CHAPTER 4

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Biological sulphate reduction in artificial acid mine drainage using different defined carbon sources

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Biological sulphate reduction in artificial acid mine drainage using different defined carbon sources

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The production of acid mine drainage (AMD) containing high amounts of sulphate, heavy metals and low pH is of increasing concern. AMD is highly corrosive and results in economic and environmental problems. The potential of different defined carbon sources to drive sulphate reduction in artificial AMD was studied. This was done in a process for developing a standard laboratory procedure for the evaluation of carbon sources for potential use in passive treatment systems of AMD. Intravenous feeding apparatus (drip-bags) were used as anaerobic bioreactors. These were filled with articicial AMD, an inoculum of anaerobic digester sludge and defined carbon sources. It was found that propionic acid, butyric acid and lactic acid were the carbon sources giving the most effective sulphate reduction, while acetic acid, pyruvate and ethanol did not result in effective sulphate reduction.

Keywords: Acetic acid, acid mine drainage, butyric acid, digester sludge, ethanol, lactic acid, methanol, propionic acid, pyruvate, sulphate reduction

INTRODUCTION

Coal mining often results in the production of effluents containing high amounts of sulphate and heavy metals, generally referred to as acid mine drainage (AMD). AMD is defined as the consequence of the metabolism of sulphur- and iron-oxidising bacteria when pyrite is exposed to atmospheric oxygen and the combination of autoxidation and microbial sulphur and iron oxidation produces large amounts of sulphuric acid (Atlas and Bartha, 1993). AMD is highly corrosive resulting in economic and environmental problems (Dill *et al.*, 1994).

AMD may be remediated by neutralising the effluent and removing the sulphate. Various sulphate removal technologies are available (Dill *et al.*, 1994). These 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 & Strydom, 1985). A high degree of maintenance and operating supervision is required with available treatment strategies. Therefore, there is an increasing demand for inexpensive, environmental friendly technologies for sulphate removal, in order to remediate AMD. One such an alternative is the use sulphate reducing bacteria (SRB) in the biological removal of sulphates from AMD (Dill *et al.*, 1994). Provided a suitable electron donor is available, SRB can oxidise organic compounds like lactate or acetate, with sulphate as electron acceptor being reduced to sulphide (Dill *et al.*, 1994).

Earlier investigations showed that mixed cultures of SRB increased 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). Sulphate can be quantitatively converted 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). Success was achieved using two separate reactors for hydrogen sulphide and sulphur production respectively.

Producer gas, consisting of a mixture of H_2, CO, CO_2 and N_2 generated from coal, has also been used as energy source for biological sulphate removal (Du Preez *et al.*, 1991). During the anaerobic treatment of sulphate rich water in a trickling filter, sulphate was reduced from 1900mg/L to less than 200mg/L.

Van Houten *et al.* (1994) used gas-lift reactors fed with hydrogen and carbon dioxide as energy and carbon source for the reduction of sulphate. It was concluded that when free H_2S concentrations are kept below 450mg/L, a maximum sulphate conversion rate of 30g/l SO_4^{2} /l/d could be achieved after 10 days operation.

In-situ treatment has been used to avoid the release of AMD in the environment (Dill *et al.*, 1994). Different organic materials have been evaluated for the passive treatment of AMD. Wood dust was investigated as a potential economical carbon source for sulphate reduction (Maree and Strydom, 1985). Sulphate removal from mine water was furthermore achieved by using either sugar, pulp mill effluent or sewage as energy

sources (Maree and Strydom, 1985). This was accomplished by providing anaerobic conditions on a solid medium and by keeping the hydrogen sulphide concentration relatively low. It was concluded that recirculation enhanced sulphate reduction, the presence of light increased sulphur production and that 1.6g sugar, 16.7ml spent liquor from a sulphite pulp mill and 172ml raw sludge was required for the removal of 1800mg sulphate. Therefore, under ideal conditions, SRB can be used for the remediation of AMD.

One other method involving SRB which should be considered, is the passive treatment of AMD (Batchelor, 1993). Nevertheless, there is a lack of local and international experience with passive treatment systems designed for the treatment of AMD. This is mainly due to the absence of methods to evaluate the potential use of different undefined carbon sources. To develop efficient passive AMD treatment, the anaerobic sulphate reduction process must be optimised. This can be done by selecting the most appropriate carbon sources, or 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 evaluation studies of undefined carbon sources.

MATERIALS AND METHODS

Bioreactor design

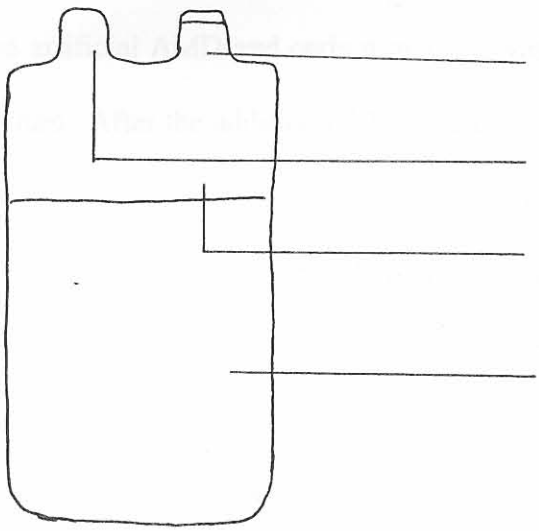

Figure 1. A Schematic representation of the anaerobic bioreactor.

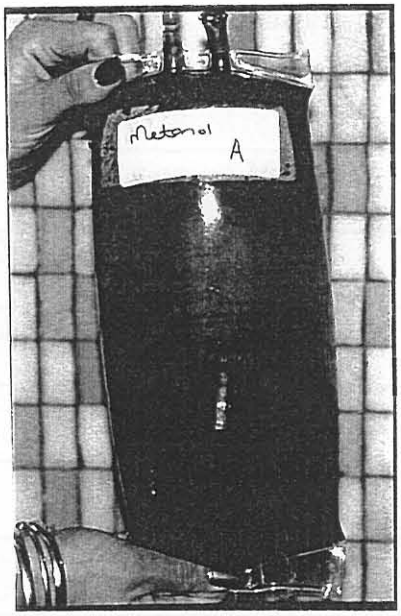

Plate 1. The anaerobic bioreactor (the expansion of the bag indicates gas production under anaerobic conditions).

Inoculum

Inoculum was obtained from an anaerobic digester at Daspoort Water Purification Plant in Pretoria, South Africa. A volume of 300ml was added to 1000ml of artificial AMD (Table 1). The respective carbon source was also added to this mixture (Table 3). The pH of the artificial AMD and carbon mixture was adjusted to 7.2 before the addition of the inoculum. After the addition of the inoculum, the pH was once again adjusted to 7.2-7.5. Of this, 900ml was added to each bioreactor (Fig 1). In order to standardise the inoculum, pH, density, moisture content, temperature, alkalinity and total solids determinations were carried out on unfiltered samples, according to standard analytical procedures (APHA, 1985).

Table 1. Characteristics of the digester sludge used as inoculum

Parameter	**Quantity**
Average pH	6.93
Average temperature (°C)	15
Average alkalinity (mg/L $CaCO_3$)	866.67
Average total solids/L (MLSS) (mg/L)	30.075
Moisture content (%)	96.26
Density	0.805
COD (mg/L)	6000
SRB (cfu)	1.8×10^{-3}

Artificial acid mine drainage

Table 2. Artificial AMD composition.

Component	**Quantity**
$MgSO_4.7H_2O$ (Merck)	1.31g
H_2SO_4 (96%) (Merck)	0.30ml
$FeSO_4.7H_2O$ (Saarchem)	4.56g
NH_4Cl (Labchem)	0.19g
H_2PO_4 (85%)(Merck)	0.02ml

The above chemicals were dissolved in one litre of distilled water. The pH was adjusted to 7.2 using 10M Sodium hydroxide (Merck). This gave an average sulphate concentration of 2500mg/L, resembling an average sulphate concentration in AMD.

Carbon sources

Table 3. Carbon sources used during this experiment.

Carbon sources	**Quantity per 1000ml AMD**
Lactic acid (Merck)	5.56ml
Acetic acid (Merck)	5.95ml
Butyric acid (BDH)	4.80ml
Propionic acid (Saarchem)	5.19ml
Pyruvic acid sodium salt (Merck)	7.64g
Methanol (Merck)	8.44ml
Ethanol (Merck)	6.05ml

The amount of carbon sources as displayed in Table 3, gave a carbon: sulphate ratio of 1:1 (w/w). Each experiment was done in triplicate.

Control experiments

Control experiments were done using 900ml of a mixture of 1000ml of artificial AMD (Table1) and 300ml of inoculum. The pH was adjusted to 7.2-7.5. No extra carbon sources were added. These experiments were also done in triplicate.

Sampling

Drip-bags were shaken every day by hand to achieve a well-mixed suspension. Samples were taken from the bioreactors through a rubber seal using syringes (Promex) with a gauge of 1.00mm (Fig 1). To exclude the possibility of H_2S inhibition on the biological processes, excess gasses were removed as required through the rubber ports (Fig 1).

Chemical analysis

Alkalinity and pH determinations were carried out according to analytical procedures as described in Standard Methods (APHA, 1985). The pH and the alkalinity of the different bioreactors were monitored every 2-3 d. A SQ118 spectroquant (Merck) was used to determine the amount of sulphate reduction using kit no. 1.14791.0001. This was done every 2-3 d. The Chemical Oxygen Demand (COD) was monitored every 2-3 d using a SQ118 spectroquant (Merck). All analyses were carried out on unfiltered samples. Experiments were monitored over a time period of 27 d.

RESULTS

Sulphate reduction, COD utilization, pH and alkalinity of AMD with different defined carbon sources

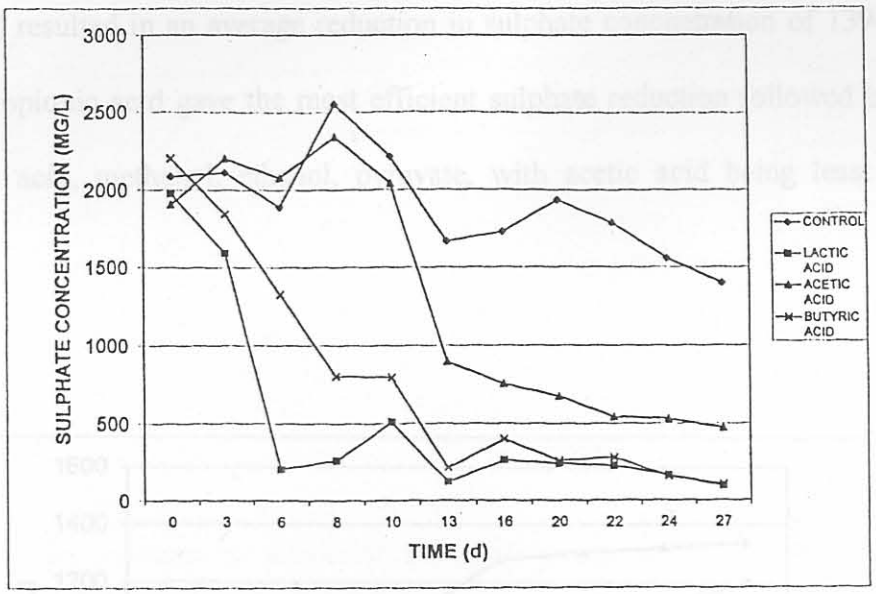

Figure 2. Sulphate reduction of AMD with lactic acid, acetic acid and butyric acid as carbon sources

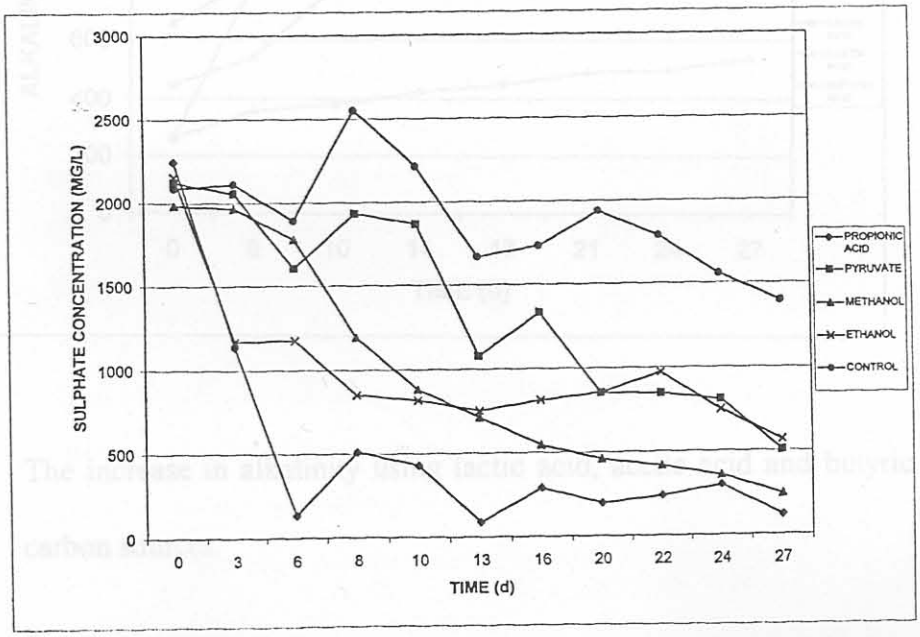

Figure 3. Sulphate reduction of AMD with propionic acid, pyruvate, ethanol and methanol as carbon sources.

The average initial sulphate concentration of the different experiments was 2084.25 mg/L (Table 4). After 6d propionic acid and lactic acid resulted in an average reduction in sulphate concentration of 2000mg/L (Fig 3) and 1770mg/L (Fig2), respectively. Butyric acid (Fig 3) resulted in an average reduction in sulphate concentration of 1394.33mg/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 4).

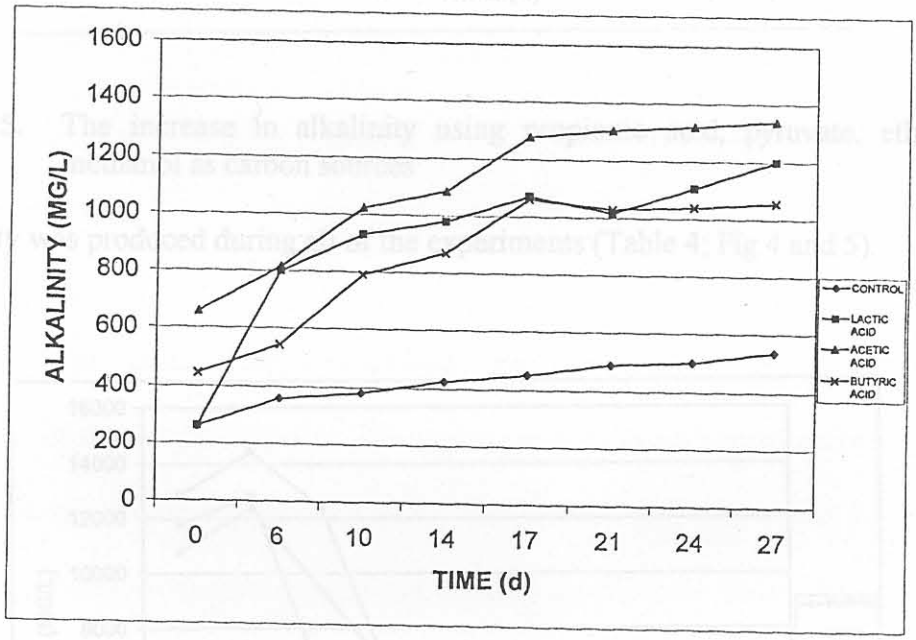

Figure 4. The increase in alkalinity using lactic acid, acetic acid and butyric acid as carbon sources.

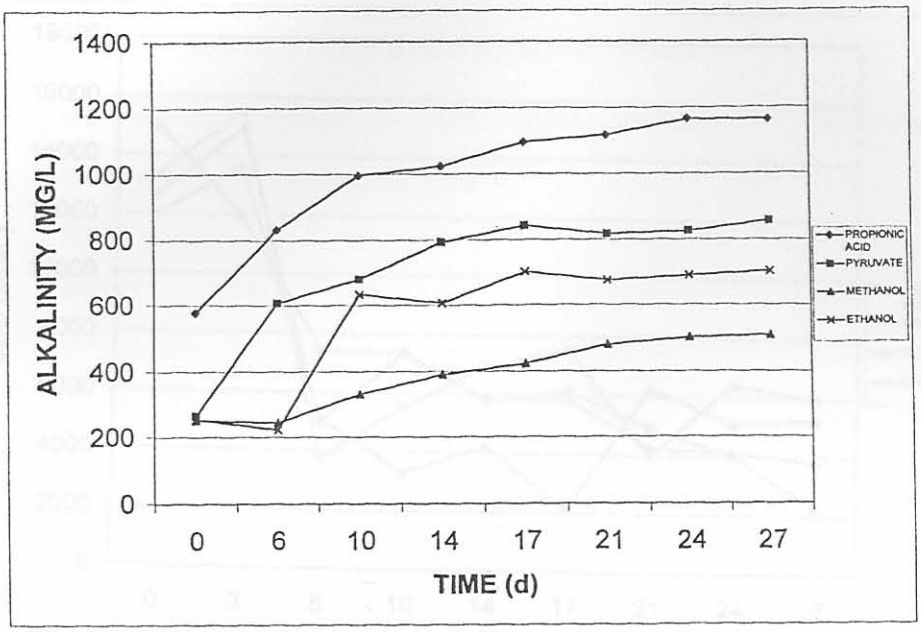

Figure 5. The increase in alkalinity using propionic acid, pyruvate, ethanol and methanol as carbon sources

Alkalinity was produced during all of the experiments (Table 4; Fig 4 and 5).

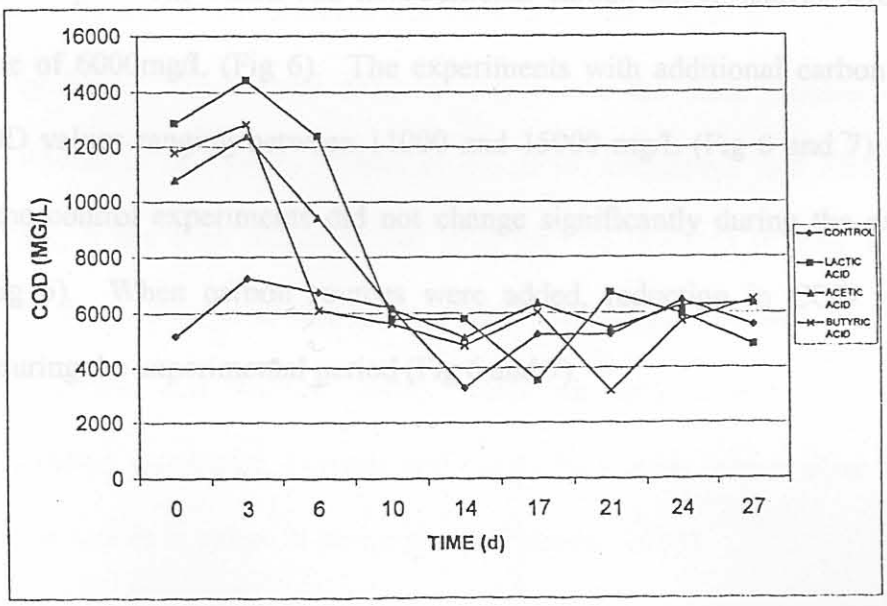

Figure 6. COD decrease using lactic acid, acetic acid and butyric acid as carbon sources

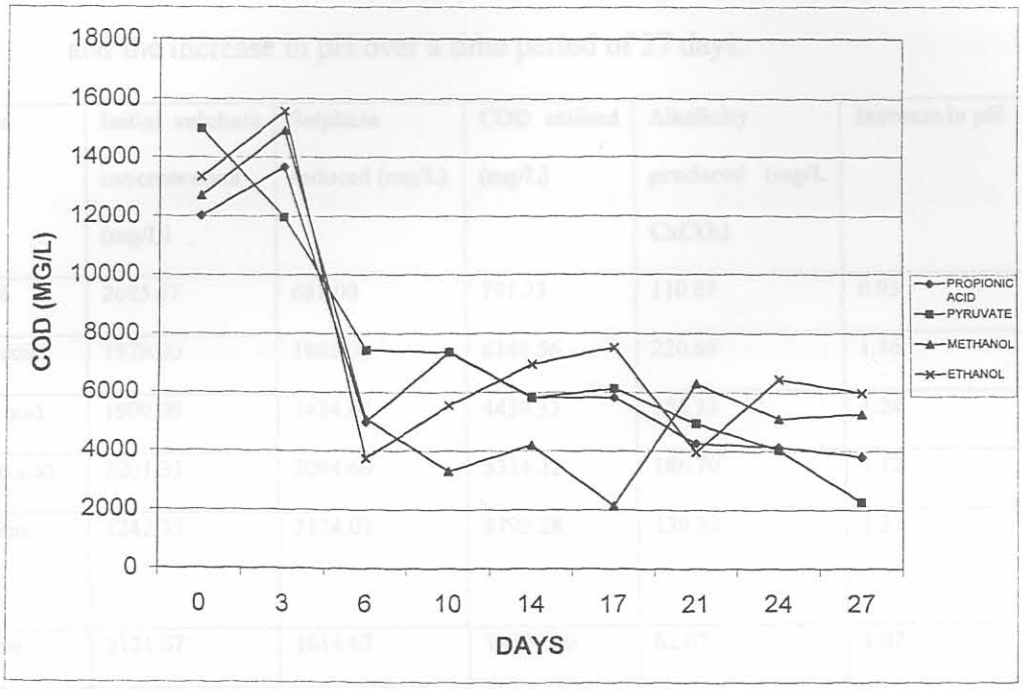

Figure 7. COD decrease using propionic acid, pyruvate, ethanol and methanol as carbon sources

The control experiment which had no additional carbon added had an average initial COD value of 6000mg/L (Fig 6). The experiments with additional carbon added had initial COD values ranging between 11000 and 15000 mg/L (Fig 6 and 7). The COD value of the control experiments did not change significantly during the experimental period (Fig 6). When carbon sources were added, reduction in COD values were observed during the experimental period (Fig 6 and 7).

Table 4. The average amount of sulphate reduced, COD utilised, alkalinity produced and the increase in pH over a time period of 27 days.

Carbon source	Initial sulphate concentration (mg/L)	Sulphate reduced (mg/L)	COD utilised (mg/L)	Alkalinity produced (mg/L $CaCO_3$)	Increase in pH	Mg COD per mg SO_4^{2-} reduced
Control	2085.67	687.00	791.33	110.67	0.93	1.15
Lactic acid	1979.00	1885.00	6144.56	220.67	1.16	3.25
Acetic acid	1909.00	1434.67	4439.33	255.33	1.24	3.09
Butyric acid	2201.33	2094.66	5314.22	186.70	1.12	2.54
Propionic acid	2242.33	2124.03	8192.28	139.33	1.21	3.86
Pyruvate	2121.67	1614.67	12706.00	62.67	1.07	7.87
Methanol	1982.67	1739.00	6391.00	116.67	1.02	3.68
Ethanol	2152.33	1585.66	7345.67	97.3	1.62	4.63

DISCUSSION

Widdel and Pfennig (1982) indicated that some short-chain fatty acids could be directly oxidized by SRB. The sulpate 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, 1982). SRB 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, from an energy perspective, was a poor substrate. This might be because, the energy required for acetate uptake at very low concentrations exceeds the energy gained from acetate metabolism, thereby limiting the acetate uptake at a certain threshold concentration. Some reports indicate a predominance of SRB growing on acetate, while a number of 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 (Qatibi *et al.*, 1990). Acetotrophic methanogens completely predominated over acetate-oxidising SRB on acetate (Qatibi *et al.*, 1990). Although sulphate reduction did occur during our studies, it was not as efficient as with 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 both 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 outcompeted 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 digestor sludge was used as inoculum during this study, which consisted mostly out 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 4).

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 4). 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 with a concomitant reduction of sulphate to sulphide, in the presence of sulphate. 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 intensely smelling organic sulphur compounds that seem to affect the SRB (Zehnder, 1988). Ethanol and pyruvate was not as sufficient as the other carbon sources tested for the reduction of sulphate (Table 4, Fig 3). 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 ehanol, they did not metabolize 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 4).

The limited sulphate reduction which occurred in the control experiments was ascribed to the carbon present in the digester sludge, used as inoculum. No significant change in the COD values of the control experiments occurred. This was not unexpected, due to the fact that most of the carbon in the anaerobic digestor sludge was already used during

the anaerobic waste water treatment processes in the digester. When additional carbon source was added, efficient sulphate reduction occurred (Table 4).

The process of sulphate reduction is accompanied by the production of alkalinity in the form of bicarbonate ions. This reacts 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 (Fig 4 and 5). This confirmed 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.

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