COD/SO_4 ratios using propionate and acetate as the energy source for the biological sulphate removal in Acid Mine Drainage

HA Greben[1], M Tjatji[1] and JP Maree[1]

[1]Division for Water, Environment and Forestry, CSIR, PO Box 395, Pretoria 0001, South Africa
Tel: + 27 12 841 2278 Fax +27 12 841 2506
E-mail Address:hgreben@csir.co.za

Abstract

To reduce the sulphate concentration in Acid Mine Drainage biological sulphate reduction technology can be applied. The purpose of this study was to determine the sulphate reduction rates when different concentrations of propionate were added to the feed water as well as to determine the residual COD concentration under these conditions. In addition the sulphate reduction rate was compared using acetate as opposed to propionate as the energy source for the biological sulphate reduction. The results of the study showed that the use of propionate as the energy source resulted in a 78% sulphate removal, while the removal was 55.5% when acetate was the energy source. Varying the propionate concentration in the feed resulted in the highest sulphate reduction rate at a feed COD/SO_4 ratio of 0.69. At this ratio, the residual COD in the treated water was 571 mg/ , while at a higher feed COD/SO_4 ratio the residual COD in the treated water was 1162 mg/ .

Introduction

Acid Mine drainage (AMD) is the result of mining activities, due to the exposure of pyrite to oxygen and water. Bacterial oxidation of sulphide minerals is the major factor in the formation of acid mine drainage, a common environmental problem in coal mining regions. To reduce the sulphate concentration in AMD biological sulphate reduction technology can be applied. In order to achieve biological sulphate reduction anaerobic conditions, favoured by the sulphate reducing bacteria (SRB) and the presence of suitable carbon and energy sources, have to be adhered to.

The selection of the carbon and energy source depends on the costs of the electron donor added per unit reduced sulphate, and on the potential for pollution due to the additive in the waste stream. When considering the affinities of SRB, Acetogenic Bacteria (AB) and Methanogenic Bacteria (MB) for substrates such as acetate and propionate it is evident that these groups of bacteria may out-compete each other for their preferred substrate. In the sulphate reducing stage, a complete reduction of sulphate to sulphide is desired. Channelling of reducing equivalents towards the SRB is enhanced by the ability of the SRB to effectively compete with other anaerobic bacteria for the available organic substrate and the sensitivity of other bacteria for sulphide (Lens et al., 1998). The anaerobic process can become very complex in the presence of sulphate, because sulphate reducers will compete with MB for compounds such as acetate and hydrogen, and with AB for compounds such as propionate and butyrate (Colleran et al., 1995). Many researchers (Lens et al., 1998, Vallero et al., 2003) have reported that acetate is the most recalcitrant VFA (Volatile Fatty Acid), and is the rate limiting factor (Visser et al., 1993) in sulphidogenic reactors. Its use results in a final COD concentration of 200-500 mg/ℓ (Greben et al., 2000). The study of Ghigliazza et al., (2000) concentrated on the treatment of gypsum-rich wastewater, using propionate as the organic carbon source. This carbon source was chosen as it is an important intermediate product, commonly found in anaerobic fermenting processes. Results of that study indicated that at a $\text{Prop}_{\text{start}}$ (g.ℓ)/$SO_4^{2-}{}_{\text{start}}$ (g.ℓ) ratio of 1.31, a 99.5% SO_4 removal at a hydraulic retention time (HRT) of 2 days could be achieved. This ratio could approach 1, after a longer acclimatization period. This finding agreed with that of other researchers indicating that sulphate removal efficiency improved with time (Visser, 1995). While good propionate utilization as well as efficient sulphate reduction could be observed, the acetate concentration in the treated water increased to constant levels as high as 1,2 g/ℓ. This observation confirmed the absence of acetate utilizing SRB and the finding of Visser (1995) that acetate is the most recalcitrant VFA to degrade under anaerobic conditions.

The aim of this study was 1) to compare the utilisation of propionic and acetic acid as the carbon and energy source in two identical sulphidogenic reactors and 2) to study the sulphate reduction rates when using different concentrations of propionate as the carbon and energy source in the feed (0.5, 0.6, 0.7 and 1.0 mℓ/ℓ feed).

Materials and Methods

Feed water

Artificial sulphate rich feed water was made up of magnesium sulphate so that the final sulphate concentration was approximately 1200 mg/ℓ. A weak $NaHCO_3$ (5 g/ℓ) solution was added to the feed water to maintain the reactor pH between 7 and 8. Macro and micro nutrients were added for biomass supplementation. The chemical composition of the feed water is presented in Table 1. The reactor feed rate was maintained at 2 ℓ/d, which resulted in a HRT of 1 d.

Table 1. Chemical composition of the feed water

Compound	**g/ℓ**
$MgSO_4.7H_2O$	2.5
$NaHCO_3$	3
Micronutrients	**µ.g/ℓ**
KCl	1
$FeCl_3.4H_2O$	0.3
$CONO_3.6H_2O$	0.7
$NiCl_2.6H_2O$	0.8
Trace elements	**µ.g/ℓ**
$MnCl_2.4H_2O$	1
$ZnCl_2$	1
$Na2MO_4.2H_2O$	1
H_3BO_3	1
$CuCl_2.2H_2O$	1

Reactors

Two packed bed reactors, R1 and R2, (Figure 1) were operated. Each had a volume of 2ℓ, and two-thirds of their total volume was filled with ceramic rings as support medium for the micro organisms. The feed water entered the reactor at the bottom, while the effluent was discharged at the top of the reactor. A recycling stream was pumped from the top to the bottom inlet of the reactor. The recycle flow was maintained at 4 times the influent flow rate.

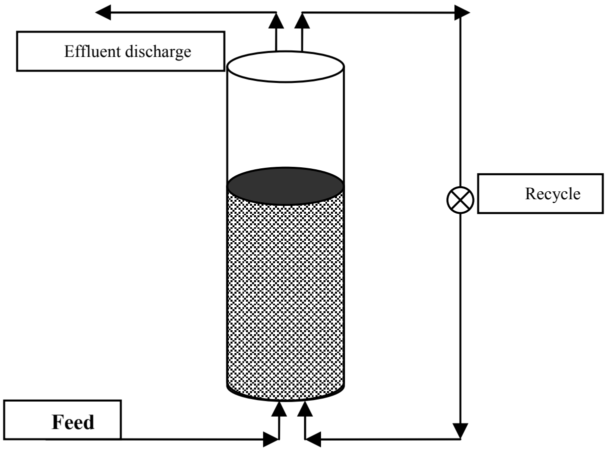

Fig 1. Schematic overview of packed bed reactor

Carbon and energy source

For reactor R1, acetic acid was used as the carbon and energy source while propionic acid functioned as the carbon and energy source for R2. Both acids were obtained from Fluka Chemika, Johannesburg, South Africa. The COD of 1 mℓ acetate/ feed is ≈1092 mg/ℓ, while for 1 mℓ propionate/ℓ feed this is ≈1512 mg/ℓ.

Experimental methods

The reactors were inoculated with sludge from the anaerobic digester at the Daspoort sewage plant, Pretoria, South Africa. During the first month of operation R1 and R2 were operated in batch mode. When sufficient sulphate reduction could be observed, the reactors were operated in continuous mode for 6 months before reported monitoring of the reactor operations commenced. R1 received 1 mℓ acetic acid/ feed and was monitored for 55 days, while the propionic acid volumes to the feed water of R2 varied, resulting in 4 different experimental periods of which the conditions are given in Table 2. The total experimental period of R2 lasted for 77 days. Both reactors were operated at 25 °C while the pH of the reactors was maintained between 7 and 8 due to the initial addition of $NaHCO_3$ to the feed water. The reason for decreasing the amount of the carbon and energy source in the feed was to decrease the feed COD/SO_4 ratio, with the aim to try to eliminate the competition for the substrate between the SRB and MB and AB, as well as to decrease the residual COD in the treated water.

Table 2. The experimental periods of R2

Period	Days	Propionate m / feed
1	1-35	1
2	36-48	0.5
3	49-64	0.6
4	65-77	0.7

Analytical methods

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, the redox and the sulphide samples. The redox potential of the samples was calculated from the mV and stabilization temperature measured with a 744 pH meter (Metrohm). The alkalinity of the samples was determined by titrating with 0.1N HCl to a pH of 4.3. The COD samples were pre-treated with a few drops of H_2SO_4 and N_2 gas to correct for the COD value caused by the sulphide concentration.

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 nm, 1 micron. The N_2 flow rate was set at 1 mℓ/min.

Results and Discussion

Sulphate removal, rates and ratios

The average sulphate concentration in the feed and in the treated water, as well as the rates and ratios resulting from the total duration of the full experimental periods in R1 and R2 are given in Table 3. The data in Table 3 show that although the feed conditions in R1 and R2 are very similar, the parameters in the treated water differed, showing an overall better sulphate removal rate in R2, as can be noted by a slightly higher pH, a higher sulphide (217 versus 176 mg/ℓ) concentration and lower redox value in R2. The percentage sulphate removal in R1 and R2 is 55.5% and 78%, respectively, while the sulphate removal rate in R1 is 0.63 g SO_4/(ℓ.d) as opposed to 0.95 g SO_4/(ℓ.d) in R2.

Table 3. The experimental data of the continuous operation of R1 and R2

Determinant	Unit	R1 (Acetate)	R2 (Propionate)	
Feed				
Sulphate	mg/ℓ	1139	1242	
COD	mg/ℓ	992	980	
Alkalinity	mg/ℓ	2214	2469	
pH		7.69	7.72	
Treated				
Sulphate	mg/ℓ	511	345	
COD	mg/ℓ	512	571	
Alkalinity	mg/ℓ	2867	3025	
Sulphide		176	217	
pH		7.88	7.93	
Redox	mV	-131	-145	
SO_4 removal	%	55.5	78	
Rate				
SO_4 reduction rt	g SO_4/(.d)	0.63	0.95	
Ratios (Experimental)				**Theoretical ratio**
Feed COD/ SO_4 ratio		0.87	0.79	
$COD_{used}/SO_{4\ rem.}$		0.84	0.42	0.67

The feed COD/feed SO_4 ratio in R1 and R2 was 0.87 and 0.79, respectively, which is slightly higher than the theoretical value of 0.67. For feed water with a COD/sulphate ratio of 0.67, there is theoretically enough sulphate available for the SRB to utilise the available COD (Rinzema & Lettinga, 1988), while when the COD/sulphate ratio in the feed water > 0.67, the utilization, and thus the competition with MB for the carbon source, increases. From the results shown in Table 3 it can be noted that the $COD_{used}/SO_{4removed}$ ratio in R1 is 0.84, which is higher than the theoretical value of 0.67. Therefore it can be assumed that the MB participated in the competition for acetate in R1. When observing the $COD_{used}/SO_{4removed}$ ratio in R2 (Table 3), it can be seen that the ratio is 0.42 i.e. < 0.67. There is no immediate explanation for this low experimental ratio, unless SRB obtain their energy from another source (such as from debris of decaying outcompeted AB). Visser (1995) has shown that at a reactor pH of about pH 8, the SRB can out-compete the MB. In both reactors the pH was 7.9, thus favouring the SRB.

The results of this study (Table 3) confirm the findings of Omil et al., (1998), that the competition for acetate in a sulphidogenic reactor is more in favour of the MB than the SRB, while the SRB gain more energy from the degradation of propionate than the AB (Harada et al., 1994).

Reactor performance

The SO_4 removal and sulphide production results of the continuous operation period of R1 and R2 are given in Figures 2 and 3. For R1, the continuous operation was monitored for a period of 55 days, while for R2, this was 77 days. During operation of both reactors at an HRT of 1 d, Figs 2 and 3 indicate that better sulphate reduction was obtained with propionate as the carbon and energy source than when using acetate,. This finding confirmed the results obtained by Omil et al., (1998), who described the competition between the SRB and MB when treating acetate and sulphate in the same reactor. When using propionate as the energy source, the competition is between the AB and the SRB. Rinzema & Lettinga (1988) stated that the outcome of the competition in an anaerobic reactor is based on the thermodynamics and kinetics of the sulphate reduction, the methanogenesis and the acetogenesis. In the case of propionate, the SRB have the advantage in the competition if no sulphate limitation occurred (Colleran et al., 1995). The results presented here show that sulphate reduction using propionate was more effective than when using acetate as the carbon and energy source. It can furthermore be observed that, for R1, sulphate reduction during the period day 1-33 was better than during the period day 34-55. This observation can possibly be ascribed to a lower COD concentration in the feed during the second period. The average COD concentration in the feed from day 1-33 was 1054 mg/ℓ, while it was 873 mg/ℓ during the period day 34-55, corresponding with COD/SO_4 feed ratios of 0.97 and 0.87 respectively.

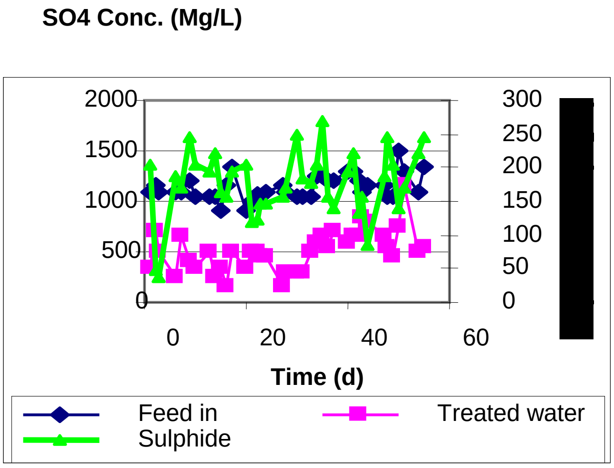

Fig. 2. Sulphate reduction in R1

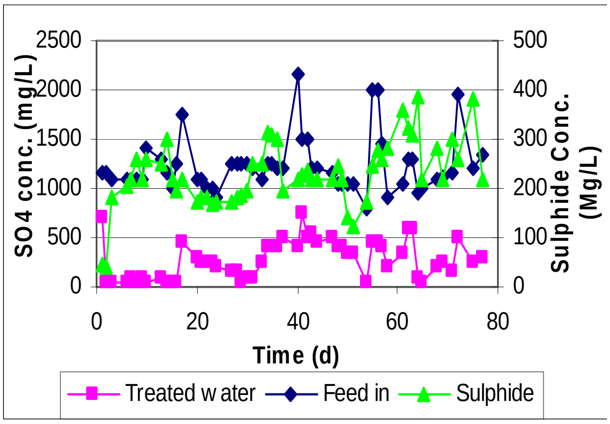

Figure 3: Sulphate reduction in R2

The effect of different concentrations of Propionate

The result of applying different feed COD/SO_4 ratios to R2, given in Table 4, is that the highest percentage removal (85%) was obtained when 1 mℓ propionic acid per ℓ feed was added, while the highest SO_4 removal rate was obtained when 0.7 mℓ propionic acid per feed was added.

It can be seen from Table 4 that the highest COD/SO_4 feed ratio (1.36) occurred during period 1, when the highest percentage sulphate removal was obtained (85%), as well as the highest residual COD concentration in the treated water (1162 mg/l). The $COD_{used}/SO_{4removed}$ ratio during period 1 was 0.66, which is similar to the theoretical value of 0.67. Although the relatively high sulphate removal is beneficial for the process, the high residual COD in the treated water is not favourable. A slightly lower percentage SO_4 removal (82%) was achieved during period 4, at which time the COD/SO_4 feed ratio was 0.69 (comparable to the theoretical value of 0.67) and during that period the residual COD in the treated water was 571 mg/ℓ. The sulphate removal rate was only slightly lower during period 4 (1.00 g SO_4/(ℓ.d), compared to 1.02 g SO_4/(ℓ.d) during period 1. Taking these results into account as well as the fact that operating at a lower feed COD/SO_4 ratio will reduce the operating costs, it can be advised to execute the biological sulphate reduction at a feed COD/SO4 ratio of close to the theoretical of 0.67. Except during period 1, the $COD_{used}/SO_{4removed}$ ratio in the other three periods is lower than the theoretical value, as was also shown in the results in Table 3. No explanation can be given. Although the COD/SO_4 feed ratio during period 4 is close to the theoretical value of 0.67, it appears that part of the COD concentration available in the feed may be used by competing micro-organisms. No gas measurements were conducted to investigate whether methane was produced.

Table 4. The results of the 4 experimental periods, when operating R2

Determinant	Unit	Period			
(Propionic acid added)	mℓ/ℓ feed	**1** (1)	**2** (0.5)	**3** (0.6)	**4** (0.7)
COD/SO_4 feed ratio		1.36	0.58	0.60	0.69
SO_4 removed	mg/ℓ	994	856	883	1024
SO_4 removal	%	85	62	72	82
SO_4 removal rate	g SO_4/(ℓ.d)	1.00	0.86	0.88	1.02
$COD_{used}/SO_{4removed}$ ratio		0.66	0.38	0.35	0.33
Residual COD	mg/ℓ	1162	443	292	571

Residual COD

The treated water was analysed for the VFA concentration and it was noticed that the residual COD in the acetate fed reactor (R1) was in the form of acetate. This was also the case for the treated water from R2, which additionally contained low concentrations of non-utilised propionic acid (varying from 10-100 mg/ℓ) during period 1. During the first period of operation of R2, when the treated water samples contained a residual COD of 1162 mg/ℓ, the average acetate concentration in the treated water was 456 mg/ℓ, while the average propionate concentration was 66 mg/ℓ. This finding corresponds with that of O'Flagerty et al., (1998). They studied the population structure of biomass from a full-scale anaerobic reactor after 5 years of operation, with the purpose of obtaining an improved understanding of long-term competition between SRB, MB, AB and other (synthropic) bacteria. The results showed that the SRB carried out incomplete oxidation of propionate to acetate. The findings of O'Flagerty et al., (1998) are similar to those of Harada et al., (1994) as they observed that propionate accumulated significantly in the reactor when low levels of sulphate were present. They deduced that the SRB strongly contributed to the degradation of propionate to acetate. It can be assumed that the SRB contribute to the degradation of propionate to acetate using hydrogen. It was also shown that the SRB were poor competitors of MPB for acetate. Only during long-term operation did SRB start to out-compete MB for acetate. Omil et al., (1998) studied the competition between acetate utilizing MB and SRB, operating two UASB reactors, at a reactor pH of 8. It was found that with an excess of sulphate (COD: Sulphate ratio < 0.67) the SRB became predominant in relation to the MB during a period of operation of 250-400 days.

Comparing the use of acetate and propionate, from this study it appears that the AB competed with the SRB for acetate. Although the feed COD/SO_4 ratios in R1 and R2 were similar, the experimental $COD_{used}/SO_{4removed}$ ratio in R1 (0.84) is significantly greater than in R2 (0.42), indicating that the AB participated in the use of acetate in the latter case.

Conclusions

Sulphate removal has been observed when using both acetate and propionate as the carbon and energy source in biological sulphate reducing reactors. These results show that when the average feed COD/SO_4 ratio using acetate and proprionate was 0.87 and 0.79 respectively, better sulphate removal could be observed. In the propionate reactor, the SO_4 removal rate changed from 1.00 to 0.86, to 0.88 to 1.02 g SO_4(ℓ/d), when the feed

COD/SO_4 ratio was 1.36, 0.58, 0.60 and 0.69, respectively. These results indicated that when operating at a feed COD/SO_4 ratio of 0.69, using propionate, the highest SO_4 removal rate was obtained. These results therefore suggest that the SRB can utilise the propionate to better advantage than the acetate. The residual COD concentration in the treated water was about 50% less at the feed COD/SO_4 ratio of 0.69 as opposed to the feed COD/SO_4 ratio 1.36. The residual COD in the treated water of both reactors comprised mainly acetate.

References

APHA, (1985) Standard Methods for the Inc. Examination of Water and Wastewater *16th Edition,* Washington DC.

Colleran E, Finnegan S and Lens P (1995). Anaerobic treatment of sulphate-containing waste streams. Antonie van Leeuwenhoek **67:** 29-46.

Ghigliazza R, Lodi A and Rovatti M (2000). Kinetic and Process considerations on biological reduction of soluble and scarcely soluble sulfates. Resources, *Conservation and Recycling,* **29:** 182-194.

Greben HA, Maree JP, Singmin Y and Mnqanqeni S (2000). Biological sulphate removal from acid mine effluent using ethanol as carbon and energy source. *Water Sci. Technol.* **42:** (3-4), 339-344.

Harada H, Uemura S and Monomoi K (1994) Interaction between Sulphate-Reducing Bacteria and Methane-Producing Bacteria in UASB Reactors fed with Low-Strength Wastes containing different levels of Sulphate. *Wat Res,* **28:** 335-367.

Lens PNL, Van den Bosch MC, Hulshoff Pol LW and Lettinga G (1998). Effect of staging on Volatile Fatty Acid degradation in a sulphidogenic granular sludge reactor. *Wat Res* **32:** (4) pp 1178-1192.

O'Flaherty V, Lens P, Leahy B and Colleran E (1998). Long-term competition between sulphate reducing and methane producing bacteria during full-scale anaerobic treatment of citric acid production wastewater. *Wat Res,* **32:** (3), pp 815-825.

Omil F, Lens P, Visser A, Hulshoff Pol LW and Lettinga G (1998). Long term competition between Sulfate reducing and Methanogenic Bacteria in UASB reactors treating Voltile Fatty Acids. *Biotechnol Bioeng* **57:** 667-685.

Rinzema A and Lettinga G (1988) Anaerobic treatment of sulfate containing wastewater. In: Biotreatment systems,.**3:** (Wise, DL, Ed), pp 65-109 CRC press, Inc., Boca Raton.

Vallero MVG, Lens PNL, Bakker C, Lettinga G, (2003), Sulfidogenic volatile fatty acid degradation in a baffled reactor, Sub-department of Environmental Technology, Wageningen University and Research Centre, Bomenweg 2, P O Box 8129, 6700 EV Wageningen, The Netherlands.

Visser A, Alphenaar PA, Gao Y, Van Rossum G, Lettinga G (1993) Granulation and immobilisation of mathanogenic and sulphate reducing bacteria in high rate anaerobic reactors. *Appl Microbiol Biotechnol* **40**: 575-581.

Visser A (1995). The anaerobic treatment of sulphate containing wastewater. PhD Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.