

Biological sulphate removal using hydrogen as the energy source.

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ABSTRACT

Large areas of polluted underground and surface Acid Mine Drainage (AMD) occur in South Africa, causing a negative environmental impact on natural vegetation, rivers, watersheds, natural habitat and aquatic life. At the CSIR, biological sulphate removal technology has been developed for the treatment of sulphate rich effluents such as AMD, using ethanol or sugar (or a combination thereof) as the carbon and energy source (Greben et al., 2000). The aim of this study was to investigate the utilisation of a mixture of H₂ and CO₂ (80%: 20%), as the energy and carbon source respectively for biological sulphate removal. In order to obtain good mass transfer from gas to liquid phase in the reactor, a column reactor was operated, containing geotextile rods as immobilisation material. The feed entered the reactor from the top, while the gas was pumped into the recycle line, thus enabling good contact between the gas and liquid.

The results of this study showed that the sulphate was removed consistently, from an average of 1250 mg/l in the feed water, to a sulphate concentration of less than 200 mg/l in the treated water, while the average Hydraulic Retention Time (HRT) was 24 h. Constant sulphate removal was demonstrated when the reactor temperature was >20°C.

KEYWORDS: biological sulphate reduction, column reactor, hydrogen, geotextile, temperature

INTRODUCTION

AMD is an acidic, metal-bearing wastewater, generated through the biological oxidation (*Thiobacillus* species) of metal sulphides (FeS) to sulphates in both active and abandoned mining operations. When the biological sulphate removal technology is applied, the Sulphate Reducing Bacteria (SRB) reduce the sulphate to sulphide. In addition, alkalinity is produced, which results in a pH increase, thus neutralising the AMD. The SRB favour anaerobic conditions for biological sulphate reduction.

When sulphate is reduced to sulphide, a carbon and energy source is needed, which functions as the electron donor. Du Preez (1992) and Van Houten (1996) showed the use of hydrogen as the preferred electron donor. An optimum of 30 g SO₄/(l.d) removal was obtained when Van Houten (1996) conducted his study. Van Houten (1996) showed that in order to create a suitable environment for the reduction of sulphate by hydrogenotrophic SRB (HSRB), the pH should be between 6.5-8.0; the temperature should be between 20 – 35°C; the H₂S concentration should be less than 450 mg/l; the system should be completely anaerobic; the biomass should be immobilized and the retention of the active biomass should be high; the gas should be in the ratio: H₂:CO₂ 80%:20%; the hydrogen mass transfer should be maximized; there should be a high gas hold-up (through the system recycle) and there should be a low bubble diameter (small gas bubbles) (Van Houten *et al.*, 1996).

Biochemically, sulphate reduction is coupled to the formation of an energy-containing compound called Adenosine Triphosphate (ATP), by a Proton motive force (PMF), derived from electron transport. Sulphate is reduced in a stepwise fashion to hydrogen sulphide gas by intracellular enzymes, necessitating transport of sulphate from the external environment by active transport, involving ATP expenditure. Inside the cell, sulphate is reduced to sulphite and hydrogen sulphide gas by a collection of enzymes (ATP sulfurylase and adenosine phosphosulphate (APS) reductase). Sulphide is formed by the action of sulphite reductase, during which action sufficient energy is released for the formation of 2 to 3 ATP moles per mole of sulphite reduced. Hydrogen serves as the electron donor for the reduction of sulphite by the action of an electron transport chain. This requires the presence of hydrogenase enzymes, which contain Ni (Daniel R. Caldwell, 1995).

The purpose of this investigation was to evaluate biological sulphate removal with H₂ and CO₂ (80%:20%) as the energy and carbon source.

The specific aims of this study were to investigate:

1. The suitability of the tall column reactor, using Geotextile as the immobilization material.
2. The effect of the H₂/SO₄ ratio on the COD concentration of the treated water.
3. The effect of different temperatures on the sulphate reduction rate.

MATERIALS AND METHODS

The Feed water contained 1050 mg/l sulphate and was made up as follows:

Table 1. Composition of Feed water and nutrients

Feed water		
Chemical compound	Unit	Amount
MgSO ₄ :	g /l	1.9
(NH ₄) SO ₄ :	mg /l	100
CaSO ₄ :	mg/l	100
FeSO ₄ :	mg /l	2
H ₃ PO ₄ :	mg /l	50
Nutrients:	ml /l	1
Nutrients		
MnCl ₂ .4H ₂ O:	µg/l	1
ZnCl ₂ :	µg/l	1
Na ₂ MoO ₂₄ :	µg/l	1
H ₃ BO ₃ :	µg/l	1
CuCl ₂ .2H ₂ O:	µg/l	1
KCl:	mg/l	1
FeCl ₃ .4H ₂ O:	mg/l	1
NiNO ₃ .6H ₂ O:	mg/l	1
CoCl ₂ .6H ₂ O:	mg/l	1

REACTOR

A tall column reactor was operated (Fig.1, Table 2) to obtain good mass transfer from gas to liquid phase in the reactor. The feed entered the reactor from the top, while the gas was pumped into the recycle line, thus providing good contact between the gas and liquid. Long Geotextile strips were used as the immobilization material, with the aim to support bacterial growth. Geotextile is a coarse, fibrous material, used in road construction. Due to its rough texture, microbial biofilms can easily grow on geotextile.

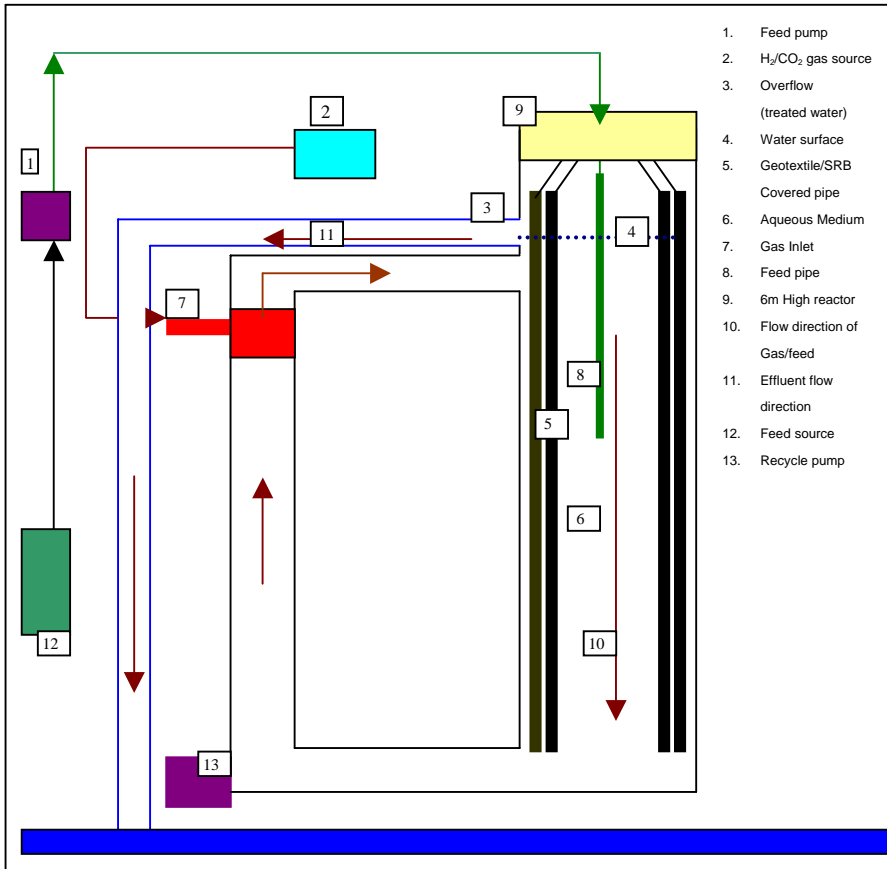


Figure 1. The hydrogen contact reactor

Table 2. Dimensions of the anaerobic column reactor

Description	Unit	Measurement
Total volume:	ℓ	190
Active volume:	ℓ	157
Length thick pipe:	m	6
Length thin pipe:	m	5
Thick pipe radius:	cm	10
Thin pipe radius:	cm	2.5
Geotextile % of active volume:	%	8.8

BIOMASS

The reactor was inoculated with the following sludges:

- Anaerobic Digester sludge, obtained from the Daspoort Sewage Plant, Pretoria, South Africa as the inoculum for the reactor.
- Sulphate Reducing Bacteria Mixture from the CSIROsure demo plant, Navigation Mine, Witbank, operating with sugar as the carbon and energy source.

EXPERIMENTAL

The column reactor was operated continuously for approximately 120 days. The HRT was kept constant at 24 h.

The following parameters were varied during the investigation:

- Ratios of Hydrogen added / the amount of Hydrogen needed to reduce the Sulphate in the Feed (5; 4; 3.5; 2.5; 2; <2)
- Temperature (23, 20 and 18°C). The reactor ran for a period of 9-19 days at each temperature.

ANALYTICAL

The sulphate, sulphide, alkalinity, COD, and pH were manually determined according to the analytical procedures as described in Standard Methods (APHA, 1985). The analyses were all carried out on filtered samples, except for the feed COD and the sulphide samples. The alkalinity of the samples was determined by titrating with 0.1N NaOH to a pH of 9.0. The COD samples were pre-treated with a few drops of H₂SO₄ and N₂ gas to correct for the COD value caused by the sulphide concentration. All VFA analyses were done using a gas chromatograph (Hewlett Packard. HP 5890 Series II) equipped with a flame ionisation detector (GC/FID), while the data analyses were done using the Chem Station, supplied by Hewlett Packard, software package. The column used was a HP-FFAP, 15 m x 0.530 mm, 1 micron. An outline of the GC/FID programme used is depicted in Table 3. The N₂ flow rate was set at 1 mL/min.

Table 3. The GC/FID programme for the detection of VFA

Parameter	Setting
Initial oven temperature (°C)	30
Initial time (Min)	2
Temperature programme: (°C)	80
Rate (°C/min)	25
Final temperature (°C)	200
Final time (min) 1	
FID temperature (°C)	240

RESULTS AND DISCUSSION

TEMPERATURE

Table 4 shows the effect of temperature on the reaction rate. It is noted that the rate increased from 0.6 g SO₄/(ℓ.d) at 18°C to 1 g SO₄/(ℓ.d) at 23°C. The indicated results were obtained with a reactor filled with 8% geotextile. If the SO₄ reduction rate is linearly related to the geotextile volume, the volumetric SO₄ reduction rate will amount to 10 g SO₄/(ℓ.d) for a reactor filled with 80% geotextile. Figure 2 shows the SO₄ removed amounted to 950, 750 and 610 mg/ℓ SO₄ at temperatures of 23, 20 and 18°C respectively.

Table 4. Effect of Temperature of the SO₄ removal rate and chemical composition of feed and treated water.

Determinand	Unit	Temperatures (°C)		
		23	20	18
Feed:				
Sulphate	mg/ℓ	1058	1286	1405
Alkalinity	mg/ℓ	n.a.*	596	205
pH		7	7	7
Treated:				
Sulphate	mg/ℓ	108	536	795
Alkalinity	mg/ℓ	1139	1311	929
pH		8.2	7.3	7.6
Sulphides	mg/ℓ	318	178	205
Rates:				
Sulphate reduction	g/(ℓ.d)	1	0.7	0.6
Ratios:				
S ²⁻ /SO ₄		0.3	0.24	0.3
Alkalinity/SO ₄		1.2	0.9	1.2

*Not analysed

The sulphate removal during the three experimental periods is presented in Figure 2.

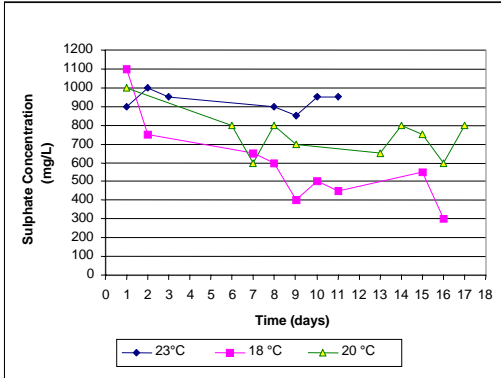


Fig.2. Sulphate removed (difference between sulphate concentration in feed and effluent) in the Hydrogen Contact Reactor when the temperatures were 23, 18 and 21°C.

Studies by Lens *et al.* (1998) showed that mesophilic SRB and Methanogenic Bacteria (MB) have similar temperature ranges, and therefore respond to similar temperature conditions; however SRB seem less sensitive to temperature changes than MB. For example, in continuous reactors, with a decrease of the reactor temperature from 35 to 25°C over a period of 30 days, the fraction of electron flow used by SRB increased from 43 to 80% (Lens *et al.*, 1998). This theory is supported in this research by good, continuous sulphate removal at ambient temperatures.

SULPHIDE PRODUCTION

During the first two periods when the temperature was 23 and 18°C, it could be seen that the theoretical ratio of Sulphide_{Produced}/Sulphate_{removed} (0.33), correlates with the ratio (0.3) obtained in practice (Table 4). During the period where the temperature was 20°C, however the S²⁻/SO₄ ratio was 0.24 instead of the theoretical value of 0.3. An explanation for this could be the influence of pH on the sulphide solubility:



The chemical equation above (1)(Greben *et al*, 2000), shows that H₂S (g) (the toxic form of sulphide that causes inhibition of SRB) is formed at pH < 6.5, while HS⁻ is formed at pH >6.5. (Lens *et al.*, 1998). In Table 4, it can be seen that the average pH during the third experimental period was 7.3, which was the lowest pH value observed of all three experimental periods. Therefore, the possibility exists that more H₂S gas was formed during this period, which can cause the partial escape of the H₂S in gas form, resulting in a lower sulphide concentration in the effluent, and thus in a lower experimental Sulphide_{produced}/Sulphate_{removed} ratio during period 3.

The average experimental alkalinity/sulphate ratio was 1.1 (Table 4), which correlates with the theoretical ratio of 1.04.

The pH of the Hydrogen Contact Reactor was kept between 7.3 and 8.2 for optimal sulphate removal (Van Houten, 1996). When deciding on the optimal pH for operating the sulphidogenic bioreactor, there are several factors of pH that should be kept in mind. The optimal pH for

ASRB is 7.3 – 7.6, while the optimal pH for AMB is 6.5 – 7.8. ASRB can also tolerate higher pH values than the AMB.

BIOFILM FORMATION AND GEOTEXTILE

Furthermore, it can be assumed that the good sulphate reduction in the reactor could be ascribed to the biofilm formation of the SRB, thus preventing washout of the microorganisms. Figure 3 shows the biofilm formation on the geotextile strip, as occurring inside the tall Hydrogen Contact Reactor.

Biofilm formation does not only favour nutrient uptake, but attachment to a surface also stabilizes the organisms and enables maintenance of community integrity (Cloete, 1994; Wolfaardt *et al.*, 1994). Syntrophy exists between the different microorganisms participating in the biofilm. The homoacetogens use the H₂ and CO₂ to produce acetate, which the SRB can then use as the carbon source (Van Houten, 1996). He used pumice particles as the immobilization material for biofilm formation in his study.

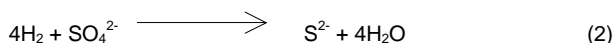


Fig.3. Geotextile used in the Hydrogen Contact Reactor before biofilm formation (top) and after biofilm formation (bottom).

METABOLIC END PRODUCTS

Hydrogen can be utilized as an energy source by SRB for sulphate reduction or by Homoacetogenic Bacteria to produce Volatile Fatty Acids (VFA) (such as Acetic Acid). Table 5 shows the effect of various H₂/SO₄ ratios on VFA formation.

The stoichiometric amount of hydrogen required for sulphate reduction is 8gH₂/96 g SO₄ (reaction 2).



It is noted that:

- An excess dosage of Hydrogen, 5 times that of which is stoichiometrically required, resulted in a COD concentration of 1226 mg/l (as O₂) in the treated water, while for an excess dosage of less than 2, the COD of the treated water was less than 50 mg/l O₂.
- Acetate was the main VFA produced. Small amounts of Propionic, Butyric and Valeric Acid were detected.

Table 5. The effect of H_2/SO_4 ratios on VFA formation

Stoichiometric excess	COD concentration (mg/l) O_2	Volatile Fatty Acids (mg/l)			
		Acetic Acid	Propionic Acid	Butyric Acid	Valeric Acid
5	1226	352	36	28	28
4	546	238	15	13	14
3.5	314	123	0	0	6
2.5	201	73	3	0	1
2	131	23	7	7	9
<2	<50	0	0	0	0

The formation of volatile fatty acids during excess H_2 dosages can be ascribed to the activity of Homoacetogenic bacteria, which use the CO_2 and H_2 to form acetate (reaction 3). The Hydrogenotrophic SRB, a heterotrophic SRB, can use the formed acetate as its carbon source. The acetate consuming SRB cannot use the CO_2 directly, as only the autotrophic bacteria can utilise the CO_2 .



This is consistent with the theory that sulphidogenic bioreactors treating sulphate rich wastewater are complex ecosystems, containing many bacterial species. In these mixed cultures, competition exists between the different species for the use of sulphate as a substrate. In bioreactors containing sulphate, competition exists for the carbon and energy source: The Methanogenic bacteria (MB) want to use it for CH_4 formation; while the SRB want to use it for sulphate reduction, and the acetogenic bacteria (AB) want to use it for acetate formation (Lens *et al.*, 1998). The outcome of this competition will determine the formation of the end products of the anaerobic mineralization processes. When using hydrogen as energy source, the most important competition is the competition between HSRB and Hydrogenotrophic Methanogenic Bacteria (HMB). The HSRB out compete the HMB, as the HSRB gain more energy from the consumption of molecular hydrogen and therefore have a higher substrate affinity, growth rate and cell yield than the HMB. The HSRB also maintain the hydrogen concentration below the threshold value of the HMB, thus preventing the HMB from using the hydrogen. This theory explains the inhibition of the HMB when sulphate forms part of the chemical components in the wastewater (Lens *et al.*, 1998).

The type of seed sludge and experimental runtime can also influence the outcome of the competition between acetate utilizing bacteria. A long period of time is needed for some types of bacterium to out compete other species, where a steady state situation needs to be reached, and where the number of one of the competing species is very low compared with the other. Therefore, the choice of seed sludge is, in addition to the applied environmental conditions, of utmost importance (Lens *et al.*, 1998). It has been shown that the Acetogenic SRB (ASRB) need 200 – 400 d to out compete the acetogenic MB (AMB), when the COD/SO_4 ratio is <1 . When an oversupply of sulphate is present, the ASRB will win the competition for acetate (Visser, 1995).

CONCLUSIONS

The following conclusions can be made from this study:

1. Good sulphate removal was observed, using a tall column reactor, operating on a mixture of hydrogen and carbon dioxide as the energy/carbon source. At a temperature of 23°C in a reactor filled with 8% geotextile, the volumetric SO₄ reduction rate amounts to 1g SO₄/(ℓ.d) (equivalent to 10 g SO₄/(ℓ.d) if the reactor is filled with 80 % geotextile).
2. When a high excess of hydrogen (5) was added, more than the Hydrogen needed for the stoichiometrical reduction of sulphate in the feed, the COD concentration was as high as 1226 mg/ℓ. The COD concentration then reduced when the hydrogen excess was decreased, until the COD concentration was <50 mg/ℓ in the reactor when the excess hydrogen was reduced to <2.
3. Geotextile is a suitable immobilization material for the SRB, as no washout of microorganisms could be observed.

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