

Comparison between sucrose, ethanol and methanol as carbon and energy sources for biological sulphate reduction

H.A. Greben, J.P. Maree and S. Mnqanqeni

Division of Water, Environment and Forestry Technology, CSIR, P.O. Box 395, 001 Pretoria, South Africa
(E-mail: hgreben@csir.co.za)

Abstract Sulphate was reduced via sulphide to sulphur when operating a single-stage completely mixed reactor configuration feeding synthetic feed. The aim of this study was to determine the volumetric and specific sulphate reduction rates using sugar, ethanol and methanol as a carbon and energy sources. The presented results indicated that sugar and ethanol were found to be suitable carbon and energy sources resulting in a volumetric and a specific sulphate reduction rate of 10.4 and 4.8 g SO₄/(l.d) and 0.8 and 2.8 g SO₄/(gVSS.d), respectively, at a Hydraulic Retention Time (HRT) of 3.6 h and at ambient temperature (21°C). Methanol induced methanogenesis rather than sulphidogenesis. The experimental sulphide/sulphate ratio was consistently lower than the theoretical value which indicated that part of the formed sulphide was oxidized to sulphur.

Keywords Biological sulphate reduction; carbon source; ethanol; single stage; sugar; sulphide oxidation

Introduction

Over the past 10 to 15 years the accent for sulphate removal from wastewater streams has moved away from the traditional chemical treatment to the biological treatment. It has been proven that sulphate can be removed biologically (Maree and Strydom, 1985, Maree *et al.*, 1986), provided that a suitable carbon source is available, such as lactic acid (Middleton and Lawrence, 1977). Omil *et al.* (1997) described the use of acetate and other Volatile Fatty Acids (VFA) but found that the competition between sulphate reducing (SRBs) and methanogenic bacteria (MB) was in favour of methane, rather than sulphide production. Visser (1995), however, showed that the reactor pH of higher than 7.5 shifted the competition in favour of the SRB. Swezyk and Pfennig (1990) indicated that ethanol can be used as a carbon and energy source, but also described competition between SRB and MB. O'Flaherty *et al.* (1997) found that ethanol and short chain VFA, such as propionate and butyrate was degraded faster by SRB when enough sulphate was present in the reactor. A cheaper possible chemical to be used is methanol (Braun and Stolp, 1985), although they described that methanol can only be used as an electron donor and that an additional carbon source is needed. Tsukamoto and Miller (1999) showed that methanol could be used as a carbon source for microbiological treatment of acid mine drainage. Van Houten (1996) proved that 30 g SO₄/(l · d) could be removed in an upflow anaerobic sludge blanket reactor, using a combination of CO₂/H₂ gases as a carbon and energy source.

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

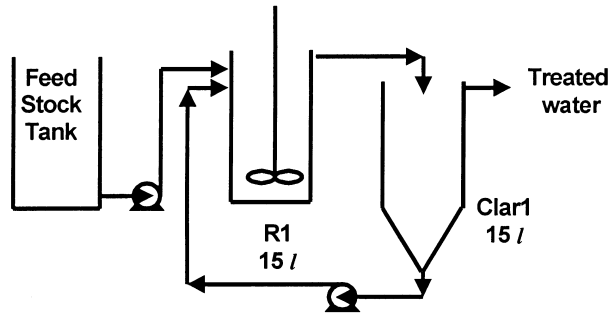
Materials and methods

Reactor configuration

Three similar completely mixed reactor systems, comprising of a reactor (volume 15 l) and a clarifier (volume 15 l) were operated (Figure 1). The reactors received synthetic feed, in which the sulphate as CaSO₄ and the COD concentrations were 1500 mg/l, each. The COD

Table 1 The experimental periods, determined by the decrease in HRT, using sugar and ethanol

Period	Number of days (sugar)	Number of days (thanol)	HRT (h)
1	8	6	24
2	15	6	12
3	3	7	7.2
4	5	7	4.8
5	7	7	3.6

**Figure 1** Flow diagram of reactor system

was added as: sugar, technical ethanol and technical methanol (1 ml per l feed, Crest Industries, Johannesburg). The feed was supplemented with both macro nutrients (75 mg/l ammonia-N and 15 mg/l orthophosphate-P) and micro nutrients (100 µg/l Fe, 210 µmg/l Co, 0.28 µg/l Mn, 0.44 µg/l V, 0.25 µg/l Ni, 0.48 µg/l Zn, 0.40 µg/l Mo, 0.18 µmg/l B, 0.37 µg/l Cu). The reactors were initially inoculated with anaerobic sludge obtained from the local municipal sewage treatment plant which thereafter became conditioned for sulphate removal. Both reactor and clarifier were open to the atmosphere to allow for air contact. The reactors were operated at room temperature (21°C). The decreased HRT determined the experimental periods, when sugar and ethanol were used (Table 1). When using methanol the HRT was constant at 24 h. When sugar was used, the feed pH was around 4. Initially a pH controller was installed to maintain the reactor pH at 7.5 by adding a NaHCO₃ solution. However, once the sulphate reduction had started, sufficient alkalinity was produced and the reactor pH could be maintained at value between 7 and 8.

Analytical

Determinations of sulphate, sulphide, COD, alkalinity, calcium and pH were carried out according to standard analytical procedures as described in *Standard Methods* (APHA, 1985). With the exception of sulphide and feed COD, all analyses were carried out on filtered samples (Whatman #1). The acidity determination of the feed was done by titrating with 0.1 N NaOH to a pH of 9.0. The COD samples were pre-treated to eliminate the sulphide contribution to the COD concentration.

Results and discussion

The results of Table 2 illustrate that sugar and ethanol are suitable carbon and energy sources for sulphate reduction but not methanol.

Table 2 Experimental conditions, chemical composition of feed and treated water, reaction rates and stoichiometric ratios between various parameters when comparing sucrose, methanol and ethanol as carbon and energy sources, feeding artificial feed

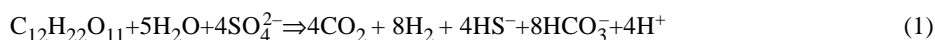
Parameter	Unit	Carbon and energy source											
		Sucrose					MeOH		EtOH				
		24	12	7.2	4.8	3.6	24	24	12	7.2	4.8	3.6	
<u>Feed:</u>													
Sulphate	mg/l	1683	1691	1550	1725	1600	1630	1550	1372	1600	1400	1320	
COD	mg/l	1500	1381	2365	188	1350	2061	1630	1326	1550	1523	1434	
Alkalinity	mg/l						–	–	79		89	85	
Acidity	mg/l	100	100	200			–	–			–	–	
pH		4.2	4.3	3.9	4.1	4	7.3	7	7.1	6.71	7.1	7	
<u>Treated:</u>													
Sulphate	mg/l	1090	850	47	83	35	1402	648	316	257	75	598	
COD	mg/l	922	640	552	109	615	563	729	526	726	714	869	
Alkalinity	mg/l	1026	728	610	920	752	140	777	543	543	641	441	
Sulphide (S)	mg/l	85	85	183	98	194	29	218	156	234	190	141	
VSS	g/l	7.9	11.3	11.7	12.5	13.2	5.2	5.3	2.6	1.9	2.5	1.7	
PH		7.2	7.3	7.6	6.9	7.3	7.6	7.7	7.68	7.5	7.7	7.3	
<u>Rates:</u>													
SO ₄ reduction	g/(l.d)	0.6	1.7	5	8.2	10.4	0.23	2.5	2.5	4.5	6.6	4.8	
COD	g/(l.d)	0.7	1.7	6	4	4.9	1.5	2	1.9	2.7	4	3.8	
Specific SO ₄ reduction	gSO ₄ /(gVSS.d)	0.1	0.15	0.43	0.66	0.79	0.04	1.21	1	2.4	2.6	2.8	
Specific COD	gO ₂ /Theor.	0.1	0.15	0.51	0.32	0.37	0.3	0.74	0.7	1.42	1.67	2.2	
<u>Ratios:</u>													
COD/SO ₄		0.67	0.86	1	1.2	0.49	0.48	6.6	0.8	0.77	0.61	0.61	0.78
S/SO ₄		0.33	0.14	0.1	0.12	0.1	0.12	0.13	0.1	0.13	0.17	0.14	0.19
Alkalinity/SO ₄		10.4	1.7	0.87	0.41	0.56	0.48	0.61	0.31	0.47	0.40	0.48	0.61

Sugar

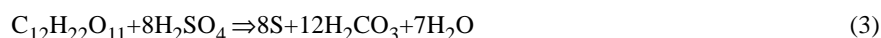
The suitability of sugar as a carbon and energy source for biological sulphate reduction in a complete-mix reactor is shown by the following:

Reaction rate. The volumetric and specific sulphate reduction rates (maximum) were determined to be 10.4 g SO₄/(l · d) and 0.79 g SO₄/(gVSS · d) respectively. The volumetric sulphate reduction rate increased from 0.6 to 10.4 g SO₄/(l · d) and the specific sulphate reduction rate increased from 0.08 to 0.79 g SO₄/(gVSS · d) when the HRT decreased from 24 to 3.6 h. The increase in the volumetric sulphate reduction rate can be ascribed to the gradual VSS increase from 7.9 to 13.3 g/l, while the increase in the specific sulphate reduction rate is due to adaptation of the biomass.

When sugar is the carbon and energy source, the SRB can utilize the sugar and produce hydrogen according to reactions (1) and (2)



When sulphate in the presence of sugar is reduced in one step to elemental sulphur, the reaction is as follows:



The sulphide oxidizing bacteria can in the presence of air oxidize the produced sulphides to elemental sulphur according to reaction (4):



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

$\text{COD}_{\text{used}}/\text{sulphate}_{\text{removed}}$ ratio

The theoretical value for the $\text{COD}_{\text{used}}/\text{sulphate}_{\text{removed}}$ -ratio (mg $\text{O}_2/\text{mg SO}_4$) was 0.67 and 0.50 when sulphate was reduced to sulphide (reaction 1) and sulphur (reaction 3) respectively. The corresponding theoretical value for $\text{sugar}_{\text{removed}}/\text{sulphate}_{\text{removed}}$ (mg sugar/mg SO_4) amount to 0.59 and 0.45 when sulphate is reduced to sulphide and sulphur respectively. The experimental determined values (as mg $\text{O}_2/\text{mg SO}_4$) were found to be greater than 0.8 (0.86, 1.00 and 1.20) for retention times greater than 5 h (24 h, 12 h, and 7.2 h respectively) and smaller than 0.8 (0.49 and 0.48) for retention times less than 5 h (4.8 h and 3.6 h respectively). The higher $\text{COD}_{\text{used}}/\text{sulphate}_{\text{removed}}$ -ratios measured at longer retention times indicated that a portion of the organic carbon was not utilised for sulphate reduction but possibly fermented to methane by the methanogens. Visser's results (1995) indicated that the SRB are more competitive at longer rather than at shorter retention times, whereas in this study the results showed that at shorter residence times lower $\text{COD}_{\text{used}}/\text{sulphate}_{\text{removed}}$ -ratios were measured, thus that the faster growing SRB out-competed the slower growing MB for the available carbon in solution. The better COD utilisation at shorter residence times can possibly be ascribed to the utilisation of H_2 (reaction 2), as hydrogen is consumed by SRB when excess sulphate is present (Visser *et al.*, 1993). The low $\text{COD}_{\text{removed}}/\text{sulphate}_{\text{removed}}$ values of 0.49 and 0.48 at HRT of 4.8 and 3.6 h, respectively, can be contributed to the fact that the sulphate was immediately reduced to sulphur (reaction 3).

$\text{Sulphide}_{\text{produced}}/\text{sulphate}_{\text{removed}}$ ratio

The theoretical value for $\text{sulphide}_{\text{produced}}/\text{sulphate}_{\text{removed}}$ ratio is 0.33 and 0.00 when sulphate is reduced to sulphide (reaction 1) and sulphur (reaction 3) respectively. Sulphide can be oxidised by aerobic bacteria (reaction 4). The experimental $\text{sulphide}_{\text{produced}}/\text{sulphate}_{\text{removed}}$ varied between 0.06 and 0.14. The fact that the actual values were between the theoretical values for sulphide and sulphur as end-products indicated that only a portion of the sulphate is reduced to elemental S via sulphide, while the balance of the reduced sulphate remained in the sulphide form. The low sulphide concentrations could be contributed to the sulphide oxidation to sulphur. This result was confirmed by the formation of a yellow-white layer (sulphur) on top of the clarifier during the experiment.

It appeared (although not clearly), that the $\text{sulphide}_{\text{produced}}/\text{sulphate}_{\text{removed}}$ ratio increased when the HRT decreased. At decreasing HRT from 24 to 3.6 h, using ethanol, the $\text{sulphide}_{\text{produced}}/\text{sulphate}_{\text{removed}}$ ratio increased from 0.1 to 0.19. These results indicated that sulphide, produced from sulphate, can completely be converted to sulphur, provided that the HRT is not too short. It can be concluded that under the specific reaction conditions, the volumetric reaction rate of sulphide oxidation to sulphur is slower than the volumetric sulphate reduction rate, resulting in un-oxidised sulphide in the effluent. The lower rate of sulphide oxidation than sulphate reduction at lower HRT may also be explained by oxygen limitation. The available air in the reactor is due to air diffusion from the top of the clarifier into the reactor system. The DO concentration in the reactor when measured was almost 0%, indicating that the reactor was anaerobic. Janseen (1997) found that under conditions when the oxygen concentration is such that the molar ratio $(\text{O}_2/\text{S}^{2-})_{\text{consump}}$ is between 0.5

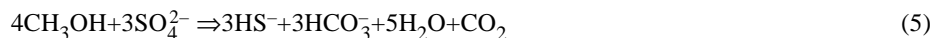
and 1.0, thiosulphate and sulphur are produced. He stated that maximal sulphur production was obtained at $(O_2/S^2)_{\text{consump}}$ between 0.6 and 1.0. Thus, a better sulphide oxidation rate could have been obtained at lower HRT if a small amount of air or oxygen had been added to the reactors. However, if excessive amounts of oxygen are added the produced sulphur will be oxidized to sulphate. Efficient sulphide oxidation is beneficial to the biological sulphate reduction process as high levels of sulphides are toxic to SRB and may cause instability in the process (Oleszkiewicz and Hilton, 1986).

Alkalinity/sulphate ratio

The theoretical alkalinity/sulphate ratio is 1.04 (Reaction 1). The actual values varied from 1.7 (when the HRT was 24 h) to less than 0.56 (when the feed rate was 4.8 and 3.6, respectively). The high value of 1.7 was due to the dosage of bicarbonate during the initial period to neutralize the acidic feed. The low values were due to the fact that acid water was fed directly. Due to the production of alkalinity and the availability of Ca^{++} (feed), $CaCO_3$ precipitated which could be observed as deposits in the reactors and tubing.

Methanol

Methanol was not suitable for sulphate reduction (reaction 5 and 6). Using methanol the sulphate reduction rate was only 0.2 g $SO_4/(l \cdot d)$. The actual $COD_{\text{used}}/sulphate_{\text{removed}}$ value of 6.6 (Table 2) was much higher than the theoretical value of 0.67 when sulphide is the end-product or 0.5 when sulphur is the end-product. This indicated that other micro-organisms were competing for the same COD in the anaerobic reactor. Oremland and Polcin (1982) showed that the MB out-compete SRB for methanol. Visser (1995) stated that in the anaerobic degradation of organic matter in the presence of sulphate, the released electrons (in terms of COD) are used by SRB and MB. When hydrogen is the available substrate the SRB will out-compete the MB. When neither substrate is present, the available methanol will be used for methanogenesis (reaction 7) and not for sulphate reduction. This finding differs from the results from Weijma *et al.* (1999) who obtained a sulphate reduction rate of 11 g $SO_4/(l \cdot d)$ at a HRT of 3 h, with methanol as the carbon and energy source. However, they operated under thermophilic conditions (65°C), whereas this investigation was carried out under mesophilic conditions (22°C). The different results can be ascribed to the difference in affinity for methanol as substrate between mesophilic and thermophilic SRB.



Sulphide_{produced}/sulphate_{removed} ratio. The actual sulphide/sulphate ratio of 0.13, which is greater than 0 (sulphide_{produced}/sulphate_{removed} ratio when sulphur is the end-product) but less than 0.33 (sulphide_{produced}/sulphate_{removed} ratio when sulphide is the end-product), indicated that both sulphur and sulphide are formed as end-products.

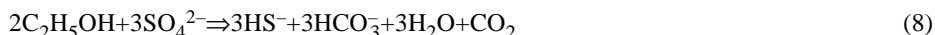
Ethanol

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

Reaction rate. The volumetric and specific sulphate reduction rates (maximum) were determined to be 6.6 g $SO_4/(l \cdot d)$ and 2.8 g $SO_4/(g \text{ VSS} \cdot d)$ respectively. The volumetric sulphate reduction rate increased from 2.5 to 6.6 g $SO_4/(l \cdot d)$ when the HRT decreased from 24 to

4.8 h and the specific sulphate reduction rate from 1.2 to 2.8 g SO₄/(g VSS · d). With ethanol the volumetric sulphate reduction rate was higher (2.8 g SO₄/(g VSS · d) than in the case of sugar (0.8 g SO₄/(g VSS · d). This is due to the lower VSS-concentration in the case of ethanol (1.7 to 2.6 g/l) than in the case of sugar (7.9 to 13.2 g/l).

Stoichiometric relationship between theoretical and actual ratios for COD_{removed}/sulphate_{removed}, sulphide_{produced}/sulphate_{removed} and alkalinity_{produced}/sulphate_{removed}. Similar observations can be made for the various ratios for ethanol as a carbon and energy source than for sugar. The theoretical value for ethanol_{removed}/sulphate_{removed} is 0.32 (reaction 8) and 0.24 (reaction 9) when sulphate is reduced to sulphide and sulphur respectively (mass to mass).



General

The specific sulphate reduction rates, using sugar (0.79 g SO₄/(g VSS · d) and ethanol (2.8 g SO₄/(g VSS · d)) are higher than the value of 0.03–0.13 g SO₄/(g VSS · d) obtained from other studies (Olthof *et al.*, 1985). This can be ascribed to the production of hydrogen, when both sugar and ethanol are degraded, resulting in a high reaction rate. Also the relative low sulphide concentration in solution in the single-stage process contributed to the obtained results, as high sulphide concentrations are toxic to SRB.

Conclusions

The following conclusions were made from the investigation.

1. Sugar and ethanol were found to be suitable carbon and energy sources for sulphate reduction. The volumetric and specific sulphate reduction rates (maximum) for sugar were determined to be 10.4 g SO₄/(l · d) and 0.79 g SO₄/(g VSS · d) respectively. The corresponding rates for ethanol were determined to be 6.6 g SO₄/(l · d) and 2.8 g SO₄/(g VSS · d) respectively.
2. Methanol was not suitable as carbon and energy source for sulphate reduction, due to the fact that the methanogenic bacteria dominated the sulphate reducing bacteria.
3. Optimum utilisation of the carbon and energy source was obtained at shorter residence times (less than 8 h). At long residence times (longer than 8 h), sulphate reducing bacteria were out-competed by methanogenic bacteria.
4. The reduced sulphate was converted to sulphide and to sulphur. Longer residence times favoured the production of sulphur as end-product over sulphide as end-product.

References

- APHA (1985). *Standard Methods for the Examination of Water and Wastewater*, 16th Edition, Washington, DC.
- Braun, M. and Stolp, H. (1985). Degradation of methanol by a sulphate-reducing bacterium. *Arch. Microbiol.*, **142**, 77–80.
- Colleran, E., Finnegan, S. and Lens, P. (1995). Anaerobic treatment of sulphate containing waste streams. *Antonie van Leeuwenhoek*, **67**, 29–46.
- Maree, J.P. and Strydom, W.F. (1985). Biological sulphate removal from a packed bed reactor, *Wat. Res.*, **19**(9), 1101–1106.
- Maree, J.P., Gerber, A. and Strydom, W.F. (1986). A biological process for sulphate removal from industrial effluent, *Water SA*, **12**(3), 139–144.
- Middleton, A.C. and Lawrence, A.W. (1977). Kinetic of microbial sulphate reduction. *J. Wat. Pollut. Control Fed.*, 1659–1670.

- O'Flaherty, V., Lens, P., Leahy, B. and Colleran, E. (1997). Long-term competition between sulphate-reducing and methane-producing bacteria during full-scale anaerobic treatment of citric acid production wastewater.
- Oleszkiewicz, J.A. and Hilton, B.L. (1986). Anaerobic treatment of high sulphate waste. *Can. J. Civ. Eng.*, 125–150.
- Olthof, M., Kelly, W.R., Oleszkiewicz, J. and Weinreb, H. (1985b). Development of anaerobic treatment process for wastewaters containing high sulphate. *Proc. Ind. Waste Conf., Purdue Univ.*, West Lafayette, 871–877.
- Omil, F., Lens, P., Visser, A., Hulshoff-Pol, L.W. and Lettinga, G. (1997). Long term competition between sulphate reducing bacteria and methanogenic bacteria in UASB reactors treating volatile fatty acids. *Biotech. and Bioeng.*, **57**(6), 676–685.
- Oremland, R.S. and Polcin, S. (1982). Methanogenesis and sulphate reduction: competitive and non-competitive substrates in estuarine sediments. *Appl. Environ. Microbiol.* **44**, 1270–1277.
- Swezyk, R. and Pfennig, N. (1990). Competition for ethanol between sulphate-reducing and fermenting bacteria. *Arch. Microbiol.*, **153**, 470–477.
- Tsukamoto, T.K. and Miller, G.C. (1999). Methanol as a carbon source for microbiological treatment of acid mine drainage. *Wat. Res.*, **33**(6), 1365–1370.
- Van Houten, R.T. (1996). Biological Sulphate reduction with synthesis gas. PhD Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.
- Visser, A., Alphenaar, P.A., Gao, Y., van Rossum, G. and Lettinga, G. (1993). Granulation and immobilisation of methanogenic 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.
- Weijma, J., Stams, A.J.M., Hulshoff-Pol, and Lettinga, G. (1999). Thermophilic sulfate reduction and methanogenesis with methanol in a high rate anaerobic reactor. *Biotech and Bioeng.*, **67**(3), 354–363.

