available. These carbon sources at the specific mine location might be available in sufficient quantities for incorporation in a full-scale passive mine water treatment system, but the suitability of such carbon sources to provide sustainable biological sulphate reduction is usually not known. This aspect should be tested prior to the decision to use these carbon sources in the passive treatment of mine water.

1.2 Objectives of the project

The primary objective of the project was to develop a laboratory method to test low-cost carbon sources for their suitability for use in passive treatment systems for mine water.

The specific aims of the project consisted of the following:

- to identify a range of carbon sources available in the country taking economical factors like available quantities and transport costs into consideration;

  A range of potentially suitable carbon sources was identified and the available quantities and transport costs for these were determined at an order of magnitude level;

- to determine test procedures regarding total carbon content, carbon available for bacterial sulphate reduction and sustainability of carbon release over time for identified carbon sources;

  This task, although identified as a requirement to the project, was not resolved to a suitable degree of satisfaction as the time and the funds for the project did not allow for extensive testing to develop a method for the determination of available carbon or the sustainability of carbon release over time. It is the opinion of the project team, based on their experience during the project, that these two questions will have to be addressed in separate projects as a follow up of this research. The answer to the questions is more complex than the stated primary objective of this project - viz the development of a quick test method for the evaluation of suitable carbon sources for the use in passive treatment systems;

- to develop a test procedure to obtain sulphate reduction kinetics for the respective carbon sources tested under passive treatment conditions;

  A test procedure for the assessment of respective low cost carbon sources was developed during

the project:

- to test the developed procedure on a model carbon source mixture, which should be able to provide carbon over a short-, medium- and long-term period; and

  This task was not achieved during the project due to time constraints. The development of the test procedure and the testing of selected defined and undefined carbon sources with the developed procedure required the full time available for the project. To test the model carbon source mixtures a different type of reactor to the one used in the test procedure would be required.

- To summarise the test procedure in preliminary guidelines for the evaluation of carbon sources for use in passive treatment systems.

  Although valuable lessons were learned during the project, too many questions remain unanswered at present to develop full guidelines for the testing and evaluation of low-cost carbon sources for use in passive treatment systems. A test protocol for the evaluation of potential carbon sources is discussed in Chapter 5.

1.3 Approach to the project

The project included four different tasks, which were undertaken by the CSIR and the University of Pretoria (UP), Department of Plant Physiology and Microbiology. The tasks undertaken were as follows:

A) Literature study

The objective of this task was to collect recently available data on carbon sources used in the passive treatment of mine waters and updated available background information that has been published in recent years. The literature review is given in Chapter 2 of this report.

B) Economic evaluation of potential carbon sources

The economic evaluation of potential carbon sources included factors such as available quantities, numbers of suppliers, location of sources, cost and transport of materials. Apart from carbon sources that have to be added to a treatment system and are replenished during treatment, the evaluation also included intrinsic carbon sources, which are generated by the treatment system itself. This evaluation is given in Chapter 3 of this report.

C) Discussion of the biological processes in the form of a conceptual model

The objective of this task was to understand the biochemical reactions and interactions of the various physiological bacterial groups making up the biocenosis in a passive mine water treatment reactor. The model is discussed in Chapter 4 of this report.

D) Experimental work

The experimental work included the development of a simple test method for the evaluation of alternative carbon sources, followed by testing the method on several defined and undefined (complex) carbon sources for sulphate reduction. The experimental work is described in Chapters 5 to 7 of this report.

The conclusions of the work undertaken for the project with recommendations for further work required are given in the final chapter of this report.

2. LITERATURE REVIEW

2.1 Introduction

Mining activities often result in the production of water containing high amounts of sulphate and heavy metals. These waters often derive in high quantities from underground works or from open cast mining operations. In most cases, these waters have to be pumped from the works to avoid flooding and the abstracted water has to be included in the mines overall water management programme. Other sources of sulphate and heavy metal rich water are seepage from waste rock or tailings dam complexes. In addition to water from operating mines, water of poor quality may arise from decanting of recharging groundwater from mines that have been decommissioned during previous decades. In the literature these mining related waters are referred to as acid rock drainage (ARD) or acid mine drainage (AMD).

Acid mine drainage (AMD) is the consequence of the metabolism of sulphur -and iron-oxidising bacteria, when pyrite is exposed to atmospheric oxygen. The combination of auto-oxidation and microbial sulphur and iron oxidation produces large amounts of sulphuric acid (Atlas and Bartha, 1993). Due to the fact that AMD is highly corrosive, it results in economic and environmental problems.

By removing sulphate and heavy metals from the water, and neutralising the water's pH, the impact of AMD to the natural environment can be reduced or prevented. Various sulphate removal technologies are available (Dill *et al.*, 1994). These include desalination processes such as reverse osmosis and ion exchange (Dill *et al.*, 1994, 1998). By using barium ions, such as barium hydroxide or barium chloride, sulphates can be chemically removed from solution (Maree and Strydom, 1985). Sulphur can be reclaimed from gypsum (calcium sulphate) by sulphate reduction followed by removal of the sulphide as hydrogen sulphide gas, which can then be chemically oxidised to elemental sulphur (Middleton and Lawrence, 1977). This process can be used to neutralise sulphuric acid wastes (Middleton and Lawrence, 1977). Considering current problems of acid mine drainage, the development of microbial sulphate reduction as a treatment process for these wastes is a desirable objective. Provided that a suitable electron donor is available, SRB can oxidise organic compounds such as lactate or acetate, with sulphate as the electron acceptor being reduced to sulphide (Dill *et al.*, 1994):

$$8CH_3COO^- + 8SO_4^{2-} + 16H^+ \text{ -----> } 16CO_2 + 16H_2O + 8HS^-$$

Growth studies conducted with pure cultures of SRB have been limited to less complex compounds, such as short chain carboxylic and dicarboxylic acids (Middleton and Lawrence, 1977). In mixed

cultures. SRB have been grown with a variety of complex carbon sources (Middleton and Lawrence, 1977) such as fishmeal, wastewater sludge, cellulose and sawdust.

Mixed cultures of SRB have been found to increase the pH of a lactic acid-mineral salts medium, containing sulphuric acid, from 5 to 8.9 in 8 days at room temperature (Tuttle *et al.*, 1969).

Du Preez *et al.* (1991) used producer gas, consisting of a mixture of H_2, CO, and CO_2 and N_2 generated from coal, as an energy source for biological sulphate removal. Sulphate was reduced from 1900mg/ℓ to less than 200mg/ℓ during the anaerobic treatment of sulphate-rich water in a trickling filter. A maximum sulphate conversion rate of 30g/ℓ SO_4^{-2}/ℓ/d was achieved by Van Houten *et al.* (1994). Gas-lift reactors fed with hydrogen and carbon dioxide as energy and carbon source were used during these studies.

Maree and Strydom (1985) studied biological sulphate removal in an up-flow packed bed reactor. Good sulphate removal was achieved by providing anaerobic conditions on a solid medium and maintaining a low hydrogen sulphide concentration by re-circulating the water through a photosynthetic reactor for sulphur production. Sugar, pulp mill effluent or sewage was used as energy sources in these experiments. Re-circulation enhanced sulphate reduction and the presence of light increased sulphur production. Therefore, under ideal conditions, SRB may be used for the remediation of AMD.

The passive treatment of AMD, involving SRB is another method, which should be considered (Batchelor, 1993). However, there is a lack of established local or international experience with passive treatment systems designed for the removal of sulphate. To develop efficient passive AMD treatment, methods must be established to evaluate the potential use of different undefined carbon sources and the anaerobic sulphate reduction process must be optimised. This can be done by selecting the most appropriate carbon sources, or a combination of various carbon sources. The primary objective of this project was to develop a standard procedure for the evaluation of carbon sources for sulphate reduction in acid mine drainage, using defined carbon sources. The information obtained from these experiments would form the basis for studies conducted on undefined carbon sources. A conceptual model for the passive treatment of acid mine drainage will also be presented.

2.2 Acid mine drainage (AMD)

2.2.1 Sources and problems associated with acid mine drainage

Atlas and Bartha (1993) defined acid mine drainage as the consequence of the metabolism of sulphur- and iron-oxidising bacteria when mining exposes pyrite to atmospheric oxygen. The combination of

auto-oxidation and microbial sulphur and iron oxidation produces large amounts of sulphuric acid, which kills aquatic life and contaminates water. Acid mine drainage (AMD) renders the contaminated stream unsuitable as a water supply or for recreational use due to the fact that it is highly corrosive. This leads to many economic and environmental problems.

The discharge of industrial effluent containing high concentrations of sulphate into surface waters contributes directly to mineralisation and corrosion potential of the receiving waters (Dill *et al.*, 1994). Some acid mine drainage originates from subsurface mining because of water flowing through the mine itself (Atlas and Bartha, 1993). The problem with subsurface coal mining is limited and easily controlled. After the coal has been removed, the areas surrounding these streams are collapsed (Atlas and Bartha, 1993). This procedure limits the amount of rock exposed to oxidation action at any one time. During strip-mining of coal, tailings are left as porous rubble which are exposed to oxygen and percolating water (Atlas and Bartha, 1993). Due to iron and sulphur oxidation, the pH drops rapidly and prevents the establishment of vegetation or stable soil cover. The recovery of the land may take from 50 to 150 years (Atlas and Bartha, 1993).

Thus, sulphate in mine water originates from at least two sources:

- bacterial oxidation of pyrite
- the spent sulphuric acid used in metallurgical or chemical plants (Du Preez *et al.*, 1991).

According to McGinness and Johnson (1993), the scale of the pollution problem associated with AMD is vast. It was estimated in 1963 that 3 million tons of sulphuric acid entered the Ohio River in the form of AMD. They concluded that besides its acidity (pH of AMD may range from <2 to 4), the toxicity of AMD to most life forms comes from its heavy metal content. Soluble iron concentrations, both as ferrous and ferric forms, are inevitably high and other metals for example copper, lead and zinc may also be present at elevated concentrations (McGinness and Johnson, 1993). Soluble iron concentrations depend upon the geochemical nature of the material being oxidised. The dominant anionic species present is sulphate and may reach concentrations up to >40 g/ℓ (McGinness and Johnson, 1993).

The water situation in South Africa is threatened both from a supply and a quality point of view (Maree, 1988). The supply and demand curves of water in South Africa will converge before 2020 due to the fact that South Africa is a semi-arid country with limited water resources (Maree, 1988). As a result of expanding populations, the total water demand in South Africa for agriculture, housing, industrialisation and mining will continue to increase rapidly.

Sulphate significantly affects the utilisation of water (Maree, 1988). Therefore, successful treatment of sulphate-polluted water will contribute considerably to the prevention of pollution of South Africa's surface water.

At present, the city councils of Johannesburg and water authorities in the Gauteng area allow the discharge of water with higher sulphate concentrations than the acceptable levels of 200-500mg/ℓ into sewer systems or rivers (Dill *et al.*, 1994). This is accepted due to the fact that the ratio of sulphate-rich water produced by industrial activities to surface water, is high in this region. As soon as proven technologies are available for the removal of sulphate at an acceptable cost, legislation will be enforced to prevent the discharge of waters with high sulphate concentrations into the receiving waters (Dill *et al.*, 1994).

2.3 The microbiology in acid mine drainage

Thiobacillus ferrooxidans, an autotrophic bacterium, is responsible for the enzymatic oxidation of ferrous sulphide minerals like pyrite, which are often found associated with coal and other minerals in nature (Tuttle *et al.*, 1969). This oxidation process leads to an accumulation of ferric, sulphate and hydrogen ions in the drainage waters from coal mines. It was reported that hydrogen ions are responsible for the inhibitory effects of AMD on heterotrophic bacteria of neutral streams (Tuttle *et al.*, 1969).

According to McGinness and Johnson (1993), the microbiology of AMD is surprisingly complex. Acidophilic bacteria are dominant; however, eucaryotes, ranging from fungi and yeasts to protozoa and rotifera, may also be found. Chemolithotrophic bacteria that obtain energy from the oxidation of ferrous iron and/or reduced sulphur are the primary producers and have been shown to form the basis of an acidophilic food web *in vitro*. The numbers of iron-oxidising bacteria decreased with increasing distance from the mine, whilst neutrophilic heterotrophic bacteria increased. McGinness and Johnson (1993) also found that the highest counts of total bacteria were found in AMD water within the mine. *Thiobacillus ferrooxidans*, was the dominant iron-oxidising bacterium at all sampling sites while *Leptospirillum ferrooxidans* accounted for between 9 and 50% of the isolates. Acidophilic heterotrophic bacteria were occasionally isolated from the downstream river, but they did not detect any iron-oxidising bacteria.

Sulphuric acid and ferric ions have a deleterious influence on the biota of streams that receive mine drainage (Tuttle *et al.*, 1968). Ecological reports have indicated that H_2SO_4 could kill the normal microflora of affected waters whilst aciduric species, notably fungi, appeared to thrive (Tuttle *et al.*,

1968). Tuttle *et al.* (1968) studied the activity of micro-organisms in acid mine water and found that acid-tolerant aerobes survived when acid entered the stream and actually increased in number to about 2×10^5 per ml until the pH approached 3. The organisms then represented the heterotrophic aerobic microflora of the streams comprised of a mixture of mine drainage and non-acid water. Similar microflora to those of the streams comprising of a mixture of mine drainage and non-acid water, were not found in a stream that consisted entirely of acid mine drainage. It was also found that most gram-positive aerobic and anaerobic bacteria died out very rapidly in acidic water, and that they comprised a very small percentage of the microbial population of the streams examined. Where mine water entered a stream, iron- and sulphur-oxidizing autotrophic bacteria were present and sulphur-oxidizing bacteria predominated over iron oxidizers.

During studies conducted on sulphate reduction in AMD, Tuttle *et al.* (1969) found that the SRB represented two different types. These were tentatively identified as a *Desulfovibrio* and a *Desulfotomaculum* species. They also isolated ten different yeasts from the ponds and examined them for sugar-fermenting capacity. This was done because of their potential production of alcohols and organic acids, which could serve as nutrients for the dissimilatory SRB. Four gram-positive and seven physiological types or groups of gram-negative bacteria were also isolated.

2.4 Treatment of acid mine drainage

Conventional water treatment techniques are ineffective for treating AMD because their original design was focussed on the treatment of organic pollution.

2.4.1 Sealing methods

According to Atlas and Bartha (1993) the best way to deal with the AMD problem is to prevent it at the source. Abandoned subsurface mines can be sealed off to prevent or restrict the availability of oxygen for pyrite oxidation. Prompt reclamation of the land can also effectively control AMD in the case of strip mining (Atlas and Bartha, 1993). This involves spreading topsoil over the rubble and establishing a vegetation cover. This technique is also effective on mounds of mine tailings.

2.4.2 Broad-spectrum antimicrobial agents

In theory, AMD can still be curbed if the sealing off of pyritic material from oxygen cannot be accomplished, by suppressing the activity of the iron- and sulphur-oxidising bacteria. However, broad-spectrum antimicrobial agents could be dangerous pollutants themselves and cannot be considered for this purpose (Atlas and Bartha, 1993). Anionic surfactants, benzoic acid, organic acids, alkyl benzene

sulfonates, and sodium dodecyl sulphate inhibit iron- and sulphur-oxidising bacteria (Bitton, 1994). Some of these chemicals or their combinations were able to reduce acidic drainage from coal refuse under simulated field conditions. However, the application of these techniques under field conditions has not been attempted. In addition, the volumes of AMD would render bactericide treatment too expensive for consideration.

2.4.3 Desalination

Other technologies that are available for the removal of sulphate include sulphate removal from water by desalination processes such as reverse osmosis and ion exchange (Dill *et al.*, 1994). Chemically, sulphates can be removed by using barium ions, such as barium hydroxide or barium chloride (Maree and Strydom, 1985).

2.4.4 Biological treatment methods

There is an increasing demand for inexpensive, environmental friendly technologies for the removal of sulphates in order to prevent the formation of AMD. Sulphate-reducing bacteria (SRB) may be used in the biological removal of sulphates from AMD. If a suitable electron donor is available, SRB can oxidise organic compounds such as lactate or acetate, with sulphate as electron acceptor being reduced to sulphide (Dill *et al.*, 1994). During this reaction, protons are consumed which leads to an increase in pH of the treated water up to a final pH of 7.0 - 7.5. Heavy metals are precipitated by produced H_2S as almost insoluble heavy metal sulphides (Dill *et al.*, 1994).

It has been shown that sulphate can be converted quantitatively to H_2S by *Desulfovibrio desulfuricans* and further conversion to elemental sulphur can be effected by the photosynthetic bacteria *Chlorobium limicola* forma specialis *thiosulfatophilum* and *Chromatium vinosum* (Maree and Strydom, 1985). On the basis of this theory, various configurations of bioreactors were developed for the removal of sulphate. Success was achieved by using two separate reactors for hydrogen sulphide and sulphur production, respectively (Maree and Strydom, 1985).

According to Atlas and Bartha (1993), Higgins and Burns demonstrated the feasibility of a novel treatment technique for AMD using the activity of *Desulfovibrio* and *Desulfotomaculum*. They combined mine effluent with large amounts of organic waste materials. The activity of aerobic and facultative anaerobic cellulolytic micro-organisms lowered the redox potential and produced degradation intermediates, which could be utilised by SRB. Even though this process restores neutral pH and removes the iron and sulphur from the effluent, the economic and environmental feasibility of the proposed process has yet to be explored.

Tuttle *et al.* (1969) studied the microbial dissimilatory sulphur cycle in acid mine water. They studied water carrying ferric, sulphate, and hydrogen ions produced from pyritic minerals associated with coal as a result of autotrophic bacterial metabolism. The water accumulated behind a porous dam composed of wood dust originating at a log-cutting mill. The water was enriched in organic nutrients as it seeped through the porous dam. This then supported growth and metabolism of heterotrophic bacteria in the water downstream from the dam. Dissimilatory SRB, which reduce sulphate to sulphide were included in the heterotrophic microflora within and below the sawdust dam. Black iron sulphide (FeS) precipitate was deposited on the pond bottom as a result of the reduction of ferric to ferrous ion by sulphide. When the pH of the lower pond water was compared with that of the upper pond water, a net increase was observed. Microbial activity in the wood dust was demonstrated, and a sequence of cellulose degradation processes was inferred on the basis of sugar accumulation in mixed cultures in the laboratory, ultimately yielding fermentation products, which serve as nutrients for SRB. Mixed cultures, which contained SRB, were also found to reduce sulphate at pH 3.0 in the laboratory with sawdust as the only carbon source, whilst pure cultures isolated from the mixed cultures did not reduce sulphate below pH 5.5. During laboratory studies, maximal sulphate reduction was found to occur in flasks containing partially degraded wood dust, and a rise in pH correlated with the removal of sulphate.

Trickling filter reactor

Du Preez *et al.* (1991) studied biological sulphate removal from mining effluents in a trickling filter, utilising producer gas (a mixture of H_2, CO, CO_2 and N_2 generated from coal) as energy source. They concluded that:

- during anaerobic treatment of sulphate rich water in a trickling filter, influent sulphate was reduced from 1900mg/ℓ to less than 200mg/ℓ
- both producer gas and pure carbon monoxide are viable energy sources for the biological sulphate reduction process, and this can be used to treat acidic mine effluents.

Gas lift reactors

Biological sulphate reduction using gas-lift reactors fed with hydrogen and carbon dioxide as energy and carbon source, was studied by van Houten *et al.* (1994). It was concluded that when free H_2S concentrations are kept below 450mg/ℓ, a maximum sulphate conversion rate of 30g SO_4^{2-}/ℓ/d can be achieved after only 10 days operation and that the gas-to-liquid hydrogen mass transfer capacity of the

reactor determines the maximum sulphate conversion rate.

In-situ treatment of water

In-situ treatment of water is another possibility to avoid the release of AMD in the environment (Dill *et al.*, 1994). In a long-term study over an 18 month period where different organic materials were mixed with dump material and flooded with AMD, an initiation of sulphate reduction in the oxygen-free layers of the soil was obtained. After the addition of sugar beet waste water as additional carbon and energy source, sulphate reduction continued over the complete experimental period (Dill *et al.*, 1994).

Up-flow packed bed reactor

Maree and Strydom (1985) studied biological sulphate removal in an up-flow packed bed reactor. Sulphate removal from mine water was achieved by using sugar, pulp mill effluent or sewage as energy sources. They optimised the environmental conditions necessary for sulphate and sulphur producing bacteria. This was accomplished by providing anaerobic conditions on a solid medium and by keeping the hydrogen sulphide concentration constantly low, by re-circulating the water through a photosynthetic reactor for sulphur production. They concluded that re-circulation enhanced sulphate reduction, the presence of light increased sulphur production and that 1.6 g sugar, 16.7 mℓ spent liquor from a sulphite pulp mill and 172 mℓ raw sewage sludge respectively were required for the removal of 1.800 mg sulphate.

It was shown that a three stage process (anaerobic - aerobic - anaerobic) employing up-flow packed bed reactors for anaerobic treatment, and an activated sludge system for aerobic treatment, could be used to produce reusable water from mining effluents (Dill *et al.*, 1994). Sulphate was reduced from 2500mg/ℓ to less than 500mg/ℓ with concomitant removal of H_2S, carbonates, complex cyanides, phenol and heavy metals. Molasses was used as energy source.

The H_2S produced during biological sulphate reduction can be oxidised to elemental sulphur only (and not oxidised totally to sulphate) provided that the oxygen level in the process is kept at low levels (Dill *et al.*, 1994).

2.5 The microbiology of the sulphur cycle

Micro-organisms play important roles in the sulphur cycle. For example, photosynthetic micro-organisms are able to transform sulphur by using sulphide as an electron source (Prescott *et al.*, 1990). Sulphur can also be assimilated in the form of sulphate by plants, algae and many heterotrophic micro-

organisms (Prescott *et al.*, 1990). Sulphate can be reduced to sulphide by assimilatory sulphate reduction. This reduction is necessary for incorporation into cysteine, methionine and coenzymes in the form of sulfhydryl (-SH) groups (Prescott *et al.*, 1990). The toxicity of H_2S prevents the direct uptake of sulphide by most micro-organisms. The toxicity is avoided by immediately reacting the reduced sulphur with an acceptor, for example FeS (Atlas and Bartha, 1993). The H_2S is subject to photo-oxidative reactions when exposed to the atmosphere, yielding sulphate. If H_2S does not escape to the atmosphere, it can be microbially oxidised under aerobic conditions or phototrophically oxidised under anaerobic conditions (Prescott *et al.*, 1990).

An example of a dissimilatory reduction process and anaerobic respiration is the use of sulphate as an electron acceptor to form sulphide, which accumulates in the environment (Prescott *et al.*, 1990).

Mineralisation of organic sulphur

Several types of micro-organisms carry out mineralisation through aerobic and anaerobic pathways. Under aerobic conditions, sulfatase enzymes can degrade sulphate esters to sulphate:

$$R\text{--}O\text{--}SO_3^- + H_2O \text{ -------> } ROH + H^+ + SO_4^{2-}$$ (Bitton, 1994).

Amino acids containing sulphur are degraded to inorganic sulphur compounds or to mercaptans under anaerobic conditions (Bitton, 1994).

Sulphur assimilation

Both oxidised and reduced forms of sulphur can be assimilated by micro-organisms (Bitton, 1994). Reduced forms such as H_2S are assimilated by anaerobic micro-organisms, whereas aerobes utilise the more oxidised forms.

Sulphur oxidation reactions

According to Bitton (1994) several micro-organisms are involved in sulphur oxidation. He made the following conclusions:

1. H_2S oxidation:

H_2S is oxidised to elemental sulphur under aerobic and anaerobic conditions.
Thiobacillus thioparus oxidises S^{2-} to S^0 under aerobic conditions.

Photoautotrophic bacteria and a chemoautotroph, *Thiobacillus denitrificans* carry out oxidation under

anaerobic conditions.

2. *Oxidation of elemental sulphur:*

This reaction is mainly carried out by aerobic, gram-negative, non-spore-forming thiobacilli, which can grow at very low pH.

3. *Sulphur oxidation by heterotrophic bacteria:*

Oxidation of sulphur by heterotrophic bacteria can occur in neutral and alkaline soils.

Sulphur - oxidizing bacteria

Sulphur-oxidising bacteria are chemolithotrophs (Prescott *et al.*, 1990), and *Thiobacillus* is probably the best-studied example (Prescott *et al.*, 1990). Sulphur-oxidising bacteria oxidise sulphur, H_2S, thiosulphate and other reduced sulphur compounds to sulphuric acid. Both oxidative phosphorylation and substrate-level phosphorylation involving adenosine-5-phosphosulphate (APS) generate ATP (Prescott *et al.*, 1990). Sulphur-oxidising bacteria can use carbon dioxide as a carbon source. However, many will also grow heterotrophically when they are supplied with reduced organic carbon sources such as glucose or amino acids (Prescott *et al.*, 1990). A few other species can grow aerobically as sulphur-oxidising bacteria, carrying out anaerobic respiration with molecular sulphur as an electron acceptor (Prescott *et al.*, 1990).

Thiobacillus can also oxidise H_2S and other reduced sulphur compounds (Atlas and Bartha, 1993). Due to their low acid tolerance, they deposit elemental sulphur rather than generating sulphuric acid by further oxidation. Other members of this genus produce sulphate from the oxidation of elemental sulphur and other inorganic sulphur compounds (Atlas and Bartha, 1993). Photosynthetic sulphur bacteria, the *Chromatiaceae*, *Ectothiorhodospiraceae*, and *Chlorobiaceae*, are capable of photo-reducing carbon dioxide while oxidising H_2S to elemental sulphur (Atlas and Bartha, 1993). In addition, H_2S can be oxidised by microaerophillic bacteria, for example: *Beggiatoa*, *Thioploca* and *Thiotrix* (Atlas and Bartha, 1993).

Purple sulphur bacteria, as well as green sulphur bacteria, also play a role in the sulphur cycle. Purple sulphur bacteria oxidize hydrogen sulphide to sulphur and deposit it internally as sulphur granules (Prescott *et al.*, 1990). Green sulphur bacteria are able to oxidise hydrogen sulphide to elemental sulphur, but the latter is deposited outside the cell (Prescott *et al.*, 1990).

2.5.1 Sulphate reduction

Bitton (1994) made the following statements about sulphate reduction:

1. Assimilatory sulphate reduction:

The anaerobic decomposition of organic matter containing sulphur amino acids such as methionine, cysteine, and cystine by proteolytic bacteria may result in H_2S production.

2. Dissimilatory sulphate reduction:

- SRB (strict anaerobes) are responsible for this reaction:

$$SO_4^{2-} + 2H^+ \text{ ------> } H_2S$$

- Sulphate is used as a terminal electron acceptor in the absence of oxygen and nitrite.

Sulphate-reducing bacteria

Sulphate-reducing bacteria are an ubiquitous group of micro-organisms. They share an ability to couple the reduction of sulphate and other sulphur compounds to the oxidation of a variety of electron donors (De Bruyn, 1992). All are all strict anaerobes and it is insufficient to only exclude oxygen from the culture medium when growing pure cultures of SRB. Redox-poising agents are generally required to maintain a redox potential of -150 to -200mV in the medium (Dasu *et al.*, 1993). Some are known to be capable of fermentative growth in the absence of sulphate, analogous to the fermentative growth of a yeast without oxygen, but none can grow with oxygen as electron acceptor, and oxygen always inhibits their growth (Postgate, 1979). These microbes are responsible for dissimilatory sulphate reduction.

During dissimilatory sulphate reduction, sulphate acts as an oxidising agent for the assimilation of organic matter. Transport of exogenous sulphate across the bacterial membrane into the cell is the initial step in the biochemical sulphate-reduction pathway (De Bruyn, 1992). Once inside the cell, sulphate dissimilation proceeds by the action of adenosine tri-phosphate (ATP) sulphurylase, which combines sulphate with ATP to produce the highly activated molecule adenosine phosphosulphate (APS), as well as pyrophosphate. The cytoplasmic enzyme APS reductase rapidly converts APS to sulphite, which can further be reduced via a variety of intermediates to form the sulphide ion. Virtually all of the reduced sulphur is released into the external environment as the sulphide ion, usually substantially hydrolysed to free H_2S (Postgate, 1979).

Sulphate-reducing bacteria play a significant role in the anaerobic digestion of complex substrates. McCartney & Oleszkiewicz (1991) have suggested that SRB:

- generate sulphides that may result in product inhibition of SRB and/or toxicity to methane producing bacteria;

- change the reactor pH via generation of alkalinity in the conversion of sulphate to sulphide;
- accelerate the oxidation of organics, such as lactate, which are normally degraded at a lower rate by incomplete oxidising non-SRB;
- reduce the rate of methanogenesis; and
- decrease the quantity of methane produced by competing for the available carbon and/or hydrogen.

A further special nutritional feature of some species of SRB is their ability to grow with reduced organic compounds that cannot be utilised in pure cultures of fermentative bacteria (Zehnder, 1988). These compounds include: propionate, butyrate, higher fatty acids or phenyl-substituted organic acids. These SRB can exploit the reduced products as energy sources by using an external electron acceptor.

Classification of sulphate reducing bacteria

According to Postgate (1979), the taxonomy of SRB is in an unsatisfactory state, having become confused in the 1920s to 1940s by the prevalence of impure cultures and the use of inappropriate culture media. Six genera of SRB exist (Table 2.1), namely: *Desulfovibrio, Desulfotomaculum, Desulfomonas, Desulfobacter, Desulfobulbus and Desulfosarcina* (Postgate, 1979; Cohen, 1993). The first two genera seem to be quite unrelated to each other, but the third genus *Desulfomonas*, is very like *Desulfovibrio*.

Desulfovibrio is usually easier to isolate and purify (Postgate, 1979). *Desulfovibrio* is mesophilic but can be halophilic and it does not form spores. Naturally occurring halophilic strains of *Desulfotomaculum* are not known, but they may be mesophilic or thermophilic (Postgate, 1979).

Table 2.1 List of known species of sulphate-reducing bacteria (adapted from Postgate, 1979; Cohen, 1993)

Desulfovibrio	*Desulfobacter*	*Desulfotomaculum*
desulfuricans	*postgatei*	*nigrificans*
vulgaris	*Desulfobulbus*	*orienitis*
salexigens	*propionicus*	*ruminis*
africanus	*Desulfosarcina*	*antarcticum*
gigas	*variabilis*	*acetoxidans*
baculatus	*Desulfomonas*	
sapovorans	*acetoxidans*	
haarsii		
thermophilus		

Several strains of oxygen-tolerant sulphate reducing bacteria are known (Cohen, 1993). Those that are resistant to high oxygen levels require hydrogen for growth. Other strains are seemingly facultative sulphate reducing bacteria: namely they may switch from heterotrophic aerobic growth to anaerobic sulphate reduction mode (Cohen, 1993). Another group of isolated sulphate reducing bacteria are oxygen sensitive when grown in axenic culture and can cope with oxygen only when grown in co-culture with thiobacilli (Cohen, 1993).

Sulphate-reducing bacteria are classified according to their oxygen sensitivity in the following manner (Cohen, 1993):

- Oxygen-sensitive strains, capable of functioning under oxygen only when grown in co-culture with oxygen scavenging bacteria such as thiobacilli;

- Oxygen-insensitive sulphate-reducing bacteria, capable of carrying out sulphate reduction activity under oxygen by elevated hydrogenase activity, which protects the oxygen-sensitive sites in the organism; and

- Facultative sulphate-reducing bacteria, carrying out aerobic respiration under aerobic conditions and shifting to sulphate reduction when exposed to anaerobic conditions. Among the metabolically related sulphur-reducing bacteria, certain types are facultatively aerobic or microaerobic. The archaeal lithoautotrophic *Acidianus* can even switch between sulphur reduction and aerobic sulphur oxidation.

Ecology of sulphate-reducing bacteria

Sulphate-reducing bacteria are widespread and active in locations that have been made anaerobic by microbial digestion of organic material and are present in almost all aquatic and terrestrial habitats (Prescott *et al.*, 1990). They thrive in habitats such as the muds and sediments of polluted lakes and streams, sewage lagoons and digesters, as well as in waterlogged soils. They have a remarkable capacity for survival in terrestrial and aquatic environments even though they grow relatively slowly compared with common soil or water organisms (Postgate, 1979). Whilst these bacteria are strictly anaerobic, they have been detected in many ostensibly aerobic regions (De Bruyn, 1992).

Growth and isolation of sulphate reducing bacteria

Most soils and waters contain SRB, but these are often outnumbered by other types of microbes (Postgate, 1979). Enrichment of the SRB population is usually necessary before isolation can be attempted. According to De Bruyn (1992), various media and modifications of these media are

available for the detection and isolation of SRB. Sodium lactate is normally used as carbon source while ferrous salt is used as an indicator of sulphide production. These media also contain redox poising agents and yeast extract. A pH between 7.2 and 7.6 is required for the growth of SRB, but the optimum temperature is SRB-species dependent (De Bruyn, 1992).

Due to the fact that SRB are strict anaerobes, handling and cultivation require techniques effectively to remove oxygen from both the medium and the gas phase in contact with the medium, as well as to lower the redox potential. A negative redox potential of -100mV (Eh) is recommended for successful growth of SRB (De Bruyn, 1992). Ascorbate, cysteine hydrochloride, dithiothreitol and titanium(III)citrate can be used as reducing agent (De Bruyn, 1992). Preparation of the reducing agents must be done anaerobically because they can react with oxygen to form toxic substances. Agar plates can be incubated in the conventional manner using an anaerobic cabinet with an anaerobic atmosphere such as argon or nitrogen (De Bruyn, 1992).

Substrate utilisation of SRB

Sulphate-reducing bacteria may be involved in fatty acid turnover either by direct metabolism of fatty acids, or indirectly because of their importance as H_2 -scavengers (Banat and Nedwell, 1982). Figure 2.3 illustrates the possible options for substrate utilisation and metabolite formation by SRB in the presence of exogenous sulphurous electron acceptors.

When separated solely on the basis of substrate utilisation, most species of the genera *Desulfovibrio*, *Desulfotomaculum* and *Desulfomonas* can be described as predominantly fermentative (De Bruyn, 1992). The remaining species of the fermentative genera, with the exception of *Desulfovibrio baarsii* and *Desulfotomaculum acetoxidans*, are lactate-utilising bacteria, which incompletely oxidise their substrates to acetate and hydrogen sulphide (De Bruyn, 1992). All incomplete substrate oxidations result in the formation of acetate. The only way that dissimilatory sulphate-reduction will be significant as a terminal oxidation process will be if acetate can be oxidised in the course of sulphate reduction (Joubert, 1987).

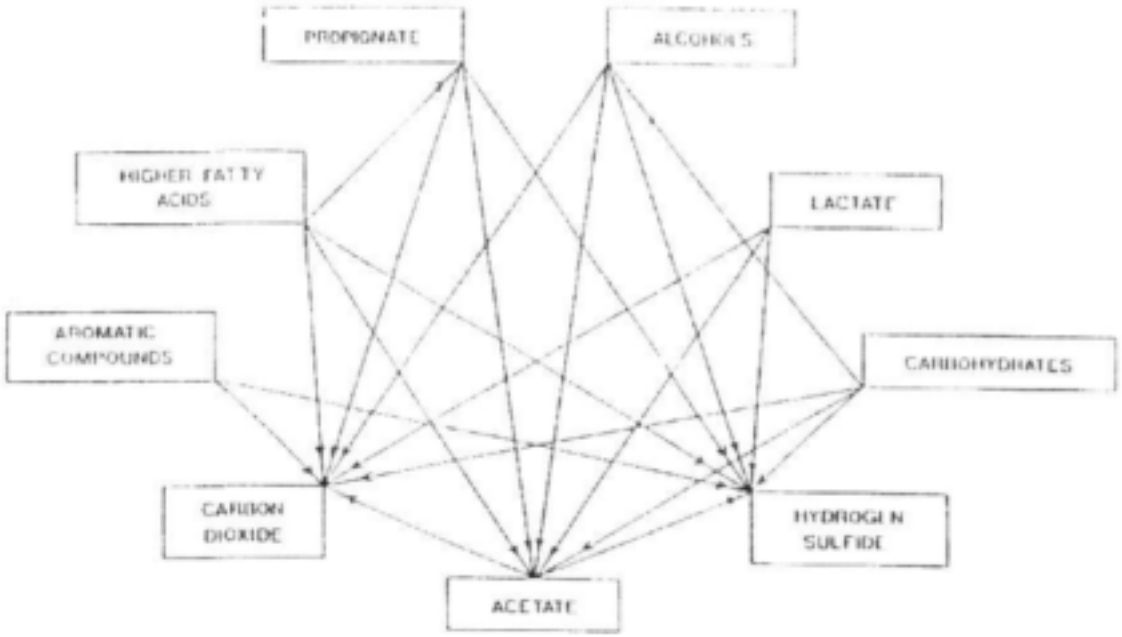

Figure 2.1 Metabolite formation by sulphate-reducers in the presence of exogenous sulphurous electron acceptors (Joubert, 1987).

Utilisation of acetate

The first real confirmation that the SRB were able to anaerobically oxidise acetate, was reported on the non-sporing sulphur reducing *Desulfomonas acetoxidans* species (Joubert, 1987). *Desulfobacter* species are nutritionally very specialised sulphate reducers and show the best growth on acetate (Zehnder, 1988).

The oxidation of acetate in *Desulfobacter postgatei* was shown to occur via the citric acid cycle (Zehnder, 1988):

$$CH_3COO^- + SO_4^{2-} \text{------>} 2HCO_3^- + HS^- \qquad \Delta g^{\circ\prime} = -47.6kJ$$

Laanbroek and Pfennig (1981) studied the oxidation of short-chain fatty acids by SRB, and concluded that, in freshwater and in marine sediments, acetate and propionate were oxidised completely with concomitant reduction of sulphate and that L-lactate was always fermented. It was also found that acetate-oxidizing SRB could only be isolated from marine sediments.

Visser *et al.* (1993), studied the anaerobic degradation of volatile fatty acids at different sulphate concentrations. It was found that at each sulphate concentration acetate was completely converted into methane and CO_2, and acetotrophic SRB were not detected. Some reports show a predominance of

SRB growing on acetate while a number of reports mention complete conversion of acetate by methanogens, even at high sulphate concentrations. During these studies it was evident that acetate is mainly consumed by methanogens, thus acetotrophic methanogens can very effectively compete with acetate-degrading SRB. Acetate degradation is independent of sulphate reduction (Qatibi *et al.*, 1990).

According to Ahring and Westermann (1987), acetate, energetically, is a poor substrate. This might result in the energy required for acetate uptake at very low concentrations exceeding the energy gained from acetate metabolism, thereby limiting the acetate uptake at a certain threshold concentration. They found that with a low concentration of acetate of less than 0.5 to 0.7mM, the degradation rate was concentration dependent, whereas the degradation was generally slow when the concentration was less than 0.15mM.

Utilisation of lactate

Lactate is a common intermediate during anaerobic degradation of complex organic matter and can be further degraded under anaerobic conditions via several pathways (Sorensen *et al.*, 1991). These include:

- formation of acetate, hydrogen and carbon dioxide, requiring a hydrogen-utilising methanogenic bacterium as hydrogen sink;
- formation of carbon dioxide or acetate by simultaneous reduction of oxidised sulphur compounds to carbon dioxide;
- formation of propionate, acetate and carbon dioxide;
- reduction of oxaloacetate to form acetate, formate and succinate; and
- fermentation to acetate, propionate and hydrogen, independent of the activity of hydrogen-utilizing bacteria.

Lactate has been used very successfully as an organic substrate for enrichment, isolation, and cultivation, or for determining cell numbers of *Desulfovibrio* and *Desulfotomaculum* species (Zehnder, 1988). Lactate is also oxidised by several completely oxidising sulphate reducers, while a number of incompletely fatty acid-oxidising sulphate reducers are unable to use lactate. The equations for incomplete and complete lactate oxidation are as follows (Zehnder, 1988):

$$2CH_3CHOH\,COO^- + SO_4^{2-} \text{ ---------> } 2CH_3COO^- + 2HCO_3^- + HS^- + H^+$$
$$\Delta G^0 = = -160.1kJ$$

$$2CH_3CHOH\,COO^- + 3SO_4^{2-} \text{ --------> } 6HCO_3^- + 3HS^- + H^+$$
$$\Delta G^0 = = -255.3kJ$$

In the presence of sulphate, lactate is generally accepted to support growth of the SRB (Joubert, 1987). Thermodynamic feasibility studies have shown that lactate will only sustain growth when it is incompletely oxidised to acetate according to the equation (Joubert, 1987):

$$2\ Lactate^- + SO_4^{2-} \text{ -----> } 2\ acetate^- + 2H_2O + 2CO_2 + S^{2-}$$

Oxidation of lactate, via pyruvate by SRB has also been demonstrated (Joubert, 1987). Apart from acetate and carbon dioxide as major products, small amounts of gaseous hydrogen may also be formed through the mediation of reversible hydrogenase.

In 1981, Laanbroek and Pfennig studied the anaerobic mineralisation of L-lactate in the presence and in the absence of sulphate in sediment samples. They found that lactate was always fermented and that lactate-oxidising, lactate fermenting bacteria and sulphate reducing bacteria, belonging to the species *Desulfovibrio desulfuricans*, were found in approximately equal amounts in the sediments. The fact that fermentation of L-lactate occurred in the presence as well as in the absence of added sulphate was rather unexpected, although fermentative bacteria were isolated in approximately the same numbers as the SRB from the highest dilution agar shake tubes. Hydrogen or formate might be more important electron donors for the reduction of sulphate by *Desulfovibrio* under natural conditions and lactate may not be the primary substrate for sulphate reduction (Laanbroek and Pfennig, 1987).

Qatibi *et al.* (1990) studied the effects of sulphate on anaerobic lactate degradation by a mixed microbial culture from an anaerobic fermenter fed with wine distillery waste water. They found that without sulphate, and with both sulphate and molybdate (inhibits sulphate reduction), lactate was rapidly consumed and propionate and acetate were produced; whereas with sulphate alone, only acetate accumulated. No sulphate was utilised in the presence of molybdate, which indicated that lactate degradation was largely due to fermentation. In the presence of sulphate, lactate degradation was accompanied by a concomitant consumption of the electron acceptor. It was also found that methane was not affected by the presence of sulphate, although less methane was produced in the presence of molybdate.

Utilisation of pyruvate

Even though pyruvate is a major intermediate during lactate metabolism, utilisation of this intermediate plays a central role in regulating the fermentation products of the SRB (Joubert, 1987). Oxidation of pyruvate, both in the presence and absence of sulphate, can be carried out by most fermentative SRB. Pyruvate is dismuted mainly to acetyl phosphate, carbon dioxide and hydrogen in the absence of sulphate (Joubert, 1987).

Utilisation of hydrogen and formate

According to Zehnder (1988), incompletely oxidising sulphate reducers using lactate are usually able to grow just as well with hydrogen as an electron donor and accordingly, *Desulfovibrio* strains isolated from natural sources with hydrogen were all able to grow on lactate. Many SRB that use hydrogen may grow with formate, but there are a few species that only use either hydrogen or formate. The first hint from nutrition physiology that SRB could conserve energy solely by electron transport phosphorylation was the utilisation of hydrogen by *Desulfovibrio* species (Zehnder, 1988). Several completely oxidising SRB that may grow autotrophically may use hydrogen but with relatively slow growth rates.

Utilisation of propionate, butyrate and higher fatty-acids

Next to acetate, propionate was shown to be quantitatively the most important product in the fermentation of organic materials by natural populations of bacteria (Widdel and Pfenning, 1982).

In the anaerobic conversion of organic matter to methane and carbon dioxide volatile fatty acids are important intermediates (Ahring and Westerman, 1987). Normally, propionate and butyrate account for 20 % of the total methane produced in a digester. One characteristic feature of *Desulfobulbus* species is the incomplete oxidation of propionate to acetate (Zhender, 1988):

$$4CH_3CH_2COO^- + 3SO_4^{2-} \text{-------} > 4CH_3COO^- + 4HCO_3^- + 3HS^- + H^+$$
$$\Delta G^{\circ} = = -150.6kJ$$

In 1993, Visser *et al.* found that at higher sulphate concentrations, oxidation of propionate by SRB became more important and only under sulphate-limiting conditions did syntrophic propionate oxidisers out-compete propionate degradation sulphate reducers. Remarkably, syntrophic butyrate oxidisers were able to compete with SRB for the available butyrate, even with an excess of sulphate.

Two groups of bacteria are involved in the degradation of propionate, butyrate and longer-chain fatty acids (Ahring and Westermann, 1987). These are the obligatory hydrogen-producing acetogenic

bacteria oxidising the acids, and the methane-producing bacteria utilising the acetate and hydrogen produced.

According to Zhender (1988), the *Desulfobulbus* species isolated so far are a physiologically homogeneous group, whose growth with lactate may be somewhat faster than with propionate, but only the latter allows selective enrichment.

Propionate is also removed under anaerobic conditions in sludge from a sulphide removing reactor (Widdel and Pfennig, 1982). No fresh water sulphur-reducing bacteria are known which are able to use propionate (Widdel and Pfennig, 1982). Therefore, it may be possible that SRB such as *Desulfobulbus propionicus* convert propionate to acetate. According to Zehnder (1988), incomplete oxidation of propionate to acetate is a characteristic feature of *Desulfobulbus* species. Nearly all *Desulfobulbus* isolates described use ethanol and propanol, whilst a few strains slowly degrade butyrate or 2-methyl-butyrate to acetate. Methanogenic and sulphate-reducing conditions lead to at least 13 possible pathways for conversion of propionate (Speece, 1996). Speece (1996) concluded that since propionate and butyrate were barely detectable in effluents at steady-state, their oxidation under high influent sulphate conditions may be completely or incompletely mediated by the fatty acid-utilising SRB. It was also shown that the presence of SRB enhanced the degradation of propionate either through direct utilisation or through interspecies H_2 transfer.

Stams *et al.* (1984) studied the pathway of propionate formation in *Desulfobulbus propionicus*. The most remarkable characteristic was the ability to form as well as to degrade propionate. In the presence of sulphate, lactate, pyruvate, ethanol, propanol and propionate are oxidised to acetate with a concomitant reduction of sulphate to sulphide. In the absence of an external electron acceptor, lactate and pyruvate are fermented to propionate and acetate.

Studies conducted by Widdel and Pfennig in 1982, showed that propionate can be oxidised by SRB without dependence on synthrophic bacteria. The new type of oval to lemon shaped SRB was able to completely oxidise propionate under anaerobic conditions when grown together with acetate-oxidising species. Such commensalism was observed in marine enrichments with propionate where, in addition to the small oval propionate-utilising SRB, larger cells of the *Desulfobacter* type were also observed.

In 1983, Banat and Nedwell studied the mechanisms of turnover of C_2-C_4 fatty acids in high-sulphate and low-sulphate anaerobic sediments. They found that the anaerobic oxidation of propionate and butyrate could be due to two possible mechanisms: fatty acids can be oxidised by proton reducing bacteria in association with a H_2-scavenger, or SRB may be capable of direct metabolism and oxidation of propionate and butyrate in a high-sulphate sediment. The addition of 20mM molybdate to a slurry of

salt marsh sediments almost entirely eliminated propionate and butyrate turnover. This suggested that the SRB were involved in their metabolism and indeed were entirely responsible for their oxidation. Molybdate did not have any apparent effect on fatty acid turnover in freshwater sediments. The presence of a H_2 atmosphere had no effect on propionate or butyrate turnover in the high sulphate salt marsh sediments, suggesting that their oxidation in these slurries did not involve proton-reduction mechanisms, or inter-species H_2 transfer, which would be inhibited by a high H_2 concentration. The metabolism of propionate and butyrate metabolism must have been due to the SRB which directly metabolise fatty acids and whose metabolism would not be inhibited by hydrogen. Butyrate turnover was clearly inhibited by the H_2 atmosphere in freshwater sediments. Although propionate turnover was extremely slow, the H_2 atmosphere seemed to further inhibit its turnover. In low sulphate freshwater sediment, it was suggested that, propionate and butyrate oxidation involves proton-reducing fatty acid oxidising bacteria linked to H_2-scavenging bacteria, probably methanogens. In high-sulphate sediments, it seemed that direct oxidation of fatty acids by SRB predominates and fatty acid oxidation involving interspecies H_2 transfer is of minor importance.

2.5.2 *Competition between sulphate-reducing bacteria and methanogens*

Both sulphate-reducers and methanogens (MPB) live under strict anaerobic conditions with similar pH and temperature ranges (Bitton, 1994). Some SRB are able to oxidise H_2 like methanogens and thus may compete with methanogens for these substrates (Figure 2.4) (Bitton, 1994). Sulphate-reducing bacteria are normally dominant in natural ecosystems such as freshwater and marine sediments and also in anaerobic digesters where methanogenesis was found to be inhibited by the presence of sulphate (Isa *et al.*, 1986). The actual description of the MPB/SRB competition within a reactor is complex.

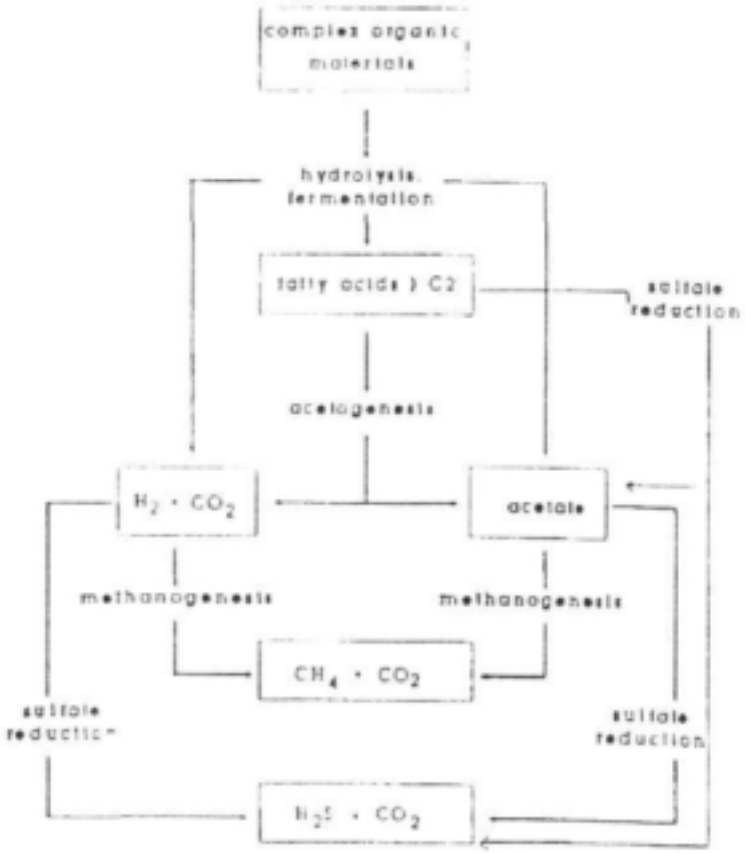

Figure 2.2 Substrate competition between sulphate-reducing and methanogenic or acetogenic bacteria (Bitton, 1994).

Thus, the main factors influencing MPB/SRB competition are:

- sulphate concentration in feed
- maximum specific utilising rate (k_{max})
- half velocity constant (k_s)
- free energy of the reaction
- nutrient availability
- adhesion properties
- proximity of cells (biofilms vs. dispersed cells)
- temperature
- substrate type
- long term shifts (Speece, 1996).

Studies conducted by Orem and Pocin (1982) showed that sulphate ions did not inhibit methanogenesis in estuarine sediments supplemented with methanol, trimethylamine, or methionine. When hydrogen or acetate was the substrate, sulphate greatly retarded methanogenesis. Acetate, hydrogen, and acetate plus hydrogen, stimulated sulphate reduction, but not methanol or trimethylamine. It was thus indicated that SRB will out compete methanogens for hydrogen, acetate, or both, but will not compete with methanogens for compounds such as methanol, trimethylamine, or methionine, thereby allowing methanogenesis and sulphate reduction to operate simultaneously within anoxic sediments containing sulphate.

Kinetic studies have shown that sulphate reducers generally have higher maximum growth rates and higher affinity for substrates (i.e. lower half-saturation constants, K_s) than methanogens (Bitton, 1994). The half-saturation constant for hydrogen is 6.6 μM for methanogens, as compared with 1.3 μM for sulphate reducers. Similarly, the K_s values for acetate are 0.07mM and 0.2 mM for methanogens and sulphate reducers, respectively (Bitton, 1994). Thus, sulphate reducers may predominate over methanogens, providing that the sulphate supply is not limiting. Despite their kinetic advantages, SRB rarely predominate in anaerobic wastewater treatment. SRB have a higher affinity for acetate (K_s = 9.5 mg/ℓ) than methanogens (K_s = 32.8 mg/ℓ) (Bitton, 1994). Thus, under low acetate concentrations, SRB will out-compete methanogens. This competitive inhibition results in the shunting of electrons from methane generation to sulphate reduction. SRB and MPB are very competitive at COD/SO_4 ratios of 1.7-2.7 (Bitton, 1994). A decrease in this ratio is favourable to SRB, whereas an increase is favourable to MPB. Little is known about the competition between acetogenic bacteria (AB) and SRB for propionate and butyrate in anaerobic digesters (Visser *et al.*, 1993). For waste-water with an excess of sulphate it is assumed that SRB will out-compete MPB because of their better kinetic growth properties.

Accordingly to Speech (1996), the following has been published about acetate and SRB/MPB competition:

- Acetate is a favoured substrate for MPB
- MPB predominant over SRB for acetate
- MPB generally solely present in low alkalinity anaerobic reactors
- MPB able to form a bio-film faster than SRB at higher acetate concentrations
- MPB primary acetate converter at high acetate/sulphate ratios
- MPB out-competing at higher acetate concentrations over several months, but SRB predominant with low acetate concentrations in biofilms during the same period
- MPB using 60% COD and SRB 40% for acetate
- MPB using 93-97% of acetate substrate at COD/sulphate ratios of 1-50

- MPB acetate utilising clearly less with increasing sulphate concentrations.

In anaerobic microbial systems acetate would be completely oxidised to carbon dioxide rather than to methane and carbon dioxide in the presence of sulphate even if acetate proves to be the major energy source for the bacteria that use it in methane production (Bryant *et al.*, 1977).

3. THE AVAILABILITY OF ALTERNATIVE CARBON SOURCES FOR USE IN PASSIVE TREATMENT SYSTEMS IN SOUTH AFRICA

3.1 Definition of a passive treatment system

A passive treatment system is defined as a system that requires very low or no levels of chemical input, with low maintenance and operational requirements. With regard to a passive treatment system for the treatment of mine water, where the treatment objectives are the removal of sulphate and heavy metals and the neutralisation of the water, the following considerations apply.

Sulphur transformation in the treatment system is microbially mediated, and the bacteria responsible for sulphur transformations require a carbon source. Hence a passive system required for sulphate reduction should:

(i) contain, or generate intrinsically, a substrate which contains carbon;
(ii) release carbon in a form that is assimilable by SRB;
(iii) release such carbon in the quantities required to meet the influent sulphate load; and
(iv) if extrinsically generated, the quantity of carbon included in the system should be sufficient to reduce the entire design sulphate load over the design lifetime of the project.

3.2 Intrinsically generated carbon

Many wetlands function as effective S sinks (Faulkner & Richardson, 1989). A bog in Massachusetts retained 77% of the atmospheric SO_4^{2-} input and a northern Minnesota peatland sequestered 56% of the annual SO_4^{2-} input (Urban *et al.*, in press). Utilising the root zone process, Winter and Kikuth, 1985) reported 87% of total S removal from wastewater effluent, while annual sulphate retention by a fern ranged from 22-77% over a four-year period. (Figure 4.2, closed circles; Bayley *et al.*, 1986).

Changes in the hydrological regime decreased S retention efficiency with the wetland becoming a source rather than a sink. Bayley *et al.*, (1986) concluded that aerobic soil conditions during dry periods oxidised reduced S to SO_4^{2-}, which was flushed from the system during rainfall events. Similar oxidation effects on SO_4^{2-} exports were reported by Wieder (1985).

The preceding discussion on S retention did not address the issues of gaseous losses, which were not cited in any of the studies. Wetlands may also function as transformers of S as opposed to true sinks if significant fluxes of S to the atmosphere occur. Quantifying gaseous emissions is difficult, but Castro and Dierberg (1987) calculated a mean H_2S release rate of 80 mg/m^2/year for some freshwater marshes in Florida, which was comparable to a value of 60 mg/m^2/year reported by Aneja *et al.* (as cited by

Castro and Dierbergh, 1987).

There is increasing evidence that H_2S may not be the primary biogenic sulphide gas; the organic gases methyl sulphide (MS) and dimethyl sulphide (DMS) can be of equal or greater importance (Krouse *et al.*, 1979). The organic sulphide concentrations were two orders of magnitude higher than H_2S in gaseous sulphur emissions from an anaerobic sewage lagoon (Rasmussen, 1974). The average DMS emission rate from some Canadian wetlands was 81 mg/m^2/yr with a range of 25-184 mg/m^2/yr (Nriagu *et al.*, 1987).

Hedin *et al.* (1989) suggested that, with proper design modifications, increased rates of net sulphide formation appeared possible for constructed wetlands that receive acid rock drainage, to immobilise iron. They based their statement on a hypothetical wetland receiving a flow of 50 ℓ/min receiving an annual load of 2.628 kg Fe, 26.280 kg SO_4^{2-}, and 13.140 kg of acidity ($CaCO_3$ equivalent).

In this hypothetical system the complete removal of all the dissolved iron by pyrite formation required the reduction of 9 010 kg of sulphate and oxidation of 2 253 kg of carbon.

$$Fe^{2+} + 4C + 2SO_4^{2-} \rightarrow FeS_2 + 4\,CO_2$$
$$2{,}628\text{ kg} + 2{,}253\text{ kg} + 9{,}010\text{ kg} \rightarrow 4{,}828\text{ kg}$$

The hypothetical wetland was constructed as a compost wetland with a substrate of mushroom compost containing initially 238,500 kg of carbon with a surface area/flow ratio of 50 m^2/(ℓ/min. Based on these criteria Hedin *et al.* (1989) suggested that the carbon in the original substrate represented 100 times the annual requirement for the precipitation of the iron, and the input of new organic carbon via net primary production would almost meet the dissimilatory sulphate reduction needs. The latter assumption was based on the average net primary production for temperate wetlands of 1,000 g carbon/m^2/year (Whittaker and Likens, 1973), with 75% being available for sulphate-reducing bacteria (Howarth et al., 1983).

One of the most important factors affecting the rate of SO_4^{2-} decomposition is the amount of organic matter available to the micro-organisms (Ramm and Bella, 1974; Nedwell and Abram, 1979). However the reactivity of the organic matter is even more important as a rate-controlling factor (Westrich and Berner, 1984). Natural organic polymers have vastly different susceptibilities to bacterial attack (enzymatic hydrolysis), and this variation can express itself in terms of different rates of sulphate reduction (Westrich and Berner, 1984). Organic matter from vascular plants is considered to be difficult to decompose because of high concentrations of biologically resistant compounds such as lignins, waxes, and resins (Goldhaber and Kaplan, 1975; Fenchel and Blackburn, 1979), compared with organic

matter of marine origin (Westrich and Berner, 1984). Although the decomposition rates of the above ground portion of some wetland plants are known (Table 3.1), very little is known or has been reported for the decomposition rates of roots or peat and other organic soil components (Kadlec, 1989).

Table 3.1 Half lives of various types of wetland biomass (Modified from Kadlec, 1989)

Type	Half-Life	Species or Subspecies	Investigators
Plants			
Phragmites	220 h to 60 d	3	4
Scirpus	260 h to 80 d	3	3
Typha	400 h to 250 d	3	5
Spartina	430 h to 280 d	2	3
Juncus	460 h to 90 d	3	5
Carex	610 h to 320 d	5	6
Misc. bog plants	850 h to 600 d	10	3
Roots			
Carex	950 d	1	21
Calluna	14 y	1	1
Eriophorum	70 y	?	1
Peat			
Highly decomposed - Oxidising	10 y	1	1
Slightly decomposed – Reducing	1700 y	1	1

Bio-polymeric materials (lignin, cellulose, and protein) have been shown to have different reactivities with regard to bacterial decomposition. For example, Crawford *et al.*, (1977) showed that lignin degrades 4-10 times more slowly than cellulose, when differentially ^{14}C - labelled lignocellulose is decomposed.

A model to determine the role of sedimentary organic matter in bacterial sulphate reduction for marine plankton has been developed and tested (Westrich and Berner, 1984). This model demonstrated that the simulated rate of sulphate reduction was in direct proportion to the amount of planktonic carbon added, and that the rate of sulphate reduction was controlled by the sequential utilisation of two types of organic matter, with decay constants equal to 7.2 d yr^{-1} and 1.0 d yr^{-1}. These decay constants should be used in conjunction with the exponential rate law (Sharp, 1987):

$$N = N_o e^{-lt}$$

where: N_o is the initial mass at time zero
N is mass remaining after time t
l is the decay constant
t is time

and imply a 99.9% and 63.2% loss per year, respectively, for the two types of organic matter present.

Similar decay constants have not been calculated/modelled for passive mine drainage treatment systems as current regulations controlling the discharge quality of mine drainage (in the USA, where most of the development of passive treatment systems has occurred), do not require the removal of sulphates, with the focus being the removal of metals, and neutralisation.

3.3 Incorporation of organic rich substrates into passive treatment systems

An alternative or complimentary approach to using wetlands is the incorporation of a carbon rich material in the reactor. Nearly all carbon is derived, either directly or indirectly, from plant material during the conversion of gaseous CO_2 to higher carbon compounds in photosynthesis. Thus potential carbon sources are various forms of vegetation including grasses, timber, or waste products derived from processing vegetation. The following is a list of materials, which could be considered as suitable for incorporation into a passive treatment system:

- Grasses, including hay and by-products such as mushroom compost, bagasse from the processing of sugar cane and manure (processed by livestock).

- Timber, including sawdust and wood chips as well as fibre recovered from waste streams as a result of the pulping process and from board mills.

The costs and indication of general availability of the above potential sources of carbon are presented in Table 3.2. Excluded from these costs is the cost of transport. Depending on the distance between source and the locality of the treatment system, it is conceivable that the landed cost of carbon could be considerably higher than that indicated.

Table 3.2 Summary of the availability and price of potential compounds containing carbon suitable for consideration in passive treatment systems. (Sources: SA Feedlot Association, SA Sugar Association, Triomf Fertiliser Ltd, and van Bredow, in lit.)

Type	Yield	Cost	Comments on availability
Hay	3-20 t/ha	R 14/ 28kg bale R 450/ton	Readily available at cost, other uses compete with proposed use
Cattle manure	?	Approximately R 20/ton	Cattle manure currently generally disposed of to land or composted
Pig manure	?	?	Readily available
Chicken manure	?	?	Some used in animal feeds
Horse manure	?	R 147.00/ton	Used for mushroom growing
Sugar cane	45-80 tons per season	R 96/ton	not generally traded
By products from sugar milling	?	?	not generally available used as a source of fuel, board, animal feed, soil conditioner and single cell protein.
Mushroom compost		R 114/m^3	
Wood & Wood Products	?	?	Not generally available, used as fuel, board or paper manufacture or exported.
Sawdust Wood chips.	?	R 60-R80/ton	Not always available
Sewage Sludge	?	?	Quantity and availability will vary depending on locality

? Yields and costs could not be determined to a sufficient degree of certainty

Each one of these carbon sources will have a different decomposition rate under the reducing conditions that prevail in the sulphate removal reactor of a passive mine water treatment system. Considering the availability of carbon, it will be possible to combine a number of different carbon sources, based on the differential rates of digestion, so as to prolong the active life of the sulphate removal reactor. However, with the total life-span of the systems being envisaged in the order of 30 years and longer, the digested substrate will ultimately have to be replenished.

The time interval between replenishments of the carbon source mix will in all likelihood be dependent on the sustainability of the carbon source for the medium-term performance of the reactor. The short-term performance will be governed by carbon sources with a high release rate of carbon within a short period of time. These carbon sources need to be readily fermentable as they have to be transformed into fatty acids, alcohol and hydrogen for uptake by the SRB. Such carbon sources could include pre-fermented plant matter such as silage or sugar- rich carbon sources such as bagasse or molasses.

It is envisaged that long-term carbon sources such as hay or wood have a limited function in the supplementation of carbon into the biological process but, more importantly, a stabilising function in the packaging of the carbon source fill in the reactor. Due to the limited biodegradability of certain

components in hay and wood, such as hemi-cellulose, the majority of those materials added to the reactor can be considered biologically inert.

Therefore, carbon sources added for medium-term performance have to fulfil the requirement that they release a suitable amount of carbon over a prolonged period of perhaps up to 9 years to sustain the sulphate removal rate required from the reactor. The short-term carbon sources are likely to support sulphate reduction only for a limited period of time of about 9 to 12 months, while the long-term carbon sources will support sulphate reduction only marginally.

3.4 Transport costs

The cost of transport for solid and semi-liquid waste materials are in the order of R 40 – 80 per ton of material within a 50 km radius and in the order of R 60 - 120 per ton within a 100 km radius. This price includes loading, unloading and transport but not the placing of the material in the treatment systems. In most cases the materials will have to be mixed, layered or handled in some form, which is an additional cost item to be included in the construction costs of the system.

3.5 Total costs of carbon sources

The cost of carbon for passive treatment systems varies considerably. The total cost of carbon in the case of cow manure is in the order of R 80 (R 20 cost + R 60 for transport) per ton, while in the case of hay the costs are in the order of R 500 per ton.

4. CONCEPTUAL MODEL OF THE MICROBIOLOGY OF SULPHATE-REDUCING UNITS (SRUs) IN PASSIVE TREATMENT SYSTEMS

4.1 Model types

A model may be defined simply as a purposeful representation or description (often simplified) of a system of interest. In terms of this definition, models are widely used in Science and Engineering. For example, microbiologists and sociologists study model organisms (e.g. *E. coli*) and model communities, engineers apply models in the design of a diverse variety of systems (e.g. wastewater treatment plants). Models can be broadly categorized as (1) physical, (2) conceptual, and (3) mathematical (Simeonov *et al., 1996*).

A physical model is a spatially scaled representation of a system. For example, the laboratory- and pilot-scale experiments used by scientists and engineers to investigate system response and behaviour are physical models.

A conceptual model is, as its name suggests, a conceptualisation of the system under consideration. The conceptual model should describe input parameters, the internal system processes which change the inputs, and the output parameters. For example, for a biological system, a conceptual model should describe (1) all incoming compounds and organisms to the system, (2) the organisms that exist in the system, (3) their aeration/oxidation in term of the compounds of interest and (4) the types of product produced by the biological behaviour of the system.

More specifically, the model should describe the major biological and biochemical interactions in the system, particularly those interactions between organisms in competition for substrate and the effects of compounds (end-products) on organism behaviour. A conceptual model can be considered as a qualitative description of a system.

A mathematical model can be considered as a quantitative description of a system. Mathematical models formulate mathematical descriptions of process rates and present stoichiometric alterations to describe changes in compounds.

Mathematical models have proved to be most useful for the design and operation of biological wastewater treatment systems (Simeonov *et al.*, 1996). By providing quantitative descriptions, they allow predictions of the system response and performance to be made. From the predictions, design and operating criteria can be identified for optimisation of system performance. Also, mathematical models can serve as very powerful research tools. By evaluating model predictions, it is possible to test hypotheses on the behaviour of the wastewater treatment system (e.g. biological processes, their

response to the system constraints, etc) in a consistent and integrated fashion. This may direct attention to issues not immediately obvious from the physical system, and lead to a deeper understanding of the fundamental behavioural patterns controlling the system's response. In essence, mathematical models can provide a defined framework to direct thinking (design, operation or research).

Mathematical models are usually of one or two types steady-state, or dynamic. Steady-state models have constant flows and loads and predict the average (constant) response of a system to a specified (single) set of inputs. In contrast, dynamic models are quite complex and predict the time-dependent response of a system to a set of time-varying input.

To develop a conceptual model for a biological system, a number of tasks need to be completed, including:

- Identification of the objectives of the model;
- Description of the conditions (or limits) within which the model should operate;
- Identification of the major compounds utilized and formed;
- Identification of the processes that produce these compounds and act on the compounds that are reduced or changed by the process; and
- Conceptual representation of the mechanisms that describe the kinetic and stoichiometric behaviour of the physical characteristics (e.g. temperature, pressure, partial gas pressures, pH, etc.) existing in the process and the compounds present.

4.2 Objectives

The objective of this chapter is not necessarily to present a complete conceptual model for a passive treatment system treating acid mine drainage, but rather to highlight those aspects that should be taken into account in conceptualising the biological component of the system (namely, the sulphate-reducing units).

4.3 Description of the system

The system under consideration is the sulphate reducing unit (SRU) for passively treating acid mine drainage (ACID). The system concept is that sulphate-rich water with principally low pH comes into contact with a sulphate-reducing bacterial system (a bio-film attached to a support material containing amongst other micro-organisms a certain percentage of sulphate-reducing bacteria that require a biodegradable organic substrate as electron donors). The biodegradable substrate under consideration would be any biodegradable organic matter that could be degraded by the microbial community, the electrons used for reduction of sulphate (SO_4^{2-}) to hydrogen sulphide (H_2S) and the organic material

(i.e. catabolic processes, carbon, hydrogen, oxygen, and nitrogen) used for construction of cell mass, i.e. anabolic processes.

It is stipulated that the system is essentially anaerobic and has a volume sufficiently large in relation to the flow rate of the AMD passing through the treatment unit to provide a hydraulic residence time (HRT) which is longer than the average growth rate of the bio-film (doubling time) to avoid loss of the micro-organisms by washout.

4.4 Organism groups present

Under anaerobic conditions, with organic material present, a number of organism groups could be present. The extent to which these are present will depend on a number of factors including their ability to withstand the environmental conditions present, the ability to metabolise the substrate, and the ability to withstand the presence of compounds produced as by-products by other organisms in the environment. The anaerobic system is conceptualised as containing the following major organism groups.

4.4.1 Acidogenics

Acidogenic bacteria conduct two major reactions in processes referred to as fermentation:

(1) the solubilisation of large molecules such as carbohydrates, proteins and lipids to sugars, amino acids and long chain fatty acids respectively; and

(2) the products described above are fermented to short-chain fatty acids (e.g. acetic, propionic and butyric acids), carbon dioxide and hydrogen gas.

In the conceptual model, it is assumed that in the first step described above, the major substrate available is carbohydrate and that the major end-product is glucose, together with some hydrogen, carbon dioxide, and ammonia. In the second step described above, it is assumed that the major end-product is acetic acid mainly dissolved in the water phase, and additionally, carbon dioxide and hydrogen, partially present in the water phase and partially present in the gas phase within the reactor.

The balanced equations describing the anabolic and catabolic reactions of the second step reaction of the acidogens are as follow:

Catabolic: $C_6H_{12}O_6$ (glucose) → $2\,CH_3COOH$ (acetic acid) + $2CO_2$ + $2H_2$
Anabolic: $C_6H_{12}O_6 + 6/5NH_3 \rightarrow 6/5C_5H_7O_2N + 18/5H_2O$

The acidogenic are fast-growing, with minimum doubling times estimated at 3.3 h, with a specific yield on glucose as substrate of 0.10 mg VSS/mg glucose utilized (Toetemeya et al.,1982). The acidogenic bacteria have a pH tolerance down to pH3.5 but in the lower pH region, product formation changes from acetic acid to ethanol and butanol.

A second factor affecting the type of end product produced is the potential pressure of hydrogen pH_2 in the system, which is a consequence of the type of initial substrate hydrolysed. Under high pH_2 ($pH_2 > 10^{-3.7}$ atm) conditions, acetic, butyric and proppionic acids are produced, but under low pH_2 conditions ($pH_2 < 10^{-3.7}$ atm), only acetic and butyric acids are produced. Butyric acid is not taken into account in conceptualising the system because it is not found in significant quantities in anaerobic systems. Yields changing from 0.03 to 0.14 mg COD/mg COD substrate have been measured and a value of 0.1mg COD has been accepted as typical.

4.4.2 Acetogenic bacteria

The acetogenic bacteria are heterotrophic and convert organic acids such as propionate and butyrate to acetate, but mediate these fractions only at low pH_2. However, as mentioned above, the system is conceptualised as having a high pH_2 and as such it is assumed that the acetogenic bacteria do not have a role in the system.

4.4.3 Hydrogenotrophic methanogens

The hydrogenotrophic methanogens are obligate anaerobes using hydrogen gas as electron donor and CO_2 as electron acceptor and carbon source in the production of methane. The catabolic reaction is as follows:

$$4H_2 + CO_2 \Longrightarrow CH_4 + 2H_2O$$

The anabolic reaction is as given for the acidogenic bacteria. These organisms perform optimally down to pH values of 5.4 and have rapid doubling times of about 6 hours under these conditions. The hydrogenotrophic methanogens compete with the hydrogentrophic sulphidogens (described in Section 4.4.6) for the hydrogen gas. Yield values between 0.03 and 0.043 mg COD/mg COD have been unassured and a value of 0.041 has been accepted as typical.

4.4.4 Acetoclastic methanogens

The acetoclastic methanogens are obligate anaerobes, and convert acetic acid to methane and CO_2 as

shown in the catabolic reaction below:

$$CH_3COOH \Longrightarrow CH_4 + CO_2$$

They grow slowly, with minimum doubling times of 2 to 3 days. As a consequence of their utilization of acetic acid they have an influence on the system's pH. They compete with the heterotrophic sulphidogens for acetate as electron donor and are adversely affected by pH values below about 5.4. Yield values of between 0.03 and 0.04 mgCOD/mgCOD have been measured and a value of 0.04 is accepted as typical.

4.4.5 Heterotrophic sulphidogens

Because the system under consideration is conceptualised as producing acetic acid as the major product of acidogenesis, the only acid product under consideration for use by the sulphidogens as electron source for the reduction of sulphate is acetate. The catabolic aeration can be considered as follows:

$$CH_3COOH + SO_4^{2-} \Longrightarrow H_2S + 2CO_2 + 2OH^-$$

It should be noted that 100 mg of alkalinity as $CaCO_3$ is generated per mole of sulphate reduced.

Sulphate reduction with acetate as electron donor and carbon source has been observed in anaerobic digestion of waters of intermediate salinity (TDS values between 2,000 and 10,000 mg/ℓ). For this conceptualisation of the system it is assumed that these bacteria are present, but that their sulphate turn-over in the low salinity water (TDS values between 1,000 and 2,000 mg/ℓ) is out-competed by the hydrogenotrophic sulphidogens in competition for sulphate as the electron acceptor under low sulphate concentrations. With high sulphate concentrations the main competition for acetate as substrate are the acetoclastic methanogens. Yield values of about 0.2 gCOD/gCOD are accepted for the heterotrophic acidogens.

4.4.6 Hydrogenotrophic sulphidogens

The hydrogenotrophic sulphidogens are obligate anaerobes, which use hydrogen as their energy source and organic carbon as the carbon source. They are pH mesophiles and depend on a stable pH balance in the system (which is partly dependant on the activity of the SRBs themselves). The SRB will be adversely affected if the alkalinity production in the system is insufficient to maintain a pH around 7 by counter-balancing the increase of the acid from the incoming acid mine drainage.

The balanced equations describing the catabolic and anabolic reactions of the hydrogenotrophic

sulphidogens are as follows:

Catabolic: $SO_4^{2-} + 4H_2 \Longrightarrow H_2S + 2OH^-$

Anabolic: $10H_2 + 5CO_2 + NH_3 \Longrightarrow C_5H_7O_2N + 8H_2O$

A yield for this group of organisms of 0.275 gVSS/gCOD has been accepted. The competitors to the hydrogenotrophic sulphidogens are the hydrogenotrophic methanogens, which compete for the dissolved molecular hydrogen, and the acetoclastic sulphidogens, which compete for the sulphate. Obviously the last competition is beneficial in that the acetoclastic sulphidogens contribute to the removal of sulphate and acetate (COD).

The features of these different bacterial groups have been summarised in Table 4.1.

It is unclear which of the two pathways described above will consume less organic substrate. The amount of substrate required for the production of molecular hydrogen from organic material by the hydrogenotrophs is more or less the amount of substrate required to produce acetate from the organic matter by the acetoclastics. It would be desirable for the dominant organism present in the system to be the one that would require the least substrate per mole of sulphate reduced.

Table 4.1 Summary of bacterial physiological groups potentially present in passive mine water treatment systems.

Organism Group	Metabolic oxidation/reduction reactions		Yield	
			(Gvss/COD)	(moles vss)/ (moles sub)
Heterotrophic Acidogens: (glucose ==> Acetate)	Catabolic:	$0.82\,C_6H_{12}O_6 + 3.28H_2O \Longrightarrow 0.82CH_3COOH + 3.28CO_2 + 6.56H_2$	0.13	0.21
	Anabolic:	$0.18\,C_6H_{12}O_6 + 0.21NH_3 \Longrightarrow 0.21C_5H_7O_2N + 0.63\,H_2O$		
Hydrogenotrophic Methanogens	Catabolic:	$0.958H_2 + 0.240\,CO_2 \Longrightarrow 0.240CH_4 + 0.479\,H_2O$	0.03	0.0042
	Anabolic:	$0.42H_2 + 0.021\,CO_2 + 0.0042NH_3 \Longrightarrow 0.0042\,C_5H_7O_2N + 0.0336H_2O$		
Acetoclastics Methanogens	Catabolic:	$0.942CH_3COOH \Longrightarrow 0.942\,CH_4 + 0.942\,CO_2$	0.04	0.023
	Anabolic:	$0.058CH_3COOH + 0.023\,NH_3 \Longrightarrow 0.023C_5H_7O_2N + 0.069\,H_2O$		
Hydrogenotrophic Sulphidogens	Catabolic:	$0.61H_2 + 0.153\,SO4^{2-} \Longrightarrow 0.153\,H_2S + 0.305\,H_2O + 0.305OH$	0.275	0.039
	Anabolic:	$0.39H_2 + 0.195\,CO_2 + 0.039\,NH_3 \Longrightarrow 0.039\,C_5H_7O_2N + 0.312\,H_2O$		
Heterotrophic Sulphidogens	Catabolic:	$0.717\,CH_3COOH + 0.717\,SO_4 \Longrightarrow 0.717\,H_2S + 1.434\,HCO_3^-$	0.200	0.113
	Anabolic:	$0.283\,CH_3COOH + 0.113\,NH_3 \Longrightarrow 0.113\,C_5H_7O_2N + 0.339\,H_2O$		

5 DEVELOPMENT OF A SIMPLE LABORATORY TEST TO DETERMINE THE SUITABILITY OF DIFFERENT CARBON SOURCES FOR SULPHATE REMOVAL FROM MINE WATER IN PASSIVE TREATMENT SYSTEMS

5.1 Introduction

The study of anaerobic bacteria has intensified tremendously over the past years. These micro-organisms, in addition to being of clinical interest, have considerable ecological importance as well as potential use in industrial processes. However, experimentation has been hampered by the lack of inexpensive, readily available equipment.

Currently a variety of techniques and equipment are used for anaerobic studies. These include custom-made bioreactors, anaerobic cabinets, Gaspack systems (Figure 5.1) and a variety of other devices. Professionally manufactured anaerobic bioreactors are prohibitively expensive and also expensive to operate. As the cultivation of sulphate-reducing bacteria (SRB) causes anaerobic corrosion of steel, even high-quality stainless steel, reactors for the cultivation of SRB are best manufactured from glass or polyethylene based materials.

Sulphate-reducing bacteria are strictly anaerobic micro-organisms and all oxygen must be excluded from the culture system (Prescott *et al.*, 1990). This can be accomplished by:

- Using special anaerobic media containing reducing agents such as thioglycollate or cysteine. The reducing agents will eliminate any dissolved oxygen present within the medium so that anaerobes can grow beneath the medium surface.

- Oxygen may also be eliminated be removing air with a vacuum pump and flushing out residual oxygen with nitrogen gas. Often CO_2 as well as nitrogen is added to the chamber since many anaerobes require a small amount of CO_2 for best growth (see Figure 5.2, Prescott *et al.*, 1990).

- Co-cultivation with facultative anaerobic micro-organisms, which will replenish the oxygen present in the reactor by oxidation of biodegradable organic matter causing a decrease of the redox potential in the reactor (only applicable for the cultivation of mixed anaerobic micro-organism cultures).

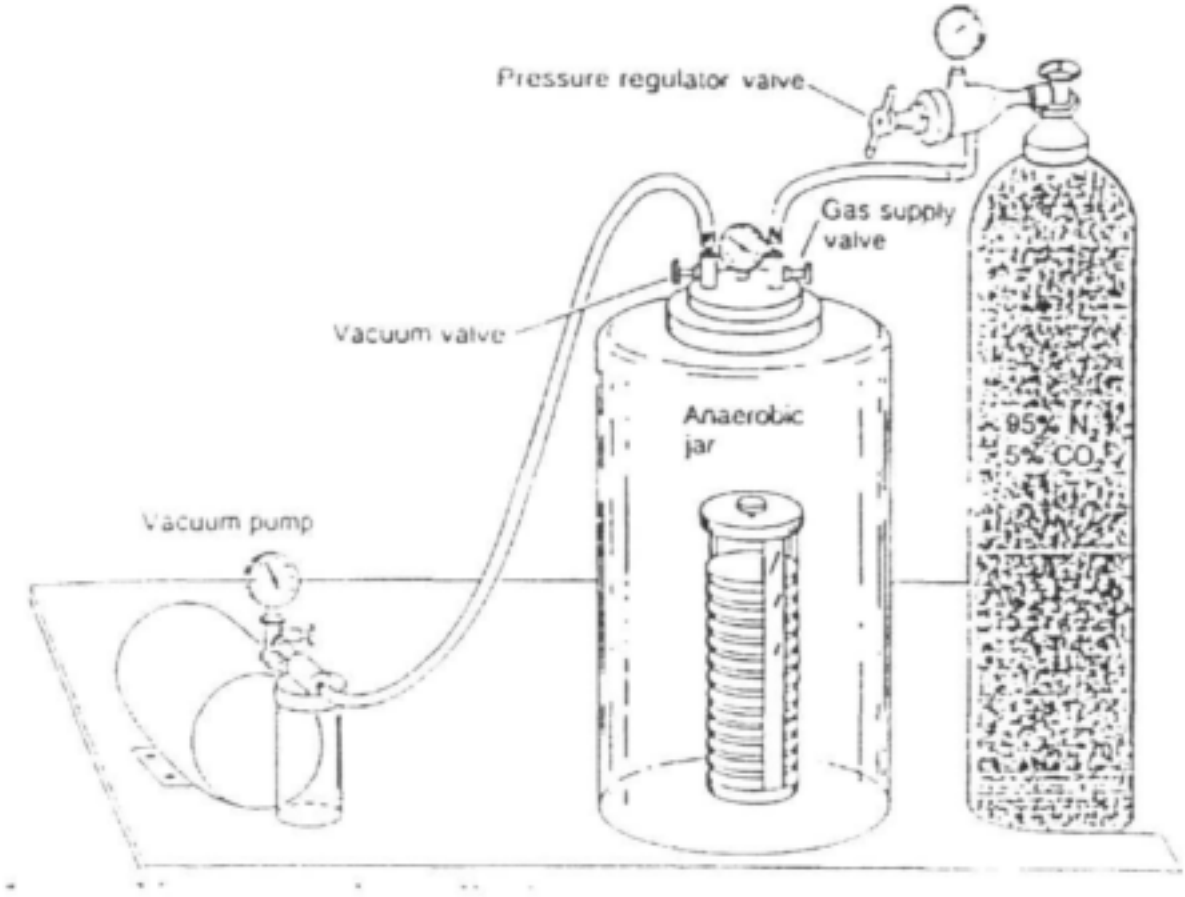

Figure 5.1 Vacuum and gas displacement method of anaerobic culture.
The anaerobic jar is evacuated at least three times and refilled each time with nitrogen or a nitrogen-carbon dioxide mixture (Prescott *et al.*, 1990).

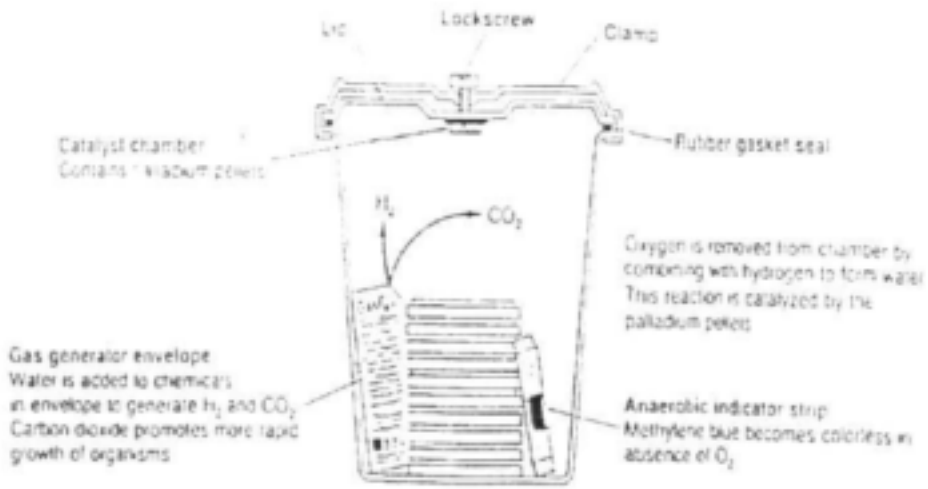

Figure 5.2 The GasPak anaerobic system (Prescott *et al.*, 1990).

One of the most popular ways of culturing small numbers of anaerobes is through the use of a GasPak jar (Figure 5.2; Prescott *et al.*, 1990). In this procedure, the environment is made anaerobic by the addition of hydrogen into the jar atmosphere, which then reacts with the oxygen present to form water. This reaction can be accelerated by the use of a palladium catalyst.

Cultivation vessels of up to 6,000 ml capacity can be constructed using Pyrex glass bottles or Erlenmeyer flasks, which are closed with 38 mm screw caps (Demain and Solomon, 1986). For larger cultures, 10-20 litre capacity carboys of Pyrex glass can be used. However, bioreactors manufactured from glass are very expensive.

Conventional anaerobic cabinets are often not readily available, they require some form of regular maintenance and the special gasses needed for operation are expensive. Anaerobic cabinets are very useful in the culturing of anaerobic micro-organisms but not practical for use as anaerobic bioreactors.

From the above it is clear that there is a lack of inexpensive, anaerobic bioreactors suited for training purposes and laboratory experimentation. The project on the suitability of carbon sources was planned with the likelihood that about 20 different carbon sources would have to be tested in triplicate for extended periods of up to several weeks. With an experimental set of three defined and three undefined carbon sources including two control experiments, 20 bio-reactors would be required at any one time for the experimental work. Therefore, an inexpensive but reliable anaerobic reactor was required for completion of the proposed work.

5.2 Materials and Methods

In this study the use of intravenous feeding apparatus (drip-bags) as anaerobic bioreactors was evaluated by conducting sulphate reduction tests on artificial mine water using sulphate-reducing bacteria and a variety of defined carbon sources. The system layout is shown in Figure 5.3.

5.2.1 *Design of a cost-effective anaerobic bioreactor*

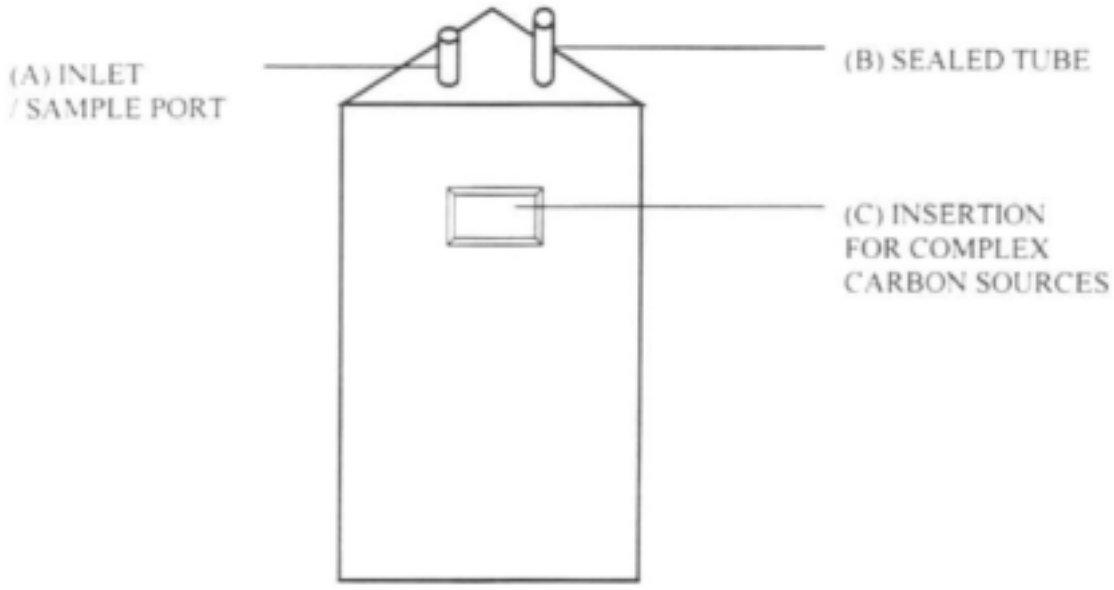

Figure 5.3 A schematic representation of the low cost anaerobic bioreactor used for the studies. The items of interest on the reactor are indicated as follows: (A) Inlet/Sampling Port; (B) sealed tube; (C) patch applied after complex carbon source has been added through an small opening cut in the bag and sealed gas tight with a patch (obtained from a bicycle puncture repair kit).

5.2.2 *Media composition*

All experiments were conducted with an artificial acid mine drainage (AMD) solution. The chemical composition of this artificial AMD solution is given in Table 5.1.

Table 5.1 Composition of the artificial acid mine drainage (AMD) solution.

Component	Quantity
$MgSO_4.7H_2O$ (Merck)	1.31g
H_2SO_4 (96%) (Merck)	0.30 ml
$FeSO_4.7H_2O$ (Saarchem)	4.56 g
NH_4Cl (Labchem)	0.19 g
H_3PO_4 (85%)(Merck)	0.02 ml

The above chemicals were dissolved in one litre of distilled water. The artificial AMD had an average sulphate concentration of 2,500 mg/ℓ. The pH of the AMD was between 2.5 and 3.5 but for the purpose of the small-scale experiments was adjusted to pH 7.2 using 10 M Sodium hydroxide solution (Merck).

5.2.3 *Standardisation of the inoculum*

Due to the limited time available, as well as the practical requirements of a laboratory test widely conducted for the suitability testing of carbon sources for biological sulphate removal, the duration of the individual experimental tests should not exceed four weeks. In order to find the most suitable concentration of inoculum (digester sludge), the following experiment was done. Mixtures of one litre artificial AMD and 5.56 mℓ lactic acid (92%) were prepared. The pH was adjusted to 7.2-7.5 before the digester sludge obtained from the Daspoort Water Purification Plant in Pretoria, was added. Volumes of 100 mℓ, 200 mℓ and 300 mℓ of inoculum were added to the AMD/lactic acid mixtures, respectively.

5.2.4 *Chemical analysis for sulphate content*

A SQ118 Spectroquant (Merck) was used to determine the sulphate concentration in the mine water using kit no. 1.14791.0001.

5.2.5 *Preliminary sulphate reduction tests on AMD*

A volume of 300 mℓ inoculum was added to 1,000 mℓ of artificial AMD (Table 5.1). The respective carbon source was also added to this mixture (Table 5.2). Of this mixture, 900 mℓ were poured into the anaerobic bioreactors. The pH was adjusted to 7.1-7.5.

Table 5.2 Defined carbon sources used during this experiment.

Carbon sources	Quantity per litre AMD
Lactic acid (92%) (Merck)	5.56 mℓ
Acetic acid (Merck)	5.95 mℓ
Butyric acid (BDH)	4.80 mℓ

All experiments were performed in triplicate. The sulphate concentration in the different bioreactors was monitored every 2-3d. As a control experiment, only AMD and inoculum was used with no added carbon source.

5.3 Results and discussion

5.3.1 Media composition

The average sulphate concentration of the artificial AMD was 2.536 mg/ℓ. Variations in the sulphate concentration of the mine water, in the order of ± 10%, were caused by minor variations in the volume of concentrated sulphuric acid used as sulphate source in the mine water.

5.3.2 Standardisation of the bioreactor inoculum

To assess the suitable size of an inoculum for the experiments, three different volumes of inoculum were added to replicate bioreactors. The inoculum added should be sufficient to allow the experiments to be undertaken with conclusive results within 30 days but should not so voluminous that it would change the mine water composition to any significant extent. A maximum addition of inoculum was therefore determined to be 33% of the total reactor volume.

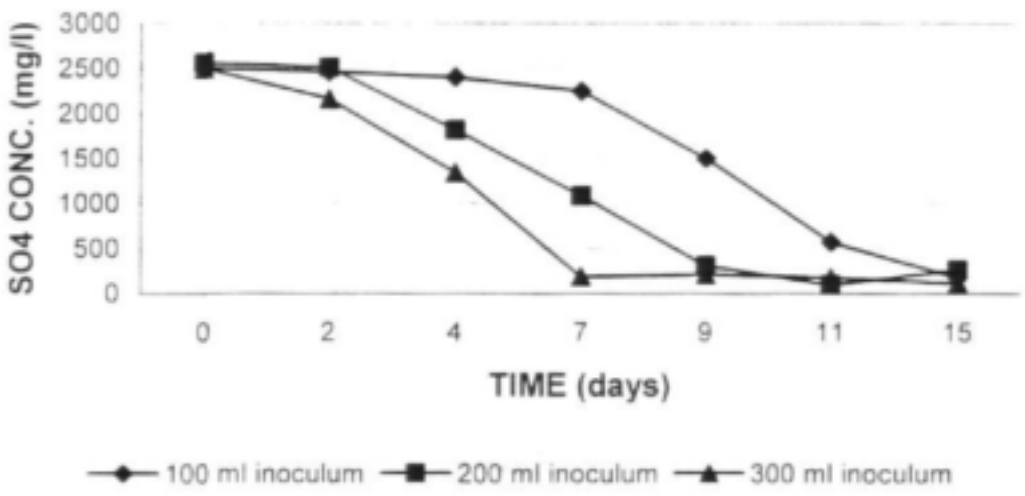

Figure 5.4 Sulphate reduction using 100 mℓ, 200 mℓ and 300 mℓ of inoculum, respectively.

Digester sludge consists mostly of fermentative bacteria and has low numbers of SRB (Zhender, 1988). In comparing the three volumes of inoculum used in the experiment, the 300 mℓ inoculum proved to achieve the fastest sulphate reduction over time. A sulphate reduction of about 90% of the initial sulphate concentration was achieved within seven days with 300 mℓ inoculum, followed by the 200 mℓ inoculum with 11 days and 100 mℓ inoculum within 15 days.

This would have been expected as the addition of the smallest inoculum will add the lowest number of

SRB that would then require a fairly long adaptation time with low sulphate removal. Significant sulphate removal with 100 mℓ inoculum started only from day 7, compared to day 2 for the 300 mℓ inoculum. This experiment has been undertaken with lactate, which is one of the most suitable carbon sources for a wide range of SRB. With the undefined carbon sources to be tested, it can be assumed that the biokinetes would be considerably slower as these carbon sources firstly have to be metabolised into suitable products readily available for consumption by the SRB and, secondly, these resulting metabolites may be compounds which are not as effective as lactate. Considering that these conditions may increase the reaction time by a factor of about 3 to 5, it was decided to undertake the remaining experiments with an inoculum of 300 mℓ inoculum.

5.3.3 Preliminary sulphate reduction tests with AMD

A preliminary comparative sulphate reduction test was undertaken with three defined carbon sources before a final decision on the inoculum volume for the remaining experiments was taken. The defined carbon sources used were acetic acid, butyric acid and lactic acid. The aim of the experiment was to determine whether or not the sulphate reduction obtained with lactic acid was similar to the previous reactor run and to assess whether or not different reduction rates would be achieved with the different carbon sources. Acetic acid is known to be a sub-optimal carbon source for sulphate reduction as most SRB are not able to synthesise acetic acid directly.

It was found that during the two week experimental period, lactic acid and butyric acid proved to be good carbon sources for the removal of sulphate, while acetic acid was not efficient (Figure 5.5). With lactic acid and butyric acid, sulphate reduction started on day 2 (as observed in the previous experiment) and rapid sulphate reduction generally occurred. A total of 90% sulphate reduction was achieved at Day 11 for lactic acid and day 15 for butyric acid. From day 4 to day 7 a loss of sulphate reduction activity was observed with both lactic acid and butyric acid, whilst sulphate reduction increased again from day 7 onwards. The reason for the drop in sulphate reduction is not known. However, if the delay had not been present, the target of 90 % sulphate reduction would have been achieved within 7 to 8 days, similar to the previous experiment.

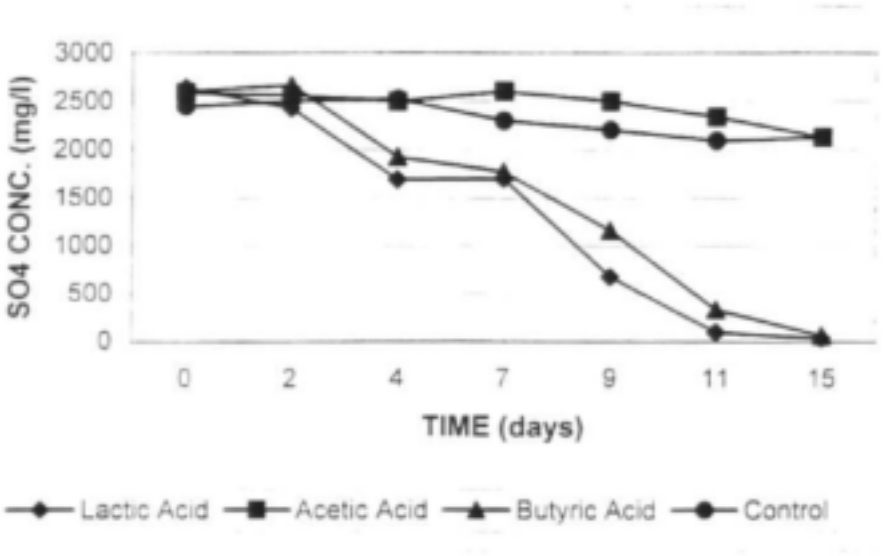

Figure 5.5 Preliminary sulphate reduction tests using lactic acid, acetic acid and butyric acid as carbon sources.

With butyric acid as carbon source, there was a smaller a decrease in sulphate reduction from day 4 to day 7, than that recorded with lactic acid. Again, the sulphate reduction rate increased from day 7 onwards to achieve 90% sulphate reduction by Day 15.

Sulphate reduction achieved with acetic acid as a carbon source was generally poor with about 20% of the sulphate being reduced within 15 days. This rate of sulphate reduction is similar to the sulphate reduction occurring in mine water by adding the inoculum only. Here, addition of the inoculum may also add small quantities of suitable carbon sources for sulphate reduction, thereby allowing limited sulphate reduction to occur.

The findings of the experiment regarding the ranking of the carbon sources according to their efficiency for sulphate reduction confirm the results obtained by other researchers (Isa *et al.*, 1986; Ahring and Joubert, 1987; Westerman, 1987; Zhender, 1988; Qatibi *et al.*, 1990; Visser *et al.*, 1993). In the presence of acetate, acetotrophic methanogens normally dominate over acetate-oxidising SRB (Zhender, 1988; Qatibi *et al.*, 1990). This would lead to the carbon being converted to methane with acetic acid being the intermediate in the process. Such carbon would subsequently be lost and not be available for sulphate reduction.

However, lactate is an excellent organic substrate for the cultivation and enrichment of SRB (Zehnder, 1988), and supports their growth in the presence of sulphate (Joubert, 1987). Qatibi *et al.* (1990) showed that SRB out-competed fermentative bacteria for lactate in the ecosystem studied. During our

experiments, lactate also proved to be the most efficient carbon source tested for the reduction of sulphate.

The experiment also confirmed that the selected experimental conditions will allow reproducible results to be achieved within the time span of the experiments. The additional three days required to achieve the target bench mark for sulphate reduction with lactic acid as a carbon source confirmed that the inoculum volume should be 300 mℓ to keep the time frame for the experiments within 30 days. Limited amounts of carbon sources may be added with the inoculum allowing for some sulphate reduction to occur. This sulphate reduction will be slow and may account for about 20% of the total sulphate removed. However, due to the low biokinetes of this sulphate reduction this is only likely to influence the result in cases were the biokinetics are generally slow. Where a suitable carbon source is present in excess, carbon added with the inoculum will only have a minor impact on the overall sulphate reduction.

5.3.4 Suitability of the anaerobic bioreactor for further experiments

In view of the above results, the drip-bag proved to be an effective and in-expensive, small-scale anaerobic bioreactor. During all of the sulphate reduction tests, some gasses were produced. The drip-bags proved to be gas-tight and no gas leakage occurred. This was illustrated by the fact that the drip-bags expanded to their full capacity. The rubber seal provided a convenient sampling port through which gasses could be removed by syringes. Only minimal contamination of oxygen, if any, occurred during sample collection. This inexpensive, easy to use anaerobic bioreactor did not require the addition of catalysts to achieve anaerobic conditions and no contamination of oxygen occurred.

5.4 Summary

During the first two months of the project the methodology for testing carbon sources was developed. The following parameters were verified during this time:

- suitability of intravenous feeding bags for use as cost-effective anaerobic bio-reactors

- standardisation of inoculum used for further experiments
  - → 300 mℓ of digester sludge per 1,000 mℓ of AMD

- standardisation of the laboratory equipment for chemical analysis:
  - → Merck SQ 118 method for SO_4 measurement;
  - → Merck SQ 118 method for COD measurement.

6 EXPERIMENTS ON BIOLOGICAL SULPHATE REDUCTION WITH ARTIFICIAL AMD USING DEFINED CARBON SOURCES

6.1 Introduction

Passive treatment is a method for the removal of sulphate and heavy metals using SRB, which should be considered as a management option (Batchelor, 1993). However, there is a lack of local and international experience with passive treatment systems that are designed for the treatment of AMD. This is due mainly to the absence of methods to evaluate the potential of different undefined carbon sources. The anaerobic sulphate reduction process must be optimised if an efficient passive AMD treatment system is to be developed. This can be achieved by selecting the most appropriate carbon sources, or combinations of different carbon sources.

The primary objective of this experiment was to test the standard procedure for evaluating defined and undefined carbon sources for sulphate reduction in acid mine drainage. The experiments with defined carbon sources are described in this chapter (Chapter 6), whilst the experiments with the undefined carbon sources are described in the next chapter (Chapter 7) of this report. The information obtained from the experiments with the defined carbon sources forms the reference point for evaluating the studies of undefined carbon sources.

6.2 Materials and methods used in the experiments

6.2.1 Defined carbon sources

For this experiment a number of defined carbon sources were used to evaluate sulphate reduction from artificial mine water. The carbon sources evaluated represent some of the potential intermediate compounds in the breakdown of the more complex carbon sources generally used in passive treatment of mine water.

Table 6.1 Defined carbon sources used during the experiment.

Carbon sources	Quantity (per litre of AMD)
Lactic acid (Merck)	5.56 mℓ
Acetic acid (Merck)	5.95 mℓ
Butyric acid (BDH)	4.80 mℓ
Propionic acid (Saarchem)	5.19 mℓ
Pyruvic acid sodium salt (Merck)	7.64 g
Methanol (Merck)	8.44 mℓ
Ethanol (Merck)	6.05 mℓ

The amounts of each carbon source, as displayed in Table 6.1, give a carbon : sulphate ratio of 1:1 (w/w) in the reactor. Each experiment was conducted in triplicate.

6.2.2 Inoculum

The carbon sources were added to the artificial mine water AMD (for composition of the artificial AMD see Table 5.1) and the pH of the artificial AMD/carbon source mixture was adjusted to 7.2 – 7.5 prior to the addition of the inoculum (as described previously). The AMD/carbon source mixture was mixed with digester sludge in a ratio of 1 : 0.3. Of the respective mixtures, 900 mℓ were poured into three bioreactors as described previously (Fig 5.3) so that the experiment could be undertaken in triplicate.

In order to standardise the digester sludge used as inoculum for the experiments with defined and undefined carbon sources, pH, density, moisture content, temperature, alkalinity and total solids determinations were carried out on unfiltered samples, according to standard analytical procedures (APHA, 1985).

The digester sludge was obtained from an anaerobic digester at Daspoort Water Purification Plant in Pretoria, South Africa.

Table 6.2 Characteristics of the digester sludge used as inoculum.

Parameter	Quantity
Average pH	6.93
Average temperature (°C)	15
Average alkalinity (mg/l $CaCO_3$)	867
Average total solids (MLSS; mg/l)	30.1
Moisture content (%)	96.3
Density	0.805
COD (mg/l)	6,000
SRB (cfu)	1.8×10^{-1}

6.3 Control experiments

Control experiments were undertaken, where the reactor content was prepared as described above, but without the addition of a defined carbon source. All carbon available in the control would derive from the inoculum added and, as seen with the previous control experiment, the sulphate reduction that occurs is expected to be limited. The sulphate reduction resulting from the carbon in the inoculum has been termed 'internal sulphate reduction' to distinguish such reduction from the sulphate reduction related to the added carbon sources to be tested. The control experiment was also carried out in triplicate.

6.3.1 Sampling

Drip-bags were shaken every day by hand to achieve a well-mixed suspension. Samples were taken from the bio-reactors through a rubber seal using syringes (Promex) with a gauge of 1.00 mm. To exclude the possibility of H_2S inhibition on the biological processes, excess gasses were removed as required through the rubber ports (Fig 5.3). Samples for analysis were collected every 2-3 days.

6.3.2 Chemical analysis

Alkalinity and pH determinations were carried out according to analytical procedures as described in Standard Methods (APHA, 1985). A SQ118 Spectroquant (Merck) was used to measure the sulphate concentration using kit no. 1.14791.0001. The Chemical Oxygen Demand (COD) was determined using a SQ118 Spectroquant (Merck).

All analyses except pH were carried out on filtered samples. The samples were filtered using WHATMAN No 1 paper filters.

6.4 Results

The specific results from the experiments that are discussed here relate to those from the sulphate reduction, the production of alkalinity, and the utilisation of COD from the defined carbon sources. The experiment for the acetic, butyric and the lactic acid has already been discussed in the previous chapter, whilst the additional defined carbon sources that were tested consisted of propionic acid, pyruvate, methanol and ethanol.

6.4.1 Sulphate reduction

The sulphate reduction in the reactors varied considerably. Good sulphate reduction was achieved with butyric acid and lactic acid (as already discussed) followed by propionic acid and methanol. Ethanol and pyruvate achieved moderate levels of sulphate reduction.

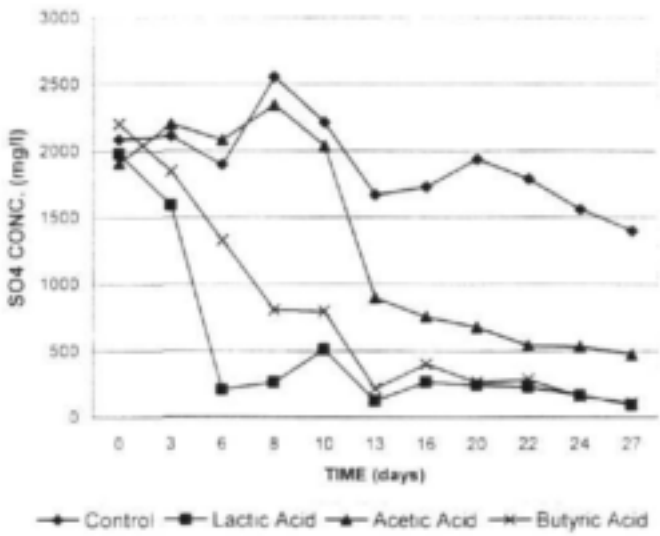

Figure 6.1 Sulphate reduction of AMD with lactic acid, acetic acid and butyric acid as carbon sources

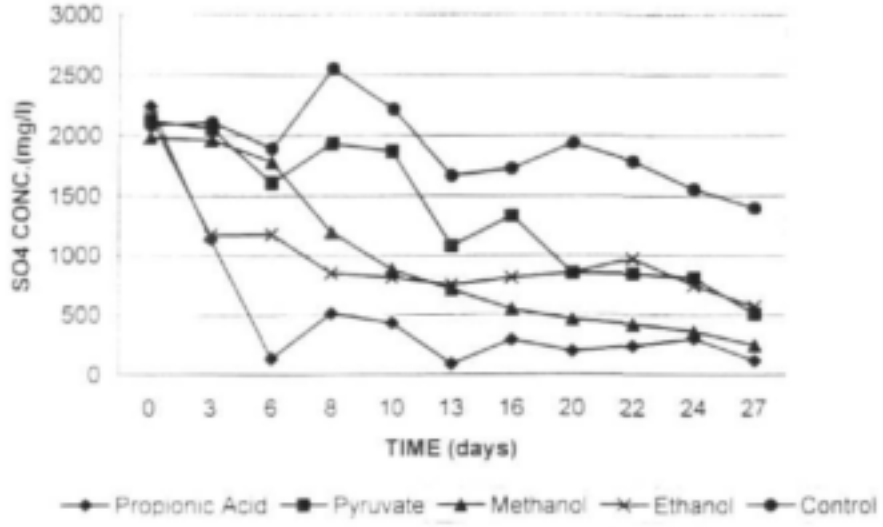

Figure 6.2 Sulphate reduction of AMD with propionic acid, pyruvate, ethanol and methanol as carbon sources.

The average initial sulphate concentration of the later experiment was 2,085 mg/l (Table 6.3). After 6 days, propionic acid and lactic acid resulted in an average reduction in sulphate concentration of 2,000 mg/l (Fig 6.2) and 1,770 mg/l (Fig 6.1), respectively.

Butyric acid (Fig 6.1) resulted in an average reduction in sulphate concentration of 1,394 mg/l after 8d. Propionic acid gave the most efficient sulphate reduction, followed by butyric acid, lactic acid, methanol, ethanol, pyruvate, with acetic acid being least effective (Table 6.3).
Alkalinity was produced during all of the experiments (Table 6.3; Fig 6.3 and 6.4).

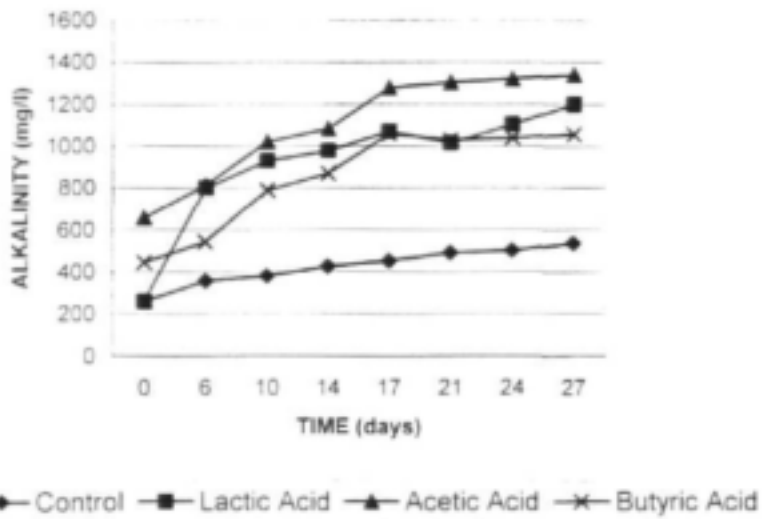

Figure 6.3 Increase in alkalinity using lactic acid, acetic acid and butyric acid as carbon sources.

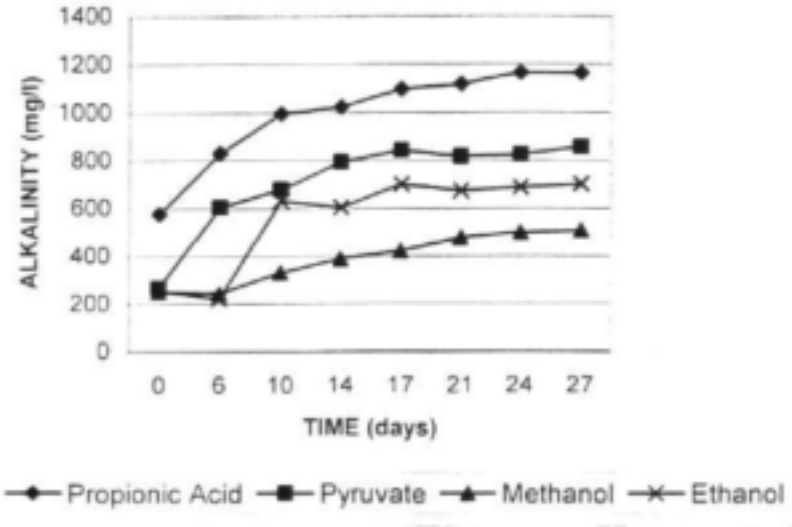

Figure 6.4 Increase in alkalinity using propionic acid, pyruvate, ethanol and methanol as carbon sources.

The control experiment, with no additional carbon, had an average initial COD value of 5,000 mg/l (Fig 6.5). The experiments with additional carbon added had initial COD values ranging between 11,000 and 15,000 m (Figs 6.5 and 6.6). The COD value of the control experiments were variable, but did not change significantly during the experimental period (Fig 6.5). When carbon sources were added, a reduction in COD values was observed during the experimental period (Figs 6.5 and 6.6).

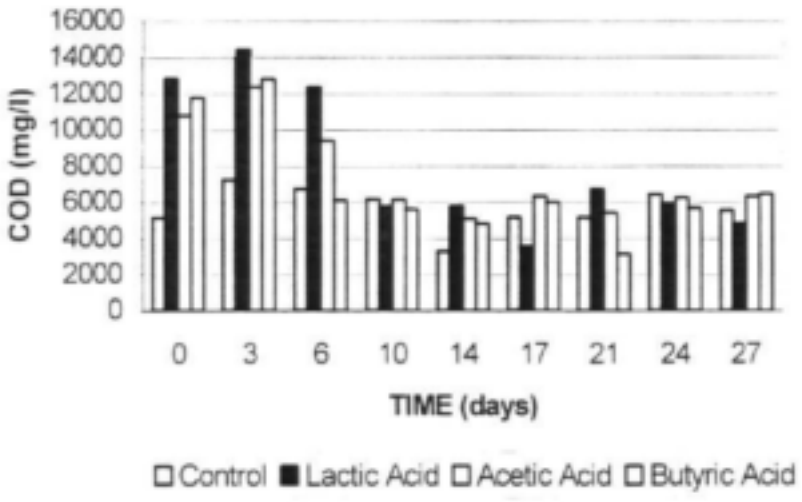

Figure 6.5 COD decrease using lactic acid, acetic acid and butyric acid as carbon sources.

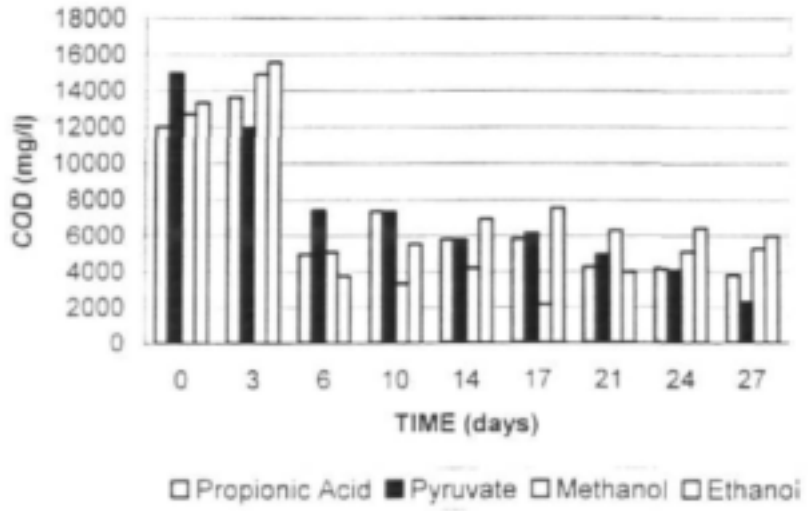

Figure 6.6 COD decrease using propionic acid, pyruvate, ethanol and methanol as carbon sources.

Table 6.3 The average amount of sulphate reduced (SR), COD utilised, alkalinity produced and the increase in pH over an experimental period of 27 days.

Carbon source	Initial SO_4 concentration (mg/l)	Sulphate reduced (mg/l)	COD utilised (mg/l)	Alkalinity produced (mg/l $CaCO_3$)	Increase in pH	mg COD used per mg SO_4^{2-} reduced	% SR
Control	2085	687	791	110.7	0.9	1.2	38.0
Lactic acid	1979	1885	6144	220.7	1	3.3	95.3
Acetic acid	1909	1434	4439	255.3	1.2	3.1	75.1
Butyric acid	2201	2094	5314	186.7	1.1	2.5	93.4
Propionic acid	2242	2124	8192	139.3	1.2	3.9	94.7
Pyruvate	2121	1614	12706	62.7	1.1	7.9	76.1
Methanol	1982	1739	6391	116.7	1.0	3.7	87.7
Ethanol	2152	1585	7345	97.3	1.6	4.6	73.6

6.5 Discussion

Widdel and Pfennig (1982) indicated that some short-chain fatty acids could be oxidized directly by SRB and the sulphate reducers responsible for these processes have been isolated (Widdel *et al.*, 1977; Widdel *et al.*, 1980; Laanbroek and Pfennig, 1981). Subsequently, it has been indicated that SRB may be involved in fatty acid turnover either by direct metabolism of fatty acids, or indirectly because of their importance as H_2 -scavengers (Banat and Nedwell, 1983). Sulphate-reducing bacteria can utilize a wide variety of carbon sources including propionate, butyrate and higher fatty acids, which other fermentative bacteria were unable to utilize in pure cultures (Zehnder, 1988).

Isa *et al.* (1986) showed that, with regard to sulphate reduction, acetate alone was not a good substrate for the SRB. Best growth on acetate was observed with *Desulfobacter* species, which are nutritionally very specialised sulphate reducers (Zehnder, 1988). According to Ahring and Westerman (1987), acetate was a poor substrate from an energy perspective. This might be because the energy required for acetate uptake at very low concentrations exceeds the energy gained from acetate metabolism, thereby limiting acetate uptake at a certain threshold concentration. Some reports indicate a predominance of SRB growing on acetate, while a number of other reports mention complete conversion of acetate by methanogens, even at high sulphate concentrations (Qatibi *et al.*, 1990). These contradicting reports suggest that acetate degradation might be independent of sulphate reduction. Acetotrophic methanogens completely predominated over acetate-oxidising SRB grown on acetate. Although sulphate reduction did occur during our studies, it was not as efficient as the other carbon sources. This may be as a result of methanogenesis predominating over sulphate reduction, as was found by Qatibi *et al.* (1990).

Lactate has been used as an excellent organic substrate for enrichment, isolation, and cultivation of certain SRB species (Zehnder, 1988). In the presence of sulphate, lactate can support the growth of SRB (Joubert, 1987). Qatibi *et al.* (1990) showed that in a bioreactor fed with wine distillery waste water, lactate was rapidly consumed with sulphate and/or sulphate and molybdate (inhibitor of sulphate reduction). The produced propionate was strongly oxidised in the presence of sulphate. They showed that SRB out-competed fermentative bacteria for lactate in the ecosystem studied. In our studies, lactate also proved an efficient carbon source for sulphate reduction. This is in agreement with the work of previous researchers (Joubert, 1987; Zhender, 1988; Qatibi, 1990).

Oxidation of pyruvate, both in the presence and absence of sulphate, can be carried out by most of the 'fermentative' bacteria (Joubert, 1987). Anaerobic digester sludge was used as inoculum during this study, which consisted mostly of fermentative bacteria and low numbers of SRB (Zhender, 1988). This may account for the fact that pyruvate was less effective as a carbon source for sulphate reduction compared to the other carbon sources during our studies (Table 6.3).

Visser *et al.* (1993) found that at higher sulphate concentrations, oxidation of propionate by SRB became more important and only under sulphate-limiting conditions did syntrophic propionate oxidisers out-compete propionate-degrading sulphate reducers. Syntrophic butyrate oxidisers were able to compete with SRB for the available butyrate, even with an excess of sulphate (Visser *et al.*, 1993). According to Zehnder (1988), many *Desufobulbus* species among the SRB oxidise propionate. Methanogenic and sulphate-reducing conditions lead to many possible pathways for conversion of propionate (Speece, 1996). Speece (1996), concluded that since propionate and butyrate were barely detectable in effluents at steady-state, their oxidation under high influent sulphate conditions may be

completely or incompletely mediated by the fatty acid-utilising SRB. The presence of SRB enhanced the degradation of propionate, either through direct utilisation, or through interspecies hydrogen transfer (Speece, 1996). Propionate and butyrate proved to be the best carbon sources for the reduction of sulphate during our study (Table 6.3). This confirms results obtained by other researchers (Visser *et al.*, 1993; Speece, 1996).

According to Stams *et al.* (1984), ethanol and pyruvate are oxidised to acetate in the presence of sulphate, with a concomitant reduction of sulphate to sulphide. The ability to grow on ethanol as electron donor is common among completely and incompletely oxidizing sulphate reducers (Zehnder, 1988). Pure cultures in batch enrichments with ethanol or higher alcohols as carbon sources sometimes cease to grow after a while and produce acrid smelling organic sulphur compounds that seem to affect the SRB (Zehnder, 1988). Ethanol and pyruvate were not as efficient as the other carbon sources tested for the reduction of sulphate (Table 6.3, Fig 6.1). This might be due to the production of organic sulphur compounds, which inhibit SRB (Zehnder, 1988).

Methanol is seldomly used by SRB (Zehnder, 1988). Even if some species grew well with ethanol, they do not seem able to metabolise methanol (Zehnder, 1988). This may account for the fact that methanol was less effective as a carbon source for the reduction of sulphate during our studies (Table 6.3).

The limited amount of sulphate reduction that occurred in the control experiments was ascribed to the carbon originally present in the digester sludge used as inoculum. No significant change in the COD values of the control experiments occurred. This was not unexpected, because most of the carbon in the anaerobic digester sludge was already used during the anaerobic waste water treatment processes in the digester. When an additional carbon source was added, efficient sulphate reduction occurred (Table 6.3).

The process of sulphate reduction is accompanied by the production of alkalinity in the form of bicarbonate ions. These react with H^+ ions to form water and neutralisation occurs with a release of carbon dioxide (Watzlaf and Hedin, 1994). Alkalinity was produced during all our experiments (Figure 6.3 and 6.4), confirming the findings of other researchers.

The results observed during our studies were in agreement with published results by other workers (Joubert, 1987; Zhender, 1988; Qatibi, 1990; Visser, 1993; Speece, 1996). Therefore, we concluded that the method developed in this study, could be used as a standard laboratory procedure, for evaluating carbon sources for potential use in the passive treatment of AMD.

7 EXPERIMENTS ON BIOLOGICAL SULPHATE REDUCTION WITH ARTIFICIAL AMD USING UNDEFINED CARBON SOURCES

7.1 Introduction

The mining industry has become increasingly aware of the critical need to address the problems associated with water quality management. Acidic sulphate-rich effluents present a serious worldwide environmental pollution problem and this is especially true of southern Africa, where water resources are extremely limited. Discharging sulphate rich effluents into surface waters contributes directly to salinisation of surface water, as well as corrosion and scaling of equipment when this is associated with the presence of calcium (Dill *et al.*, 1994). The sulphate ions in acid mine drainage (AMD) originate from three main sources, namely the bacterial oxidation of pyrite, the spent sulphuric acid used in metallurgical and chemical plants and from the cooling systems in these plants due to evaporation (Du Preez *et al.*, 1991). In 1963, it was estimated that three million tons of sulphuric acid entered the Ohio River in the form of AMD (Dugan and Lungren, 1964).

Generally sulphate levels below 200 mg/ℓ to 500 mg/ℓ are acceptable for discharge into public streams (Dill *et al.*, 1994). Although the City Council of Johannesburg allows the discharge of effluents containing more than 2,000 mg/ℓ sulphate into local sewer systems or rivers it is only due to the fact that the ratio of sulphate-rich water produced by industrial activities to surface water is high in that region. It is expected that as soon as proven technologies become available, legislation will be enforced to ensure that the receiving water bodies will be protected from such polluted water (Dill *et al.*, 1994).

The average sulphate content of AMD varies widely, normally ranging between 2,500 – 5,000 mg/ℓ. The range of the sulphate-rich effluent is of the order of 500-7,500 mg/ℓ depending on the underlying geology of the material mined and the mining methods applied. The pH of AMD is typically in the region of 2.3 - 2.5 and this is caused by the oxidation of pyrite, most often associated with coal and gold mining ore bodies. SRB are responsible for the enzymatic oxidation of ferrous ions and reduced sulphur compounds, with the concomitant production of ferric sulphate and hydrogen ions. The increase in acidity and the resultant decrease in pH, mobilizes other metals, which are present in the surrounding rock. These metals then dissolve in the surrounding water and thereby contribute to the formation of AMD.

The microbial ecology of surface waters is severely affected by the addition of AMD. Sulphuric acid and ferric ions in AMD have a deleterious effect on the heterotrophic biota of receiving streams (Tuttle *et al.*, 1968). AMD is toxic to most life forms due not only to its low pH but also because of its high metal content, including copper, lead and zinc. Most Gram-positive bacteria die out very rapidly in acidic water although fungi such as *Rhodutorula* appear to thrive (Ehrlich, 1968). The most numerous

components of the microbiota of AMD are the acidotrophic bacteria but other eukaryotes such as fungi, yeasts, rotifera and protozoa are also found. The basis for this acidophilic food web are the chemolithotrophic bacteria that obtain their energy from the oxidation of ferrous iron and reduced sulphur (McGinness and Johnson, 1993)

In order to alleviate the problem of acid mine drainage and acidic mining effluents, the characteristic water quality problems associated with AMD should be considered. Low pH values, elevated salinity (with high sulphate concentrations) and high metal concentrations are the most common characteristics of AMD. Sulphate can be removed from water by desalination processes such as reverse osmosis and ion exchange; however, these methods are costly (Dill *et al.*, 1994). Chemical methods include the use of barium ions, such as barium hydroxide and barium chloride (Maree & Strydom, 1995). In comparison to the costs of chemical purification, biological sulphate reduction by SRB seems to be more economical. Provided that a suitable electron donor such as lactate or acetate is included, the SRB oxidize the electron donor with the sulphate as electron acceptor being reduced to sulphide (Dill *et al.*, 1994). Moulton *et al.* (1957) demonstrated that the SRB were able to increase the pH of a mineral salt medium that contained lactic acid as a carbon and energy source, from a pH of 5.0 to pH 8.9 in a period of eight days at room temperature. Whilst similarly good sulphate reduction was obtained in the experiments of Butlin *et al.* (1960) and Tuttle *et al.* (1969), a retention time of 5-10 d was required. Maree and Strydom, (1985) showed that a three stage process (anaerobic-aerobic-anaerobic) employing up-flow packed bed reactors for anaerobic treatment and an activated sludge process for an aerobic treatment could produce water of reasonable quality from mining effluents. Sulphate was reduced from 2.5 g/ℓ to less than 0.5 g/ℓ with concomitant removal of H_2S, carbonates, cyanides, phenols and heavy metals. By utilizing an up-flow packed bed reactor instead of a completely mixed bed reactor, the increased surface area of the packed bed reactor exposed to the sulphate rich medium obtained a higher reduction rate (Maree and Strydom, 1985). The by-products produced in this process, particularly hydrogen sulphide, were also removed by using a second bacterial reactor for the reduction of sulphate to sulphur by the green and purple photosynthetic bacteria, *Chlorobium* and *Chorella*.

A major disadvantage associated with the addition of organic carbon sources is the high residual organic carbon content or the release of 'coloured' water, which would require downstream treatment (Maree *et al.*, 1991). Coloured water is caused by humic substances, which are difficult to biodegrade under the short hydraulic retention times of several hours.

In the past, several undefined carbon sources have been utilized to determine the sulphate reducing ability of substrates or to treat the waste water by means of an anaerobic bioreactor, that utilizes the biological processes of methanogenesis, for sulphate reduction, or both. Producer gasses (Dill *et al.*, 1994), sewage digest (Kaufmann *et al.*, 1996), and polysulphide rubber manufacturing waste waters

(Obarsky *et al.*, 1984) have all been used in the anaerobic treatment of waters containing sulphate ions.

The objective of this study was to investigate the potential of inexpensive complex carbon sources such as waste products from the agriculture and food industry, and domestic waste products, for biological sulphate reduction of mine waters in passive treatment systems.

7.2 Materials and Methods

7.2.1 Reactor design

An intravenous feeding apparatus, more commonly known as a drip-bag was used in these experiments (Fig. 5.3). The bags are made of durable plastic and have a capacity of 1 ℓ. The bags have one tube and one port fitted with a rubber stopper. The tube was used to insert the AMD, inoculum and the carbon source.

In the case of the undefined carbon sources, which were too bulky for the port, a small insertion was cut into the reactor bag in the shape of a cross. The carbon source was added and the hole was sealed by means of a bicycle tire puncture kit, which was able to withstand the pressure of any gas being formed and provided a gas tight seal for the reactor. Once the reactors were filled, the port was sealed under heat and excess air was removed with a 1.00 x 40 mm needle inserted into the rubber-sealed port, while the reactor bag was de-pressurised until no visible gaseous phase was present in the bioreactors.

7.2.2 Artificial Acid Mine Drainage

All experiments were conducted with an artificial acid mine drainage (AMD). The detailed composition of the artificial mine water is given in Table 5.1.

7.2.3 Undefined carbon sources

The choice of carbon sources for the experiments depended on criteria such as availability and cost. The solid carbon sources used included composted cow manure, fresh cow manure, citrus compost, chicken manure, and silage, whilst the liquid sources used were: whey, digester sludge and molasses.

In order to allow the maximum amount of carbon to be made available to the SRB, the Kikuyu grass cuttings and the silage were pre-treated by blending at high speed, in artificial AMD in a Waring blender for 5 min and 2.5 min respectively.

7.2.4 *Inoculum*

Anaerobic digester sludge obtained from the Daspoort Water Purification Plant in Pretoria was used as bacterial inoculum. The inoculum was added to the medium after the pH had been adjusted to pH 7.5. In each case, 300 mℓ of inoculum was used for these experiments, as described previously.

7.2.5 *Experimental set-up*

Each bioreactor was filled with artificial AMD, a carbon source and inoculum up to a level of 900 mℓ thereby allowing enough space for gas production. The amount of carbon source added was in excess of the optimum carbon: sulphate ratio of 0.67:1 (Bhattachyra *et al.*, 1996). The pH of this mixture was adjusted and 300 mℓ of inoculum (anaerobic digester sludge) per litre of artificial mine water was added. The pH of the final solution was also adjusted where necessary to keep within a range of pH 7.1 to pH 7.8, as this is the range within which sulphate-reducing bacteria perform at optimum levels of sulphate reduction (Postgate, 1968).

In each run, three carbon sources were tested and a control (in triplicate) was also prepared. Because the total organic carbon content was not known, and it was not feasible to determine this beforehand, an excess of carbon was used in all cases. In most instances, 10 g of the wet substrate was used as carbon source; in the case of aqueous substrates such as whey, 10 mℓ were used. At the start of each experiment the dissolved COD of the mixture in the reactor was determined.

7.2.6 *Sampling*

The bioreactors were incubated at a temperature of 37°C and were agitated every two to three days by hand to mix the solution before taking a sample. Samples were taken by extraction of liquid from the sampling port using a 1 mℓ syringe (Promex) and a 1.00 x 40 mm needle (Promex). In the initial stages of the experiment (in the first 24-48 hrs) the pH of the reactors was adjusted where necessary in order to maintain the pH at approximately pH 7.4.

7.2.7 *Control experiments*

The control experiments were prepared in triplicate, and each replicate consisted of the 300 mℓ inoculum added to 1,000 mℓ of the artificial AMD. Of the mixture 900 mℓ were added into each of three bioreactors. The experiment was undertaken in order to measure the 'internal sulphate reduction' due to the carbon added with the inoculum.

7.2.8 Chemical analysis

The reduction of sulphate in the bioreactors was monitored spectrophotometrically every two to three days by means of the Spectroquant SQ118 (Merck) and the sulphate kit (Number: 1.14791.0001) especially supplied by Merck for use with the SQ118 Spectroquant. Each carbon source was measured in triplicate.

The alkalinity was monitored twice a week by using the titration method (APHA). A sample of 5 mℓ was added to 45 mℓ of distilled water and titrated with 0.1 N HCl to a pH of 4.5. The volume of HCl used in the titration was recorded and expressed as alkalinity (mg/ℓ $CaCO_3$). The pH was measured potentiometrically by use of a pH electrode (Beckmann) twice a week.

The Chemical Oxygen Demand (COD) was also measured photometrically on the Spectroquant SQ118. This was performed at least once a week.

7.3 Results

7.3.1 Experiment 1

The carbon sources used in this part of the study were sewage sludge, molasses and composted cow manure. Sulphate concentrations were measured over a period of 10 days.

In the case of molasses as substrate, the sulphate reduction rate was 52.6 mg/ℓ/d (Fig 7.1). When using cow manure as carbon source, the sulphate concentrations in the bioreactor fluctuated (Fig. 7.2) between 3,110 mg/ℓ and 2,215 mg/ℓ during the experimental period. This was probably due to the release of bound sulphate in the inoculum or carbon source by microbial action. Using digester sludge as carbon source also showed fluctuations between 3,219 mg/ℓ and 1,946 mg/ℓ (Fig 7.1). No sulphate reduction was observed: however, this is probably due to the short experimental period.

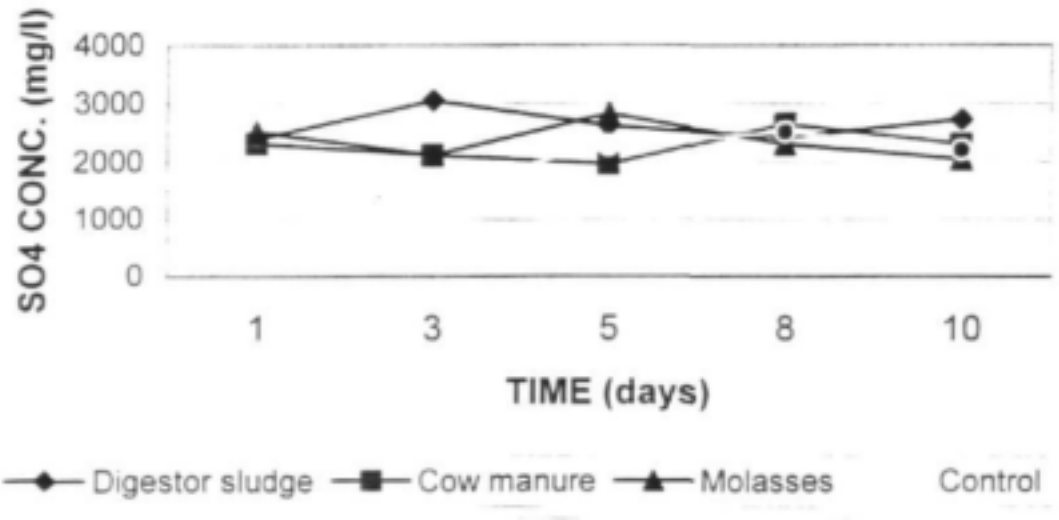

Figure 7.1 Sulphate reduction utilizing digester sludge, molasses and composted cow manure as carbon sources.

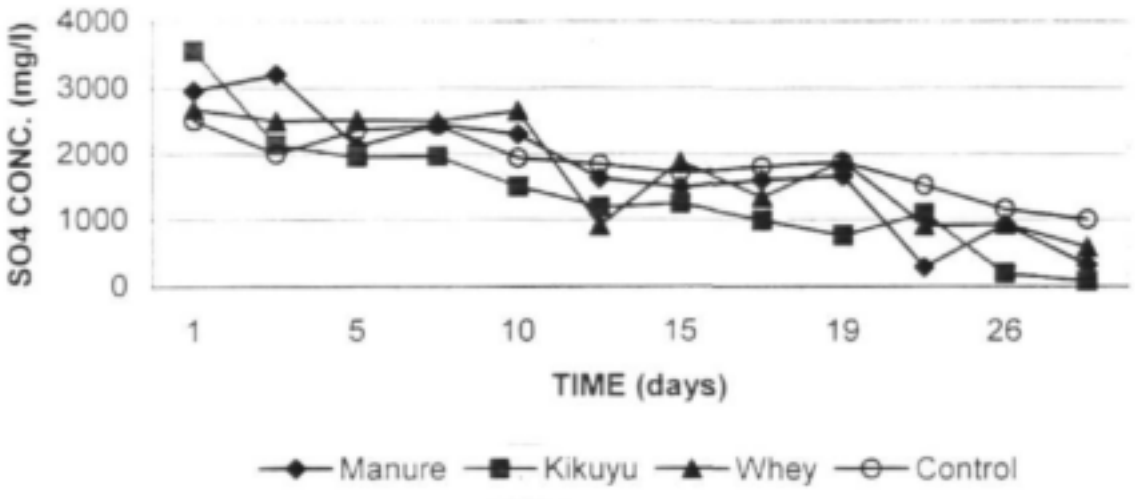

Figure 7.2 Sulphate reduction using Kikuyu grass cuttings, whey and fresh cow manure as carbon sources.

7.3.2 Experiment 2

The carbon sources chosen for this experiment were fresh cow manure, fresh Kikuyu cuttings and whey obtained from cheddar vats. Sulphate reduction rates of the Kikuyu and the manure were 138.25 mg/l/d and 104.6 mg/l/d, respectively, for the 28 d of the experiment. The sulphate reduction rate decreased over time and this was attributed to the sharp decline of the carbon present in the bioreactor (Fig. 7.2). Following a second addition of the respective carbon sources on Day 19, improved sulphate reduction

was observed (Fig 7.2). This suggests that the microbial community in the bioreactors had adapted to the experimental conditions and had become carbon-limited prior to the dosage of additional carbon. This is also reflected in the increased COD values in week 4 (Fig. 7.4).

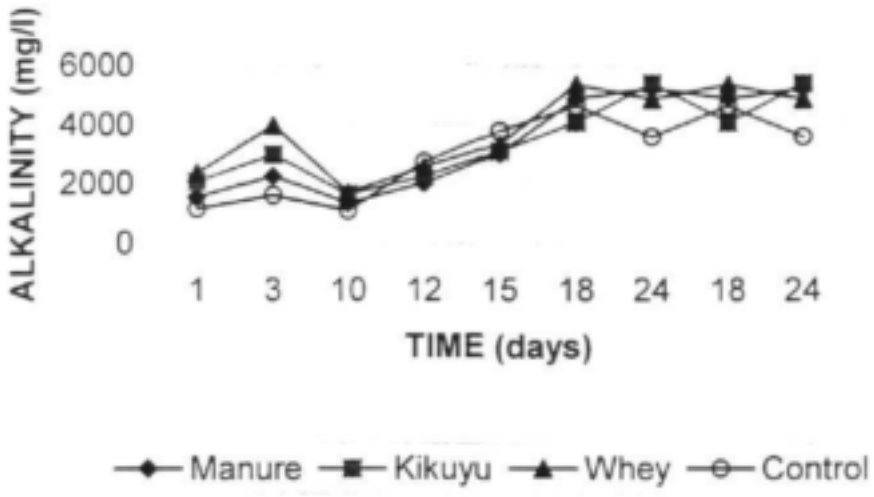

Figure 7.3 Alkalinity production using Kikuyu grass cuttings, cow manure and whey as carbon sources.

Alkalinity increased in all the reactors during the experiments with the concomitant reduction of sulphate. The alkalinity production was most evident in the Kikuyu bioreactor, which produced 3313 mg alkalinity in the 28 d of the experiment - a total of 118 mg alkalinity/d (Fig. 7.3). Manure produced 131 mg alkalinity/d and whey produced 89 mg of alkalinity /d. The control experiment produced 86 mg alkalinity/d. The pH of the reactors increased as the sulphate concentration decreased due to the production of alkalinity by the SRB.

The good performance of Kikuyu is probably due to the presence of other micro-organisms, such as lactic acid bacteria, which are able to digest the sugars and cellulose in the grass cuttings and make the simpler sugars available for the SRB (Fenton, 1987). A similar situation probably exists for the fresh manure substrate in which the microbial inhabitants of the rumen digest nutrients, which in turn were then available for the SRB.

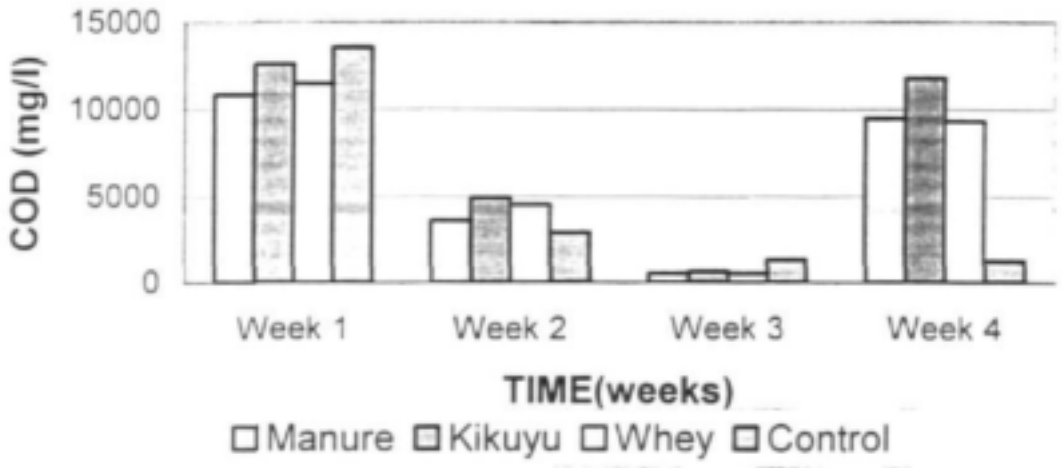

Figure 7.4 The COD values of the experiment, showing a rapid decrease in carbon present in the reactors during the first three weeks of the experiment.

7.3.3 Experiment 3

Chicken manure, Citrus compost and silage were used as substrates in these experiments. Here, as expected from the previous experiment where Kikuyu cuttings fared well, the sulphate reduction rate was best in the silage substrate. A sulphate reduction of 92.7 mg/l /d sulphate was achieved in the 30 days of the experimental run (Fig 7.5). It is possible that the lactic acid bacteria present in silage are able to digest those parts of the silage that the SRB are not able to degrade directly. It is also possible that these bacteria utilize at least part of the substrate made available, a possible explanation for the lower reduction rate. Citrus compost, which obtained a reduction rate of 45.7 mg/l/d, was not a suitable substrate for good sulphate reduction. This substrate may therefore only be considered as a long-term carbon source as it may be able to maintain low sulphate reduction in a passive treatment system for an extended period of time. Chicken manure fared similarly and reduced carbon at an average rate of 41.8 mg/l/d. The control experiment showed a reduction of sulphate of less than half (23.2 mg/l/d) of that of the bioreactors containing a carbon source. This indicates that the SRB require a carbon source in order to reduce sulphate in AMD.

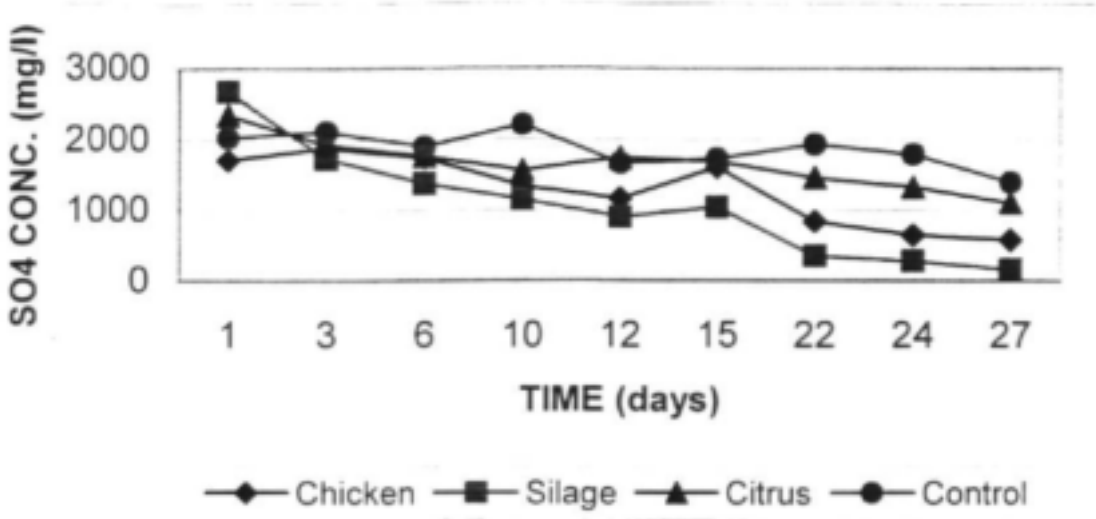

Figure 7.5 Sulphate reduction using chicken manure, citrus compost and silage as carbon sources.

Alkalinity production showed a marked upward trend in all systems. The bioreactors with silage produced an average of 17.3 mg alkalinity/l/d, whilst the citrus compost and chicken manure bioreactors produced an average of 45.7 and 41.8 mg alkalinity/l/d respectively (Fig. 7.6). Minor changes in COD were also recorded (Fig. 7.7).

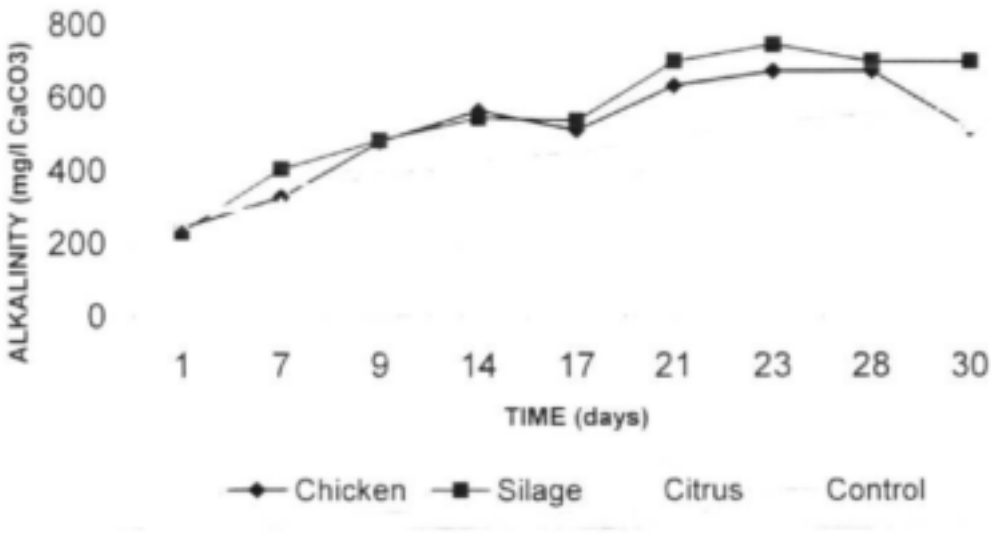

Figure 7.6 Alkalinity production with chicken manure, citrus compost and silage as carbon sources.

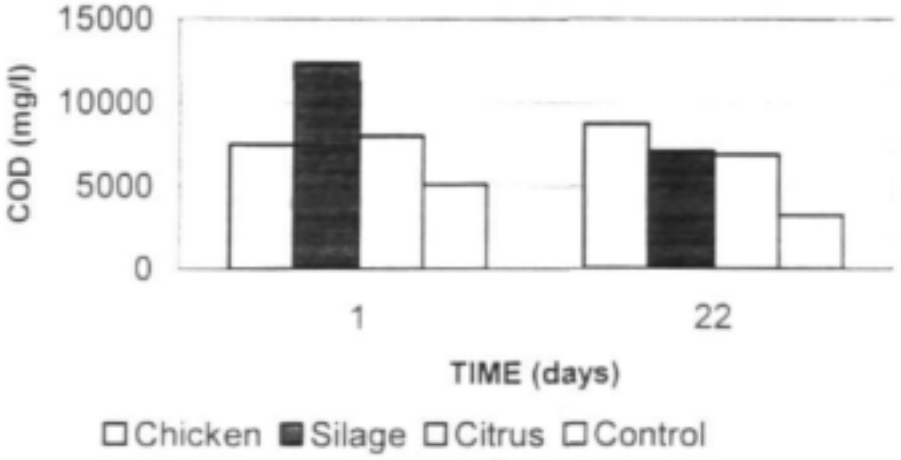

Figure 7.7 The COD values of the experiment, showing a decrease in carbon present in the reactors over a period of 28 days.

7.3.4 Experiment 4

Mushroom compost, hay and fly ash were used as carbon sources in this experiment. Hay reduced sulphate at a rate of 148.67 mg/l/d over a 17 d period. The fly ash (30% carbon) reduced sulphate at a rate of 86.34 mg/l/d (Fig. 7.8). In the same experiment, alkalinity values increased very slowly from day 17 (Fig. 7.9).

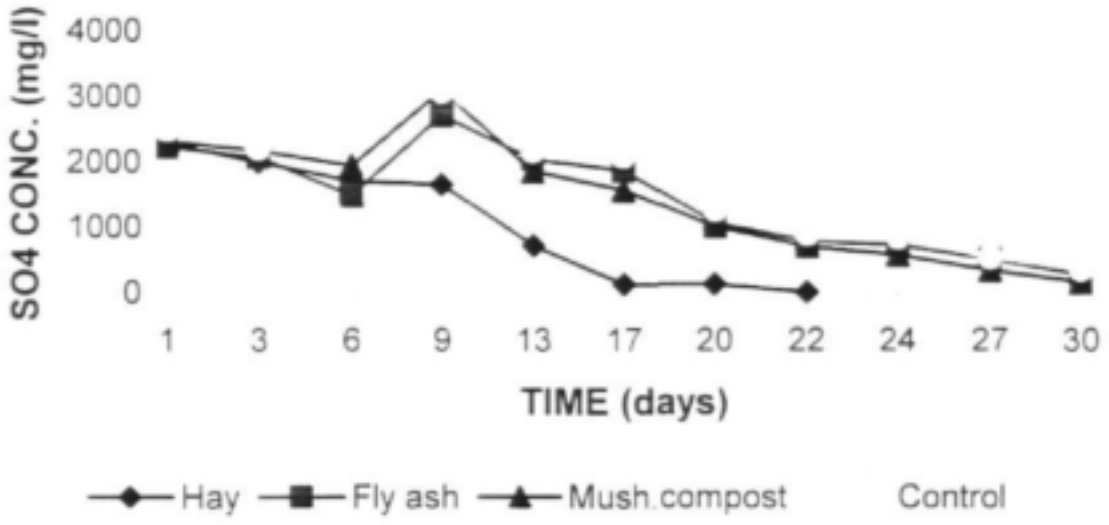

Figure 7.8 Sulphate reduction rates of hay, mushroom compost and fly ash.

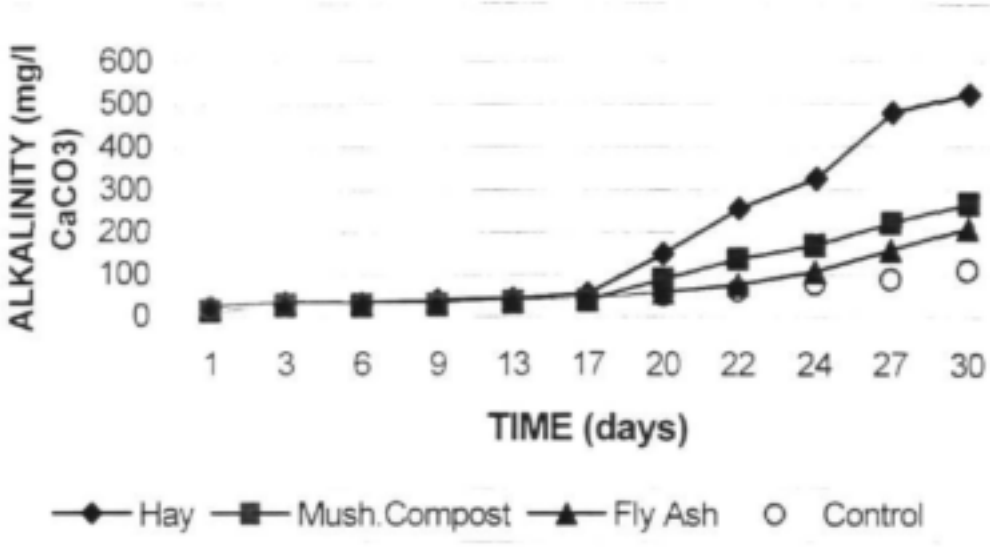

Figure 7.9 Alkalinity values of the carbon sources hay, mushroom compost and fly ash.

Table 7.1 Summary of the experimental results obtained during the four experiments.

Carbon sources	Experimental Period (d)	Sulphate reduction rate (mg/l/d)	SO_4 removal (%)	Alkalinity production rate (mg/l/d)	VSS (g/kg)
Digester sludge	10	128.5	20.4	-	5.45
Molasses	10	52.6	12	-	230.00
Cow manure (C)	10	1.96	0	-	408.82
Kikuyu	28	138.25	97.8	118	340.76
Whey	28	82.71	77.9	89	n.a.
Cow manure (F)	28	104.6	89.1	131	305.04
Chicken manure	30	41.8	66.2	41.8	100.68
Citrus compost	30	45.7	52.8	45.7	769.91
Silage	30	92.7	93.7	17.3	59.51
Mushroom Compost	25	95.23	92.2	-	225.66
Hay	17	148.67	99	-	75.48
Fly ash	25	86.34	86.7	-	739.23

7.4 Discussion

The concept of passive treatment for polluted waters is relatively new. The removal of sulphate from mine water is very important in South Africa, whilst treatment of polluted water in the USA is concerned with the removal of heavy metals and decreasing the acidity of effluents. Therefore, South Africa is faced with a unique situation. Passive treatment systems utilize natural processes and resources to drive the overall treatment of contaminated waters. These systems are almost self-sustaining, with the requirement for regular maintenance being kept to a minimum. Bacterial sulphate reduction reactions in a passive treatment system require a carbon source for growth. However, the volume (and type) of the carbon source may form a significant portion of the cost of the system. The ultimate aim of the sulphate removal process is to maximize the sulphate removal whilst using the minimum quantity of carbon.

Several passive treatment systems in the United States are already using inexpensive carbon sources to treat polluted waters. The Howe Bridge system utilizes spent mushroom compost underlain by limestone gravel. The water flows over the mushroom compost at a depth of 1-2 meters before flowing over the mushroom compost and limestone gravel. The resulting pH of the effluent is increased from pH 4.5 to approximately 6 or 7 and the sulphate concentration decreased by about 200mg/l. Several other researchers have used mushroom compost as a carbon source in the field as well as in the laboratory. Reduction rates ranging from 0.092 Mol/m^3/d (Hammack and Edenborn, from Eger 1991) to 1.2 Mol/m^3/d (Reynolds *et al.*, from Eger 1991) were obtained. Mushroom compost obtained a sulphate reduction rate of 95.23 mg/l/d with an overall sulphate reduction of 92.2 % (Table 7.1).

Hay reduced the sulphate concentration in the artificial AMD by 98.97% in our batch cultures. Rabenhorst *et al.* (1992) also found that straw and straw-manure mixtures consistently produced the highest levels of sulphide. The sulphide production levels in the hay mixture were also lower than that of straw. This may be due to the production of alcohol during early fermentation.

Cow manure, horse manure and chicken manure have also been used in passive treatment systems. The poor performance of the composted materials is attributed to the fact that the materials had already undergone substantial decomposition and therefore contained only very small amounts of readily decomposable carbon. Sewage digest has also been used as an inexpensive and readily available carbon source.

Maree and Strydom, (1985), used an up-flow packed bed reactor as a sulphate removing system. Re-circulation of the water increased sulphate reduction due to the increased diffusion rate between the biological film layer and the water, and because high localized hydrogen sulphide concentrations could

be transported faster to the vicinity of a sulphide-reducing colony. The removal of heavy metals, such as lead and nickel is attributed to their low solubility (as sulphides), while the earth metals, (Ca and Mg) and the alkali-earth metals (Na and K) are not influenced to a great extent. This is an indication that the heavy metal peaks present in industrial effluents will not easily disturb the biological activity (Maree and Strydom, 1985).

In order for passive treatment of AMD to become a viable alternative it is necessary to develop a system, that requires minimal maintenance. The period of passive treatment may be made longer by using a variety of different carbon sources over an extended time. The concept of mixing different carbon sources of varying sulphate-reducing capabilities may be used to ascertain if sustained carbon release is possible and how long sulphate reduction would theoretically occur in a passive treatment system. It must be taken into consideration that these experiments were done under optimum laboratory conditions and with an artificial AMD. Further studies are required with "actual" acid mine drainage collected from problem areas to determine how well these carbon sources will reduce sulphate under sub-optimal conditions. It may be that the carbon sources that reduce sulphate at a lower rate, may provide a more sustained reduction that is suited to the passive treatment of acidic effluents.

8 CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH WORK

8.1 Conclusions from the current project

Despite the fact that the interest in biological mine water treatment by use of sulphate-reducing bacteria has increased world wide over the past five years, the literature review revealed very little information with respect to recent practical work that might be applicable to the objective of our project. Whilst the fundamental knowledge about sulphate-reducing bacteria, their preferred defined carbon sources and their respective band of existence in the 'ecological net' of anaerobic bacterial processes is well researched and documented, very little basic experimental work on the consumption of more complex carbon sources is described in literature.

The experiments that consider the use of complex carbon sources such as molasses or saw dust are mainly laboratory or pilot-scale reactors that have been operated with one or more prospective low-cost carbon sources. However, the experiments often did not happen under strictly controlled environmental conditions. In addition, the experiments were usually conducted with a variety of different reactor types and a direct comparison of the bacterial sulphate reduction rates (if given) from these literature data is often difficult or impossible to ascertain.

In the evaluation of alternative carbon sources with respect to economic factors such as costs of carbon, its availability and the cost of transport, it has become apparent that, at this time, the availability of large amounts of carbon located close to the main mining areas is extremely limited. This would, in turn, limit the possibility to construct large-scale passive treatment systems with a potential life-span of several decades in close proximity to the mines.

The cost of carbon is therefore the most expensive single item in the construction of a passive treatment system. Passive treatment systems using intrinsically generated carbon such as reed beds or wetlands overcome the expense problem by generating their own carbon. However, the treatment capacity of these systems is limited to the rate of carbon generation within the specific system.

The experimental work conducted at the University of Pretoria developed a simple method for the evaluation of alternative carbon sources and tested the method by the evaluation of defined and undefined (complex) carbon sources. This work can be considered as a first step in the direction of establishing sulphate removal kinetics under strictly controlled environmental conditions for both defined carbon sources and for undefined (complex) carbon sources.

The objectives of the project that have been set out have only been met in part. This is largely due to a

very ambitious programme that had been established for the project. The project has been developed as one which has been closely linked to the project on passive treatment of acid mine drainage and aimed to answer a number of questions which arose after the operation of the pilot scale systems for a period of about 12 months, when the initial sulphate reduction in the treatment systems declined considerably.

The primary objective of the project was to develop a laboratory method to test low-cost carbon sources for their suitability for use in passive treatment systems for mine water.

The specific aims of the project included the following:

- to identify a range of carbon sources available in the country taking economical factors such as available quantities and transport costs into consideration:

  A range of potentially suitable carbon sources was identified and the available quantities and transport costs for these were determined at an approximate order of magnitude. The number of carbon sources identified that are available in large enough quantities for full-scale mine water treatment systems, however, is limited and their distribution suggests that even where such carbon sources are free of charge (because they are considered waste material), a significant cost factor will be related to transport of this material to site.

  Further work:

  It is recommended that a map be developed for the main mining areas of South Africa such as the East/West Rand, the Klerksdorp area, the Freestate Goldfields and the coal mining areas in Mpumalanga and KwaZulu Natal indicating the mines with the expected mine water discharge volumes that have the potential to be treated with passive treatment technology. In addition, the types of low-cost carbon sources available within a radius of 100 km, the respective volumes available and their potential transport costs should also be indicated. Such information will help to establish whether or not an operational period of a passive treatment system in certain areas has to be limited to a given time period based on limited volumes of carbon source available. Such systems may have to be replenished with carbon sources at an earlier point in time than would normally be required from an operational and technical point of view.

  Based on the information available, an analysis should be undertaken as to whether or not sufficient low-cost carbon sources are available in the respective mining areas to render the process of passive mine water treatment cost competitive against high-rate sulphate reduction processes. Such a system could be based on a GIS platform for easy access, management and

interrogation of the data collected.

- to determine test procedures regarding total carbon content, available carbon for bacterial sulphate reduction and sustainability of carbon release over time for identified carbon sources;

This task, although identified as a requirement to the project, was not resolved to a suitable degree of satisfaction as the time and the funds for the project did not allow for extensive testing of the carbon sources to develop a method for the determination of available carbon or the sustainability of carbon release over time. At present only the carbon available during the short-term experiments over a period of up to 30 days has been determined.

It is the opinion of the project team, based on the experience gained during the project, that these two questions will have to be addressed in separate projects following this research because the only way to answer the questions conclusively is more complex than the primary objective of this project - the development of a quick test method for the evaluation of suitable carbon sources for the use in passive treatment systems.

Further work:

To understand the release of carbon from carbon sources in general, it is recommended that a series of experiments be set up to allow monitoring of the carbon release from selected carbon sources over time. To simulate the environmental conditions in a passive treatment system the carbon sources should be subjected to the bacterial conglomerate present in the treatment system, but carbon-consuming processes such as sulphate reduction and methane production should be inhibited by addition of metabolic inhibitors for the respective physiological bacterial groups concerned.

The carbon output from the reactor should be measured in terms of COD and a number of the potential breakdown products such as fatty acids and alcohols. Ideally, the identification of the breakdown products should account for at least 80% of the COD deriving from the reactor. Gas analysis should include CO and CO_2.

For the long-term prediction and sustainability of carbon release a similar approach as discussed above is suggested, with some form of pre-treatment of the respective carbon sources simulating the slow process of the breakdown of complex organic carbon compounds such as lignin and cellulose. Pre-treatment of the carbon sources may consist of acid/alkaline digestion

or enzymatic treatment.

- to develop a test procedure to obtain sulphate reduction kinetics for the respective carbon sources tested under passive treatment conditions;

  A test procedure for the assessment of respective low cost carbon sources was developed during the project. For the purpose of meeting the primary objective of the project the test procedure is considered sufficient.

  It is important to understand that this test procedure will only give a relative indication of how well a given carbon source, compared with other defined and undefined carbon sources, is suited to support sulphate reduction in mine water. The method has not been fully developed and is not yet suitable to predict the medium- or long-term suitability of the carbon source. However, in pre-treating the respective carbon sources of interest as described above, these can be tested with the procedure in the same manner to give a relative indication of how the carbon source would perform over an extended period of time and how the pre-treated and the untreated carbon sources differ with regard to sulphate reduction efficiency.

- to test the developed procedure on a model carbon source mixture, which should provide carbon over a short-, medium- and long-term period;

  This task was not achieved during the project due to time constraints. Within the time allowed for the project of some 10 months of practical work, the development of the test procedure and the testing of selected defined and undefined carbon sources with the developed procedure required the entire time available for the project. To properly test the model carbon source mixtures, a different type of reactor to the one used in the test procedure would be required.

Further work:

Once the release of carbon from the different carbon sources is better understood, a model of carbon source mixtures should be tested in bench scale reactors for their potential to support sulphate reduction in the short-, medium- and long-term. As general problems with the pilot scale systems in the passive treatment project have occurred after 9 to 12 months it is estimated that the scaled down reactors will have to operate for between 6 to 18 months to prove conclusively that sustainable sulphate reduction can be achieved with a given carbon source mixture.

Once suitable carbon source mixtures have been identified, these can also be 'aged' as described above to assess their sustainability further into the life of the treatment system.

- To summarise the test procedure in preliminary guidelines for the evaluation of carbon sources for the use in passive treatment systems.

Although valuable lessons were learned during the project, too many questions remain unanswered at present to develop a full set of guidelines for the testing and evaluation of low-cost carbon sources for use in passive treatment systems. However, a test protocol for the evaluation of potential carbon sources is discussed in Chapter 5.

More experimental work is needed to substantiate the results obtained in this study during the nine months that were available for the development of the method and execution of the experimental work. The figures will then be available as base data for the design of biological sulphate removal systems for the treatment of mine water and other sulphate-rich effluents.

The development of the test method for evaluating the suitability of carbon sources for the biological treatment of mine water was initially aimed specifically at the use of these carbon sources in passive mine water treatment systems. This objective has been achieved, though only for the short-term release of carbon, because the current test method addresses the carbon availability and the concurrent sulphate reduction in the short-term. This covers a time period of several days up to several weeks, with the maximum period of one month. As discussed above, the requirement of passive mine water treatment systems for carbon sources that last for several months or years and the testing of these carbon sources for their release of carbon in the medium- and long-term is currently not addressed with the test procedure. It is foreseen that the development of such test will have to simulate extended time spans by some form of physical, chemical or biological pre-treatment of the prospective carbon sources, which then might be followed by the developed test procedure for comparative evaluation of the carbon sources. However, the development of such a test will require a project, which is conducted over several years rather than several months.

8.2 Recommendations for further research work

In discussing the conclusions from the project, a number of tasks to be addressed in the future have already been discussed. The current project can be considered as a starting point and the outstanding

objectives and any further work proposed in this regard should be followed up as a separate project that should be in the order of a three year project.

In addition, it has become clear that more fundamental knowledge is required with regard to the anaerobic degradation of complex organic carbon sources with a specific focus to obtain selected degradation products in the process. The aim of the research should be to understand the anaerobic degradation process to the extent that the operation of the process in a reactor will allow the production of degradation products such as butyric or lactic acid that, in turn, will allow the sulphate-reducing bacteria to achieve maximal sulphate removal rates. This knowledge would determine if this can be achieved in the same reactor used for the sulphate reduction, or if it would be more beneficial to have pre-fermentation of the complex organic carbon sources in a separate reactor upstream of the biological sulphate removal and to feed the effluent of the digester to the sulphate removal reactor.

It is also advisable to refine the developed test method further and to refine the protocol for the description of the complex carbon sources. The carbon sources that have been tested during this project should be repeated in several replicates. In addition, the test work should be extended to include more potential carbon sources. The intent behind this work, apart from verifying the proposed methodology, is the collection of a broader base of verified kinetic data for sulphate removal under strictly controlled environmental conditions, and to use these data in the development of a dynamic model that describes the biological processes. Results obtained from such a model would allow us to refine our understanding of the complex biological processes and the design of more efficient (and therefore more cost-effective) sulphate removal reactors. The findings of this work would not just be confined to the passive treatment of mine water, but would also be applicable in the wider field of mine water treatment such as low-maintenance and active reactor systems, as well as 'in-situ' treatment of mine water.

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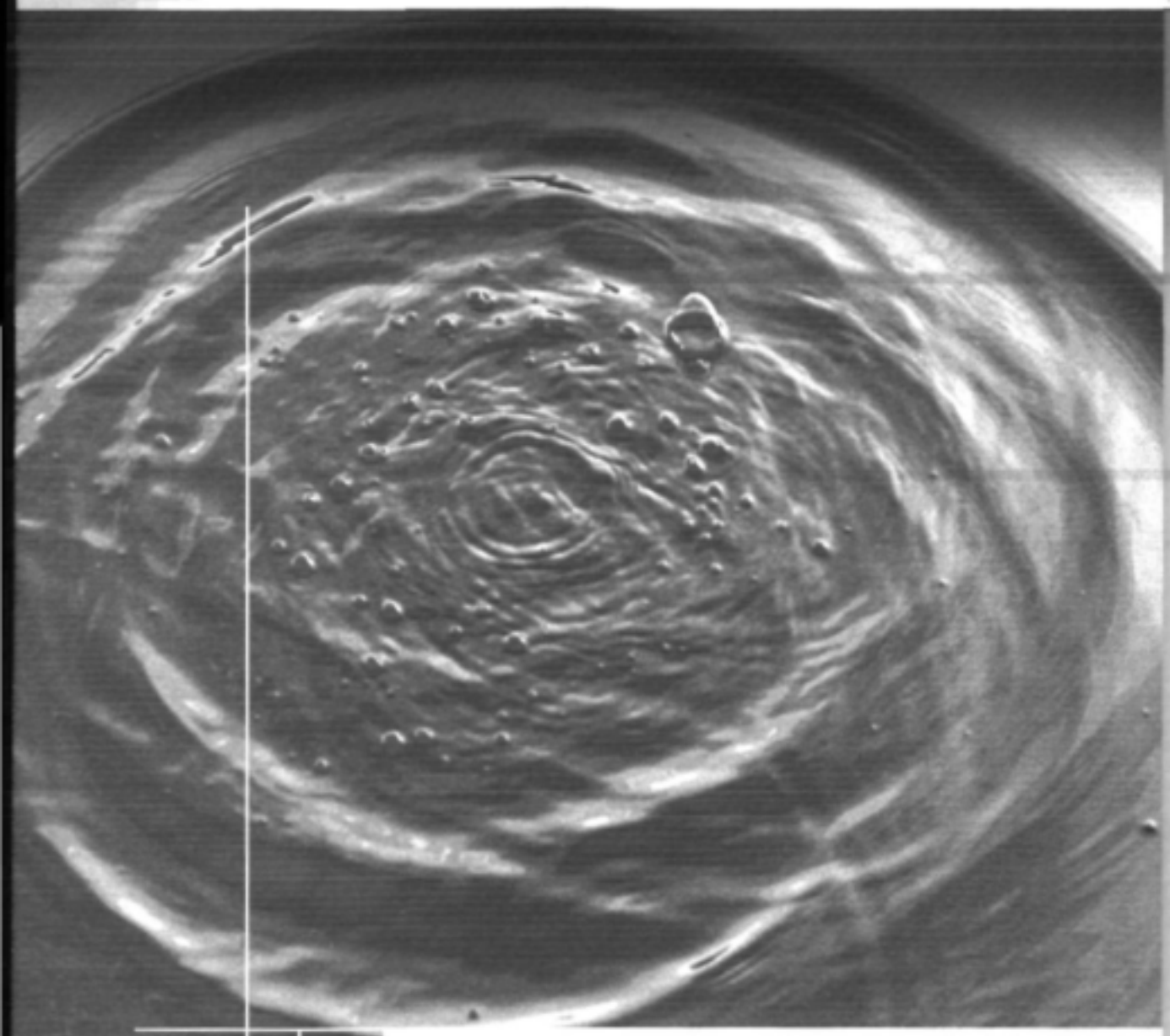

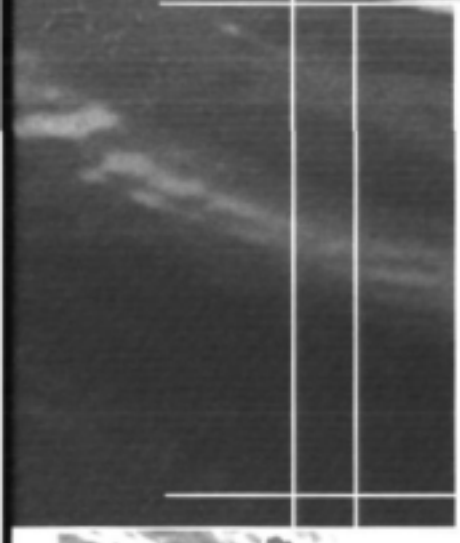

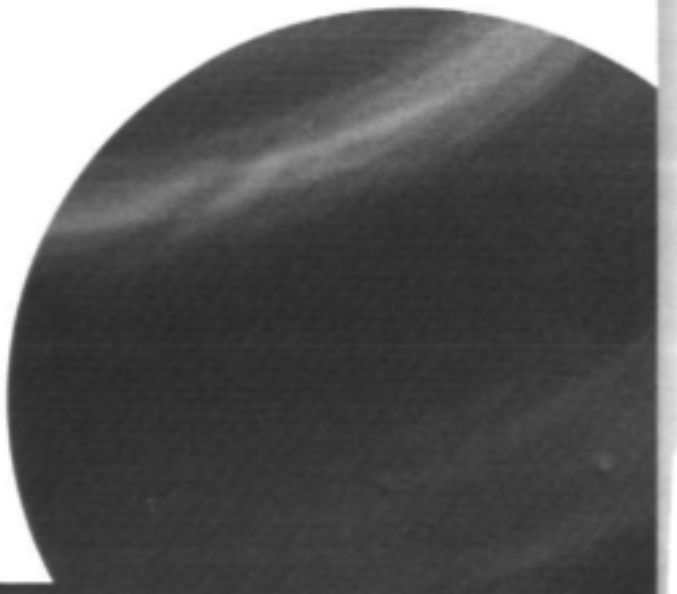

Water Research Commission
PO Box 824, Pretoria, 0001, South Africa
Tel: +27 12 330 0340, Fax: +27 12 331 2565
Web: http://www.wrc.org.za

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