CHAPTER 5

VFA PRODUCTION AND CONTINUOUS BIOLOGICAL SULPHATE REMOVAL OPERATING A TWO AND THREE STAGE REACTOR SYSTEM FEEDING SYNTHETIC FEED WATER AND DILUTED ACID MINE WATER

5.1 INTRODUCTION

Biological sulphate reduction is a competitive alternative to other sulphate removal technologies utilized for the full scale treatment of mine and other industrial effluents (Colleran *et al*. 1995). The major disadvantage of applying the biological process for the treatment of sulphate and acid rich effluents is the operational costs associated with the carbon and energy source. In order to save on operational costs, the biological sulphate removal process can be coupled to limestone and lime neutralisation of acidic mine water (Maree, 2003a; Geldenhuys *et al*. 2002; Maree *et al*. 2003b). When the sulphate rich water is firstly treated with limestone, the sulphate concentration is reduced to 2300 mg/ℓ, and after lime treatment the sulphate concentration is further reduced to 1500 mg/ℓ, the solubility level of gypsum. After lime treatment, the pH of the treated water is between 11and 12. This pH level can be reduced to pH 7.5 by bleeding CO_2 gas, generated in the limestone treatment phase, through the lime-treated high pH water. After contact with CO_2, the partially treated water with a near neutral pH is fed to the biological treatment system to further lower the remaining sulphate to concentrations below 200 mg/ℓ. This integrated chemical-biological treatment of acid and sulphate rich mine effluents saves considerably on operational costs compared to treating the total mine water stream biologically (Maree *et al*. 2004).

The utilization of a range of low molecular weight substrates for biological sulphate removal such as lactate (Laanbroek *et al*. 1983), ethanol (Greben *et al*. 2000; De Smul *et al*. 1997) and hydrogen (Du Preez *et al*. 1992, Van Houten, 1996; Eloff *et al.* 2004) has been demonstrated. A possible way of treating mine water more cost effectively is by using a cellulose-containing bio-waste product (e.g. plant biomass) as the carbon and energy source. When cellulose, a polysaccharide, is fermented, polymers, oligomers, monomers (sugars), fatty acids and other fermentation products are formed, which can be utilized as energy sources by the SRB to remove sulphate from industrial waste waters, such as mine effluents.

It was shown in Chapter 4 that the fermentation of a bio-waste product containing cellulose can effectively be mediated by microorganisms originating from rumen fluid

obtained from ruminants. The products generated in this fermentation process are, amongst others, fatty acids, such as butyric acid, propionic acid and acetatic acid. When adding tryptone to the fermentation reactor, the rumen microbes utilise this protein based, nutrient supplement to produce more propionic acid than butyric acid. The SRB can utilise the C4 and C3 acids in the sulphate reducing process, producing C2 acid, which is the rate limiting factor in the sulphidogenic reactor (Visser *et al*. 1993) and may lead to failures of anaerobic wastewater treatment systems. The oxidation of propionic acid in the sulphate reducing process is more beneficial than that of butyric acid as only one mol of acetate is formed by oxidizing propionate, whereas butyric acid oxidation results in two moles of acetic acid.

The results in Chapter 4 showed that the degradation of GC produced sufficient organic products to sustain biological SO_4 removal, in laboratory batch test reactors. The objectives of the studies in this chapter were to investigate whether continuous sulphate reduction can be achieved using GC as carbon and energy source, operating the process in a continuous mode. The study comprised of two parts: Part 1 describes the feeding of synthetic influent, into a two and a three stage reactor system, while in Part 2, pre-treated AMD from a closed mine in the Witbank area was used as feed water to the same reactor system.

5.2 MATERIAL AND METHODS

5.2.1 Study 1a. Two stage reactor system

In Study 1a, a two stage reactor system, comprising a fermentation reactor (FR) and a sulphate reducing reactor (SR), was operated and fed continuously with synthetic sulphate rich water. The effluent from FR was fed to SR, which also received synthetic feed water, the objective being that the cellulose degradation products formed in FR should function as the carbon and energy sources in SR.

5.2.1.1 Feed water

The feed water for FR and SR had a SO_4 concentration of ≈ 2500 mg/ℓ, (Na_2SO_4, Crest Chemicals, Johannesburg). Macronutrients consisting of (w/w): 6.5% N, 2.7% P, 13.0% K, 7.0% Ca, 2.2% Mg and 7.5 % S) and micronutrients (0.15% Fe, 0.024% Mn, 0.024% B, 0.005% Zn, 0.002% Cu and 0.001% Mo were made up in a stock solution (1g/5ℓ) of which 1mℓ/ℓ was added to the feed water (Hydroponic nutrient powder, Kompel, Chemicult). Both reactors received feed water at a rate of 5 ℓ/d.

5.2.1.2 Carbon and energy source

The fermentation products of the cellulose containing GC, produced in FR, served as the carbon and energy source for sulphate removal in FR and SR.

5.2.1.3 Reactor system

A two stage reactor system was operated, consisting of a fermentation reactor (FR) and a sulphate removal (SR) as depicted in Figure 5.1.

5.2.1.3.1 Fermentation reactor (FR)

For the fermentation reactor (FR), a 20 ℓ reactor was used, operated at 39 °C using a water jacket surrounding the reactor, which was connected to a waterbath set at the required temperature. FR was operated as a "hybrid" reactor. The lower part of the reactor contained ceramic rings as packing material for biofilm formation. Biomass (250 mℓ), obtained from the sulphate removal CSIR Demonstratoin Plant Navigation Colliery (Witbank) was added to promote SRB growth on the ceramic rings. The upper part of the reactor contained 1000 g GC at the start of Study 1a. The GC were replenished on a weekly basis, added to the top of the reactor. Due to its light weight, cut grass floats on water and therefore stayed at the upper part of the reactor. Initially 250 mℓ rumen fluid, (VSS of 10.6 g/ℓ) obtained from fistulated sheep (University of Pretoria, South Africa) was added to the GC. The pH of the reactor was maintained between 6.6-6.9, to accommodate the optimum conditions for rumen microorganisms. The SRB prefer a reactor pH of 7.5, but can function at pH 7. The feed water (5ℓ/d) entered FR at the top (HRT = 4 days), to allow it to make contact with the grass cuttings. A recycle stream (360 ℓ/d) was fitted from the lower GC part of the reactor to the top, with the rationale that the polymers and monomers produced could further be fermented to the substrates required for the SRB in the bottom part of the reactor. The effluent left FR at the bottom, from where it entered SR. The FR effluent contained partly removed sulphate, alkalinity, sulphide, as well as residual COD concentration, obtained from the fermentation process in FR.

5.2.1.3.2 Sulphate removal reactor (SR)

For the biological sulphate reduction a packed bed reactor (SR) was used (5 ℓ). The active volume was 2 ℓ, due to the fact that the reactor was packed with a geotextile blanket (material product used in road construction) for SRB biofilm formation. Geotextile is a coarse material, ideally suited for biofilm formation, due to the many threads per cm^2 of fibrous cloth to which the bacteria can attach. SR was inoculated with 150 mℓ biomass already adapted to sulphate removal and 100 mℓ anaerobic

sludge, obtained from Daspoort Sewage Works, Pretoria. The Anaerobic sludge was added with the rational to introduce a wider diversity of SRB, since the Demo plant SRB were adapted to ethanol as the carbon and energy source. SR received two feed streams, each of 5 ℓ/d: the effluent of FR and SO_4 rich synthetic feed water, which resulted in a HRT of ½ d. The reactor was operated at room temperature (22-25 ˚C). A recycle stream (20 ℓ/d) was installed between the upper and lower parts to improve mixing inside the reactor.

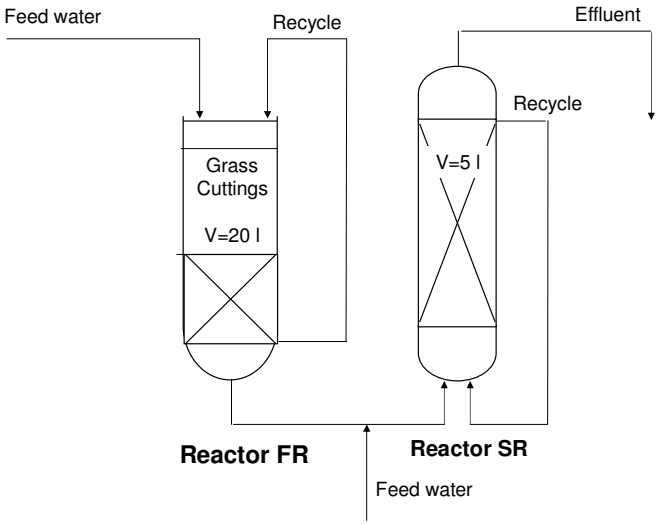

Figure 5.1 Schematical representation of the two stage reactor system.

5.2.1.4 Analytical

For all described experiments, daily samples were taken from feed and treated water from the reactors and analysed on the same day. The same analytical procedures as described in Chapter 3 (3.2.4) were followed.

5.2.2 Study 1b. Three stage reactor system

During Study 1b, a third reactor was added to the reactor system as described in *5.2.2.2* (Figure 5.3). The aim of Study 1b was to investigate whether further sulphate removal occurred by the acetate consuming SRB (ASRB), using the acetate produced in the other two reactors, as the carbon source, which would possibly be more beneficial than removing the residual COD (acetate) concentration in an additional aerobic reactor, prior to discharge in receiving water bodies.

5.2.2.1 Feed water

The feed water used in Study 1b was identical to the feed water in Study 1a.

5.2.2.2 Reactor system

The same procedure as for Study 1a was followed, except that the two stage reactor system was changed to a three stage reactor system, comprising FR and SR as well as a third reactor, the Acetate Sulphate Reactor (ASR, Figure 5.2). ASR was exactly the same as SR, also with a volume of 5 ℓ and was similarly packed with strips of geotextile (road construction material, see 5.2.1.3.2). The feed rate of the synthetic SO_4 rich feed water to ASR was 5 ℓ/d, while ASR also received the effluent of SR at a feed rate of 15 ℓ/d, resulting in a HRT of 1/3 day.

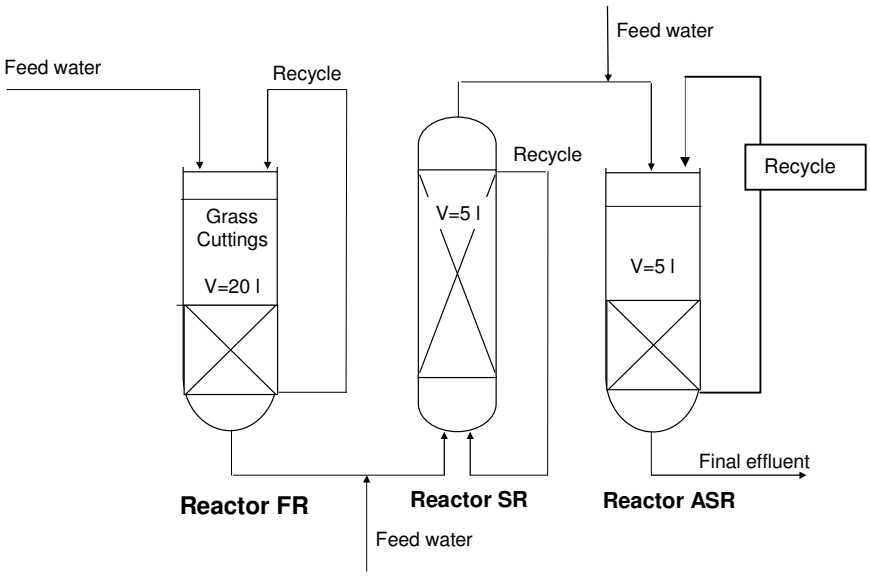

Figure 5.2 Schematic representation of the three stage reactor system

5.2.2.3 Biomass

ASR received the same biomass (SRB and anaerobic digester sludge from Daspoort, see 5.2.1.3.2) as FR and SR in Study 1a as well as 100 mℓ acetate utilising SRB (ASRB). These microorganisms were prepared on Postgate medium, with acetate as the carbon and energy source, isolated from anaerobic digester sludge, obtained from the Pretoria Sewage Works.

5.2.2.4 Carbon and Energy Source

As in Study 1a, grass cuttings were added to FR to be fermented by the rumen microorganisms to degradation products, providing SRB with the electron donor. In this study, 150 g grass cuttings were added on days 1, 32, 46 and 62.

5.2.2.5 Analytical

Daily samples were taken of feed water and treated water from the reactors. The same analytical procedures as described in Chapter 3 (3.2.4) were followed.

5.2.3 Study 2a. Effect of AMD on activity of rumen microorganisms

This study was conducted to investigate whether the rumen inoculum were affected by diluted AMD as feed water, before it was decided to feed this diluted AMD to FR in the following study. To reach the objective, a short batch reactor test was conducted, operating two reactors. The first reactor received diluted AMD as feed water (SO_4 concentration ≈ 1300 mg/ℓ), while the second reactor was fed with synthetic feed water (SO_4 concentration ≈ 950 mg/ℓ). All other conditions in the two reactors were the same.

5.2.3.1 Feed water

AMD effluent (chemical composition Table 5.1), which originated from a closed coal mine in the Witbank (South Africa) area, was used as feed water for reactor R1. The mine effluent was diluted prior to use with sulphide rich effluent from the biological sulphate removing reactor SR (ratio 1:1) so that the sulphide and the produced alkalinity present in the effluent could precipitate the metals and increase the pH of the AMD, respectively. The SO_4 concentration of the diluted feed water was approximately 1500 mg/ℓ. Artificial SO_4 rich water (SO_4 ≈1500 mg/ℓ) was used as feed water for reactor R2. Metal removal due to pre-treatment of AMD is presented in Table 5.2.

5.2.3.2 Reactors

Two completely mixed Perspex batch reactors, R1 and R2 (Vol: 2.5 ℓ), were operated under anaerobic conditions at 37 °C by means of a water jacket surrounding the reactor.

5.2.3.3 Biomass

Both R1 and R2 were seeded with100 mℓ rumen bacteria (VSS: 10g/ℓ), 100 mℓ SRB (VSS: 9.7 g/ ℓ), 50 g GC, 1 mℓ/ℓ macro nutrients (as described for Study 1a: 5.2.1.1).

5.2.3.4 Experimental

The pH in R1 and R2 was controlled (by pH controller) between pH 6.6 -6.9, which is the required pH for the rumen bacteria. In total 8.3 mℓ/ℓ of 0.1 N HCl was used to lower the increased pH due to biological sulphate reduction. The total experimental period was 10 days.

Table 5.1. Chemical composition of AMD obtained from Witbank South Mine

Parameter	Units (mg/ℓ, except for pH)
Ph	2.5
Acidity	1 200
SO_4	2 600
Cu	0.75
Total Fe	76
Pb	0.25
Mg	77
Mn	9.3
Ni	0.61
Zn	4.0
Na	19
K	7

Table 5.2. The metal concentration of AMD before and after dilution with sulphide rich effluent

Metal (mg/ℓ)	AMD	Diluted AMD
Al	11.7	5
Cu	0.75	0.04
Fe	41	0.2
Mn	9.3	5.7
Zn	4.0	0.27
Ni	0.61	0.24

5.2.4 Study 2b. Operating the two stage reactor system using pre-treated AMD as feed water

5.2.4.1 Feed water

Mine seepage water from a closed coal mine situated in the Witbank area functioned as feed water for the two stage reactor system, as described under 5.2.1.3 (Figure 5.1). The chemical composition of this mine water is presented in Table 5.1. Only FR received feed water in this study at a feed rate of initially 15 ℓ/d and later at 30 ℓ/d, while the effluent from FR was the only feed water to SR. The pH of this mine water of 2.5 was too low and the acidity concentrations too high (1 200 mg/ℓ) to use untreated AMD as feed water to the hybrid reactor FR (Table 5.1). This AMD therefore required treatment prior to feeding it to the reactor system. The biological pre-treatment method was used as described in 5.2.3.1 (Greben *et al*. 2000, 2003).

5.2.4.2 Carbon and energy source

The fermentation products of the cellulose present in grass cuttings (GC), formed in FR, served as the carbon and energy source for both FR and SR.

5.2.4.3 Reactor system (FR and SR)

The two stage reactor system FR and SR was used (Figure 5.1). In this study, feed water only entered FR, while SR received the effluent of FR as the only feed water.

5.2.4.4 Analytical

The same analytical procedures as described in Section 3.2.4 were followed.

5.2.4.5 Experimental

The same procedure as in Study 1a was followed. The feed rate was 15 ℓ/d from day 1-37 and 30 ℓ/d from day 38-78. Grass cuttings were added according the following pattern: 25 g on day 2, 100 g on days 6, 12 and 34 and 1000 g on day 27. From day 41-60 it was 20 g/d and from day 61-78, it was 40 g/d. There were three experimental periods: d 1-37, d 41-49 and d 50-78.

5.3 RESULTS AND DISCUSSION

5.3.1 Study 1a

5.3.1.1 Sulphate removal

The chemical compositions, based on the averages of the daily analyses during the total experimental periods of the treated waters of FR and SR, is given in Table 5.3. The effluent of FR was the feed stream to SR at a rate of 5 ℓ/d, while SR also received sulphate-rich feed water at 5 ℓ/d, thus the total feed-rate to SR was 10 ℓ/d. The average SO_4 load to FR was 1.7 g/ℓ, while the average SO_4 removal in FR was 1.6 g/ℓ (Table 5.4) and the average SO_4 removal in SR was 1.1 g/ℓ. Thus the combined SO_4 removal in FR and SR amounted to 2.7 g/ℓ. The data in Table 5.4 furthermore showed that the total average removal was 19 g/d SO_4 over the two reactors during the experimental period (Table 5.4).

Table 5.3. The chemical composition of the treated water from FR and SR

Parameter (mg/ℓ)	**Treated FR (Feed SR)**	**Treated SR**
SO_4	895	597
pH (Value)	7.31	7.49
COD	1417	1228
Sulphide	294	295
Alkalinity	1818	1894
C2	530	977
C3	248	98
C4	123	-
S^{2-}/SO_4	0.18	0.13
Alk/SO_4	1.13	0.68

Table 5.4. The SO_4 removal pattern in FR and SR

Reactor	SO_4 Removed(mg/ℓ)	Qfeed (ℓ/d)	SO_4 removed (g/d)	SO_4 RR* gSO_4(ℓ.d)
FR	1 614	5	8	0.8
SR	1 105	10	11	2.2

***RR=reduction/removal rate**

The sulphate concentrations in the feed- and treated water and the reactor COD concentrations in FR and SR are presented in Figures 5.3 and 5.4, respectively. The graphs in Figure 5.3 showed that initially the COD concentration in the treated water of FR is >1000 mg/ℓ, which resulted in a SO_4 concentration generally < 500 mg/ℓ in the effluent. This result showed that when the residual COD concentration is high, a sustained SO_4 removal can be achieved. When the COD concentration decreased on days 32-60 (to concentrations <1000 mg/ℓ) the SO_4 concentration in the effluent increased to values >1000 mg/ℓ, respectively. The reversed pattern was observed from day 60-70: high COD concentrations in the effluent, resulting in a lower SO_4 concentration. These results clearly indicated the relationship between a high residual COD concentration and high SO_4 removal rate.

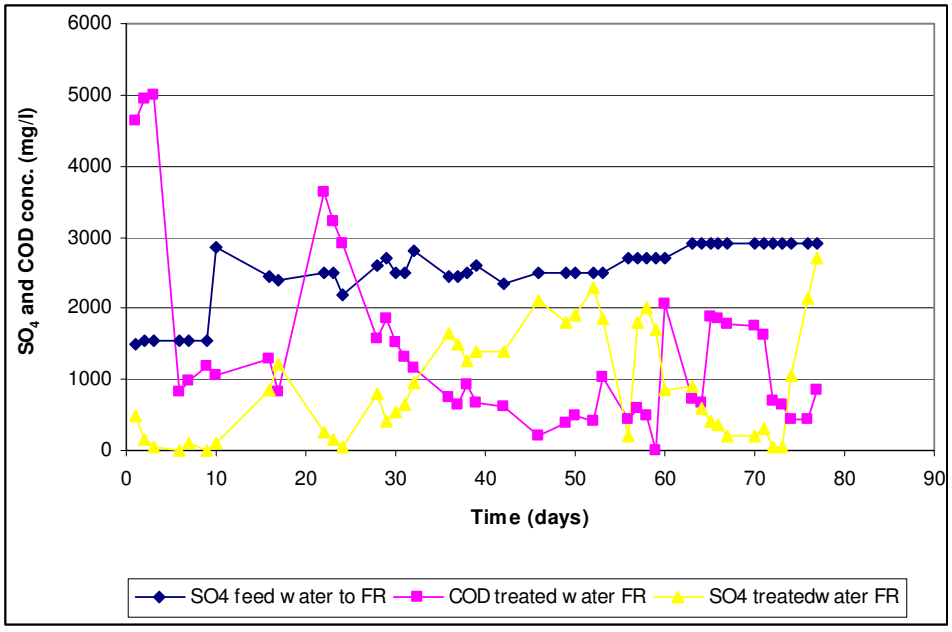

Figure 5.3. The SO_4 and COD concentrations relating to operation FR

A similar pattern can be noted for SR (Figure 5.4), where high COD concentrations (± 5000 and 4000 mg/ℓ) resulted in low SO_4 concentrations in the treated water. When the experiment was conceived, it was hypothesized that the fermentation process would occur in FR, followed by SO_4 removal in SR, utilizing the fermentation products from FR for biological SO_4 removal in SR. However, as shown in the results of study 1a, SO_4 removal already occurred in FR.

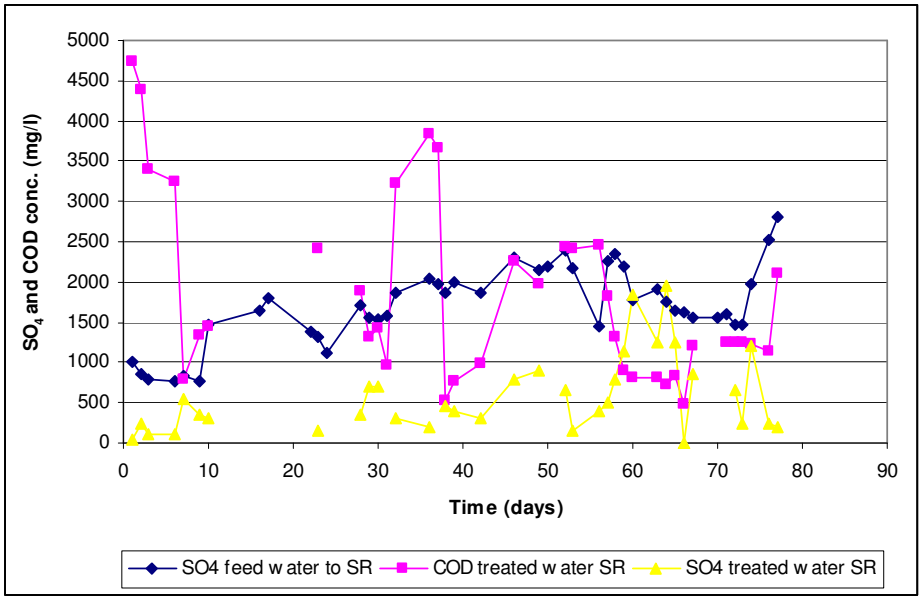

Figure 5.4. The SO_4 and COD concentrations relating to SR

5.3.1.2 VFA utilisation

The results in Table 5.4 showed that the C4 and C3 acids were utilised, producing C2 acid, since the C2 acid concentration increased with 447 mg/ℓ, while the C3 acid concentration decreased by 150 mg/ℓ and the butyric acid concentration by 123 mg/ℓ. From these and the sulphate removal results (total removed 2688 mg/ℓ SO_4) it became evident that the SRB, in addition to VFA, utilised other intermediate products from the fermentation process, e.g. hydrogen and alcohols. Hydrogen is a major intermediate in the degradation of organic matter and is used by SRB as soon as it is produced by fibrolytic and fermentative microorganisms (Hungate, 1966; Wolin and Miller, 1988). Interspecies hydrogen transfer between H_2-producing and H_2-utilising microorganisms allows growth and activities of the fermentative and sulphate reducing microorganisms. During the total experimental period of 60 days, 95 g sulphate was removed, while 447 g acetate was produced (based on the daily results). Acetate production can be ascribed to the weekly addition of 1000 g of grass cuttings. When utilising the C3 and C4 acids, the amount of acetate produced can

be the rate limiting factor and potentially cause reactor failure. Hill *et al*. (1987) proposed that an acetic acid concentration > 800 mg/ℓ or a C2:C3 ratio > 4:1 can have an adverse effect on the reactor processes. It was therefore decided to add less grass cuttings to FR during Study 1b.

5.3.1.3 Sulphide production (Table 5.3)

During the biological sulphate removal process, sulphide is produced according to reactions 5.1-5.2. This can be biologically oxidised to elemental sulphur in the presence of oxygen (reaction 5.3):

$$\text{Propionate}^- + \tfrac{3}{4}\ SO_4^{2-} \rightarrow \text{Acetate}^- + HCO_3^- + \mathbf{\tfrac{3}{4}\ HS^-} + \tfrac{1}{4}\ H^+ \quad (5.1)$$

$$\text{Butyrate}^- + \tfrac{1}{2}\ SO_4^{2-} \rightarrow 2\ \text{Acetate}^- + \mathbf{\tfrac{1}{2}\ HS^-} + \tfrac{1}{2}\ H^+ \quad (5.2)$$

$$H_2S + \tfrac{1}{2}\ O_2 \rightarrow H_2O + S^\circ \quad (5.3)$$

From these reactions, for every mole of SO_4 (96 g), one mole of sulphide is formed (32 g), which results in the experimental S^{2-}/SO_4 ratio of 0.33. Although small amounts of air could diffuse into the reactor, the Dissolved Oxygen (DO) concentration was always measured at zero, while the redox potential was on average -190 mV. Dilling and Cypionka (1990) described the aerobic respiration of SRB. They found that cultures of *Desulfovibrio desulfuricans* (strain CSN) reduced 5 mM O_2 with H_2 as electron donor. Aerobic respiration was not coupled with growth, but resulted in ATP formation. Besides H_2, organic electron donors, such as formate, lactate, ethanol and pyruvate, as well as inorganic sulphur compounds, e.g. H_2S, thiosulphate, sulfite, were utilised for aerobic respiration. Sulphite and thiosulphate were completely oxidized to sulphate.

The sulphide production in FR was 294 mg/ℓ, while the SO_4 removal was 1614 mg/ℓ. Therefore the S^{2-}/SO_4 ratio in FR was 0.18, which is lower than the theoretical value of 0.33, showing that part of the S^{2-} formed was not accounted for. This was explained by the biological oxidation of sulphide to sulphur (reaction 6.3) as well as by the fact that part of the sulphide escaped in the gaseous form, due to the lower reactor pH. Weast (1981) described that the pK_a value of the dissociation equilibrium of H_2S is 7.04 at 18 ℃. Above pH 8.0-9.0 virtually all dissolved sulphide is present in its ionised form, while at neutral pH values 20 to 50% of the dissolved sulphide is present as H_2S, depending on the reactor temperature (O'Flaherty & Colleran, 2000).

Furthermore, part of the sulphate is reduced to intermediate products, such as thiosulphate and sulphite, which are not analysed in the sulphide analysis, during the biological sulphate removal process. The increase in reactor pH after sulphate reduction (due to alkalinity production) is therefore beneficial in lowering the sulphide toxicity. In most cases it is advisable to keep the pH of the sulphidogenic reactor between 7.5 and 8.5. However, this higher reactor pH is not advisable when rumen bacteria are present in the same reactor, since they prefer a pH of 6.6-6.9.

The sulphide rich effluent from FR entered SR, which also received sulphate-rich feed water. The sulphide load to SR was 147 mg/ℓ. The sulphide concentration in the treated water of SR was 295 mg/ℓ, thus sulphide produced in SR was 148 mg/ℓ. The SO_4 removal in FR was 1105 mg/ℓ, thus the S^{2-}/SO_4 ratio in FR was 0.13, which is lower than the theoretical ratio of 0.33. The lower ratio was partly explained by the sulphur formation at the top of the reactor. Air diffusion was possible at the top of the reactor, resulting in sulphide oxidation according to reaction (5.3).

5.3.1.4 Alkalinity production

The average alkalinity production in FR was 1818 mg/ℓ $CaCO_3$, while the sulphate reduction was 1614 mg/ℓ, resulting in an Alkalinity/SO_4 ratio of 1.13, which is higher than the theoretical ratio of 1.04. This was ascribed to pH correction in FR using $NaHCO_3$, as initially the fermentation of the grass resulted in high VFA concentrations and thus in the reactor pH decrease. The alkalinity concentration entering SR was diluted by the feed stream into SR, which resulted in an average alkalinity concentration of 909 mg/ℓ. The treated water from SR contained an average alkalinity concentration of 1669 mg/ℓ, which implied an average alkalinity production of 760 mg/ℓ in SR. The average sulphate removal in SR was 1105 mg/ℓ, thus the Alkalinity/SO_4 ratio was 0.69, which is << the theoretical value of 1.04. The lower experimental value (0.69) was ascribed to addition of 0.1N HCl to correct for the increased reactor pH (values > 7), as a result of the biological sulphate removal in FR. The reactor pH in FR needed to be maintained between 6.6 and 6.9 to accommodate the rumen microorganisms.

5.3.1.5 Reactor pH

The average pH of the FR treated water was 7.31, while the average pH of the SR treated water was 7.39. The reactor pH increased due to sulphate removal, followed by alkalinity production in FR and SR.

5.3.2 Study 1b

A three stage reactor system was used (Figure 5.2). All three reactors received synthetic feed water at 5 ℓ/d. In addition SR received the effluent of FR (total feed rate 10 ℓ/d) and ASR received the effluent of SR (total feed rate 15 ℓ/d), thus FR had a HRT of 4 days, SR of ½ day and ASR of ⅓ day. Study 1b was divided into four periods, during which 150 g grass was added to FR on days 1, 32, 46 and 62. The duration of the four periods was 19, 15, 15 and 14 days, respectively. The monitoring of the reactor system started on day 14.

5.3.2.1 Sulphate removal in FR, SR and ASR

The chemical composition of the average results of the feed and treated water from reactors FR, SR and ASR during the four periods are given in Table 5.5. The highest sulphate removal took place in FR, followed by SR and ASR (Table 5.6). Sulphate removal was followed by sulphide production as deduced from the data in Tables 5.5 and 5.6. The $S^{2-}_{produced}/SO_{4\ removed}$ ratios were 0.19, 0.21, 0.19 and 0.20 during periods 1-4 in FR. The graphs in Figures 5.5-5.8 show the relationships between the available COD concentrations and the sulphate reduction in the three stage reactor system (FR, SR and ASR). The SO_4 removal pattern in FR was initially irregular, however, it improved after day 35. During the periods that the COD concentration was lower than 1000 mg/ℓ, the sulphate reduction was less efficient (≈ day 45). Fresh GC (150 g per addition) was added on days 32, 46 and 62 (arrows). It was observed from Figure 5.5 that after each grass addition, the COD concentration increased, while it decreased during the periods of sulphate reduction.

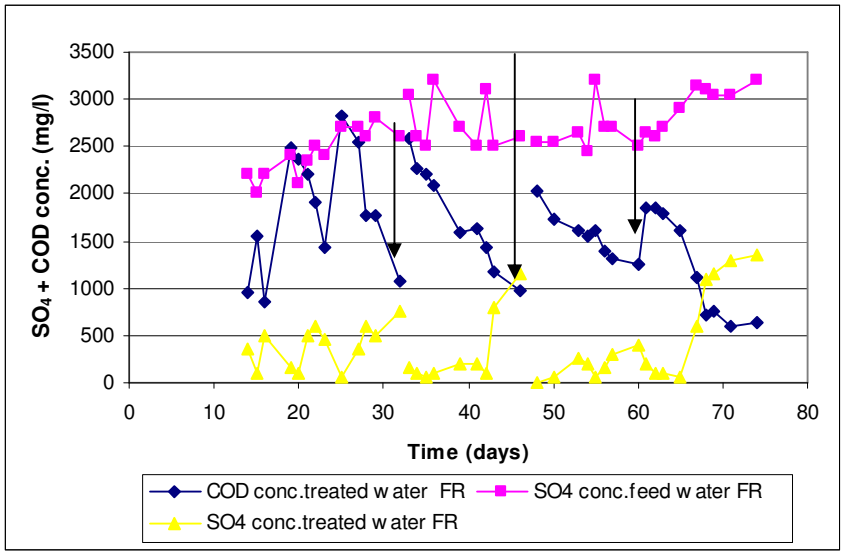

Figure 5.5. Sulphate removal and COD concentration in FR

A similar relationship between the COD and SO_4 concentration pattern was observed in Figure 5.6. When the COD concentration was high, the SO_4 concentration was low and on days when the COD concentration decreased to values < 1000 mg/ℓ, the SO_4 concentration in the reactor increased. When the COD concentration was lower than 500 mg/ℓ, the sulphate reduction seemed to come to a halt. This can be clearly seen from the graphs in Figure 5.6, after day 56. Sulphate was most efficiently removed in FR (volume: 20 ℓ), in which the HRT was 2 days for the fermentation process and 2 days for the sulphate removal process (total HRT: 4 d). The SO_4 removal in SR (volume: 5 ℓ) was not as efficient as in FR, which was ascribed to the addition of fresh sulphate feed water, to the lower COD concentration entering SR and to the shorter HRT of ½ day.

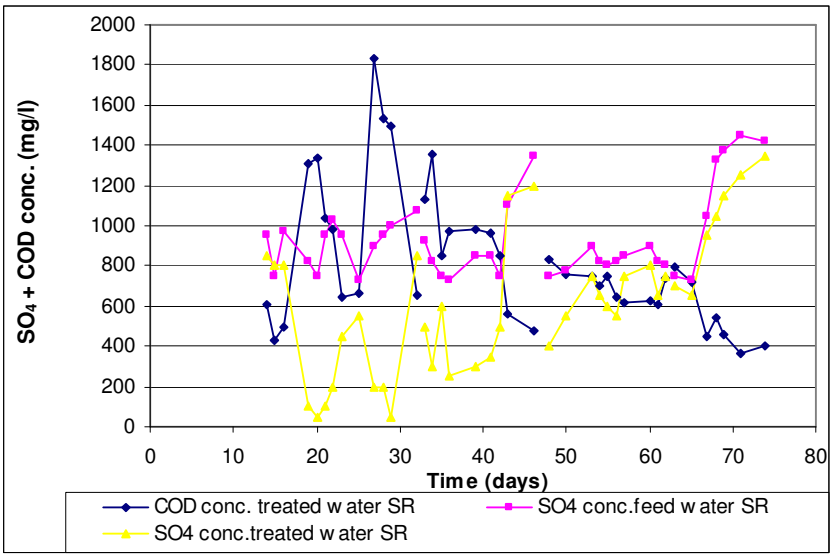

Figure 5.6. Sulphate removal and COD concentration in SR

Figure 5.7 showed that the SO_4 removal was the least efficient in ASR (volume: 5 ℓ), (HRT: ⅓ d) since the COD concentration was the lowest in ASR compared to FR and SR. The overall sulphate removal was the highest in FR, followed by SR, while it was the lowest in ASR (Table 5.4).

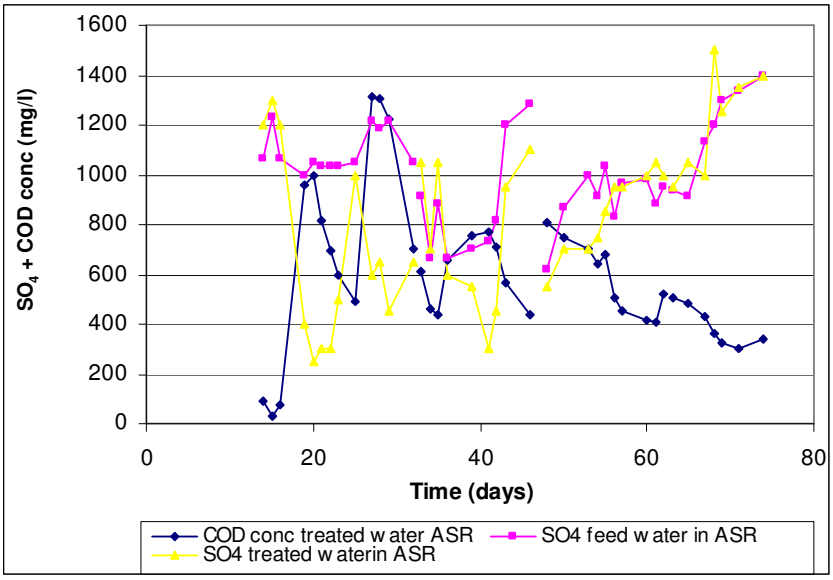

Figure 5.7. Sulphate removal and COD concentration in ASR

There were four experimental periods during study1b, determined by adding fresh GC (150 g) on days 1, 32, 46 and 62. The monitoring of the reactor system started on day 14. The chemical compositions of the feed and treated water from FR, SR and ASR during the four periods are given in Table 5.5, indicating that the highest sulphate removal took place in FR, followed SR and ASR. Sulphate removal was followed by sulphide production as deduced from the data in Table 5.5. The $S^{2-}_{produced}/SO_{4removed}$ ratios were 0.19, 0.21, 0.19 and 0.20 during periods1-4 in FR. Although these ratios were lower than the theoretical value of 0.33, it was noted that the ratios throughout the four experimental periods were stable. The sulphate removal values that occurred in the three stage reactor system, during the four experimental periods, are presented in Table 5.6. The results showed that during each period a total of 435, 245, 223 and 190 g SO_4 was removed using 150 g grass per period. It was noted from this data that the amount of sulphate removed decreased over periods 1-4.

The results from FR (Table 5.6) indicated that the average SO_4 removal in FR was similar at 176, 175 and 172 g over a period of 14 and 15 days during periods 2, 3 and 4. The higher total sulphate removal of 194 g in the first period can be ascribed to a longer period of 19 days.

It was calculated from the total sulphate removal over the four periods that from 1 g grass, in the first period, 3 g sulphate was removed; in the second period it was 1.6 g, while it was 1.5 g and 1.3 g in the third and fourth period, respectively. This

removal yield was relatively high compared to the results obtained from the batch tests as described in Chapter 4, which showed that in order to remove 1 g SO_4, 8 g grass was needed. The decrease in the sulphate removal during the four periods was ascribed to the fact that as no further GC was added, cellulose became depleted. The sulphate removal efficiency in FR during the four periods was 84, 91, 88 and 80%, respectively, while in SR the SO_4 removal decreased from 36 to 22 to 12 and to 9%, respectively, while little additional SO_4 removal was observed in ASR. The results in FR compared well to the experiments where commercial propionate was used as the carbon and energy source, since during that study, the percentage sulphate removal was 78% (Greben *et al*. 2004). The low sulphate removal in the third reactor prompted the decision not to continue with the three stage reactor design. When taking into account the SO_4 removal efficiency in FR and SR, the question also arose whether a second reactor (SR) adds sufficient value to the reactor system to warrant its use. This observation will be discussed later.

Table 5.5. The chemical compositions of the feed and treated water during the four periods in FR, SR and ASR.

Period 1						
Parameter	**Feed FR**	**Treated FR**	**Feed SR**	**Treated SR**	**Feed ASR**	**Treated ASR**
COD (mg/ℓ)		1724		922		649
pH (value)	7.15	7.23	7.21	7.46	7.30	7.66
SO_4 (mg/ℓ)	2367	383	910	490	1095	747
S^{2-} (mg/ℓ)		386		352		290
Redox Potential (mV)		-173		-168		-143
Period 2						
COD (mg/ℓ)		1965		928		604
pH (value)	7.20	7.26	7.39	7.48		7.55
SO_4 (mg/ℓ)	2761	244	864	544	859	750
S^{2-} (mg/ℓ)		522		459		364
Redox Potential (mV)		-174		-163		-152
Period 3						
COD (mg/ℓ)		1519		633		583
pH (value)	7.30	7.45	7.43	7.63	6.57	7.70
SO_4 (mg/ℓ)	2650	315	903	730	968	870
S^{2-} mg/ℓ)		446		302		225
Redox Potential (mV)		-171		-158		-147
Period 4						
COD (mg/ℓ)		1276		590		416
pH (value)	7.33	7.46	7.36	7.52	7.34	7.86
SO_4 (mg/ℓ)	2895	600	1040	905	1087	1095
S^{2-} (mg/ℓ)		467		239		159
Redox Potential (mV)		-154		-143		-138

Table 5.6. The sulphate removing data in the three reactor system

Period 1				
Parameter	*FR*	*SR*	*ASR*	*TotalSO_4 (g) removed over each period*
Av SO4 removal (g/ℓ)	2.04	0.51	0.42	
Av SO4 removal (g/d)	10.21	6.10	6.27	
Av SO_4 removed during period 1 (g)	194	122	119	435
% SO_4 removal efficiency	84	36	n.a	
Period 2				
Av SO4 removal (g/ℓ)	2.52	0.33	0.11	
Av SO4 removal (g/d)	12.58	3.25	1.64	
Av SO_4 removed during period 2 (g)	176	45.5	23	245
% SO_4 removal efficiency	91	22	n.a	
Period 3				
Av SO4 removal (g/ℓ)	2.33	0.17	0.10	
Av SO4 removal (g/d)	11.67	1.73	1.48	
Av SO_4 removed during period 3 (g)	175	26	22	223
% SO_4 removal effieciency	88	12	n.a.	
Period 4				
Av SO4 removal (g/ℓ)	2.29	0.14	No removal	
Av SO4 removal (g/d)	11.49	1.35	No removal	
Av SO_4 removed during period 4 (g)	172	20	No removal	190
% SO_4 removal efficiency	80	9	n.a.	

5.3.2.2 COD profile in reactors FR, SR and ASR

The graphs in Figure 5.8 show that the highest COD utilisation occurred in FR (difference in COD concentrations between FR and SR), which is in agreement with the sulphate reduction in FR. The residual COD concentration of FR was available for further SO_4 removal in SR, while the COD concentration in the effluent of SR was required for further SO_4 removal in ASR. This latter reactor was coupled to the reactor system, because during Study 1a, 1000 g GC/week were added to FR, which resulted in high concentrations of acetate in the final effluent. Due to the fact that less grass was added to the reactor system during this investigative period, ASR was no longer needed. The residual COD concentration in the final effluent was most likely in the form of recalcitrant COD (e.g lignin), as the VFA profile in Table 5.7 showed that most VFA was utilised, except for small concentrations of acetate.

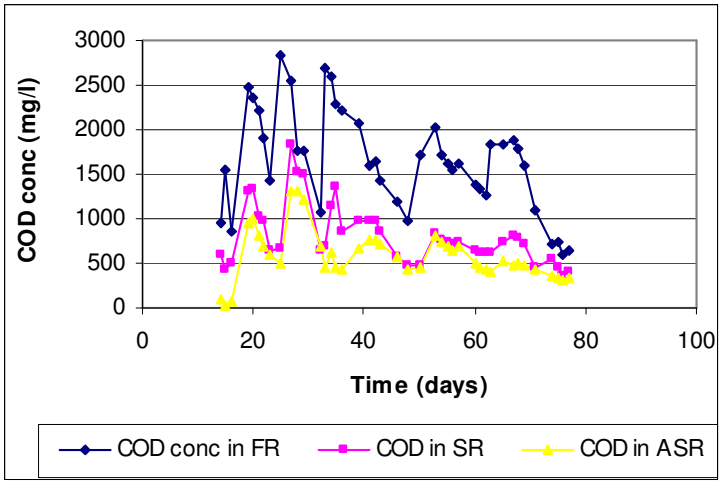

Figure 5.8. COD profile in the three reactor system

Anaerobic digestion is mediated by a complex system of various microbial populations and pathways. Several cultures coexist that derive energy from degradation of various substrates (Ahring 2003). The results of this study showed that the fermentation products required for biological sulphate reduction were obtained from the fermentation reactor (FR), which received grass cuttings on a regular basis.

5.3.2.3 VFA profile in FR, SR and ASR

The data in Table 5.7 indicated that the C3 and C4 VFAs were absent in FR, SR and ASR, whereas acetic acid was present in all reactors. During Periods 1-4, the C2 acid concentration was 649 mg/ℓ, 449 mg/ℓ, 88 mg/ℓ and 27 mg/ℓ, respectively. Thus either less butyric and propionic acids were utilised, thus less acetic acid was

produced or due to the low butyric and propionic acid concentrations, the acetic acid was utilised for sulphate reduction in ASR. The ASRB will use propionate and butyric acid, when available and only use acetic acid, when no other carbon source is obtainable. Acetate utilisation was reported in the previous chapters, when insufficient C3 and C4 acids are available for SO_4 reduction.

Table 5.7. The VFA profile in the three stage reactor system over the four experimental periods

Period 1			
Parameter (mg/ℓ)	FR	SR	ASR
Acetate	649	227	73
Propionate	16	0	0
Butyrate	3	0	0
Period 2			
Acetate	449	126	0
Propionate	3	0	0
Butyrate	1	0	0
Period 3			
Acetate	88	150	79
Propionate	0	0	0
Butyrate	0	0	0
Period 4			
Acetate	27	121	63
Propionate	2	0	0
Butyrate	0	0	0

The finding that the VFA concentrations in the three reactors decreased over the consecutive experimental periods agreed with the correspondingly decreasing COD concentrations in the reactors during the same periods. The COD and VFA results indicated that most of the available COD was utilised in FR followed by SR.

5.3.3 Study 2a

5.3.3.1 Sulphate removal

In this study the results of using pre-treated AMD and synthetic SO_4 rich water as feed water, respectively, operating batch test reactors, are shown in Figure 5.9. It was observed that the sulphate concentration in R1 decreased from 1350 mg/ℓ to 150 mg/ℓ over a period of 10 days. Initially, the sulphate removal in R2 was faster than in R1, however, the overall sulphate reductions in R1 and R2 were similar. The results, as presented in Figure 5.9, seemed to indicate that feeding diluted AMD had no adverse effects on the rumen microorganisms, as in both cases adequate COD was present for the biological sulphate reduction to take place (Figure 5.10).

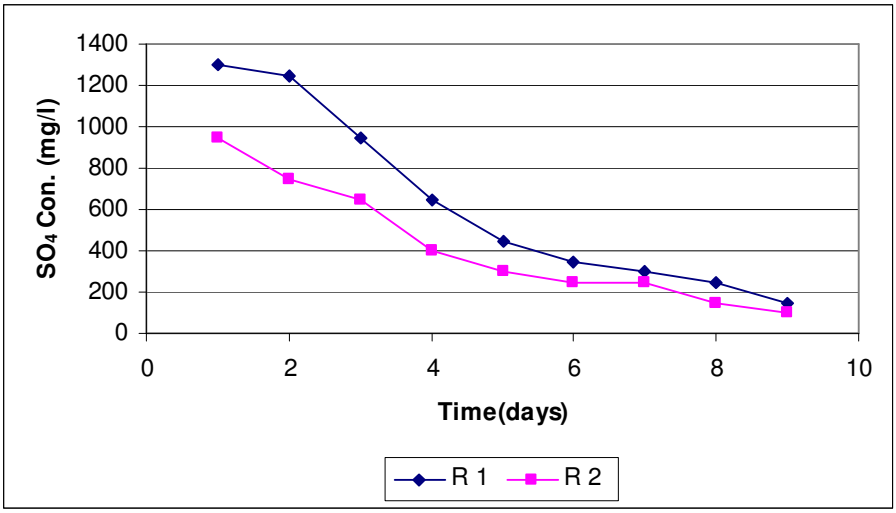

Figure 5.9. The SO_4 removal patterns in R1 and R2
R1 received pretreated AMD
R2 received synthetic sulphate-rich water

5.3.3.2 COD/Sulphate

The results in Figure 5.10 show the COD concentrations in R1 and R2. From the start of the experiments, the COD concentrations in both R1 and R2 increased to more than 5000 mg/ℓ COD, which demonstrated the good cellulose fermentation potential of the rumen bacteria. The available COD concentrations in both reactors were used for the sulphate reduction and, as was observed for the sulphate removal

pattern (Figure 5.9), the COD concentrations in both reactors were similar. These results indicated that pre-treated AMD does not affect the rumen microbes in the cellulose degrading process. It was concluded from Study 2a, that Study 2b could be executed.

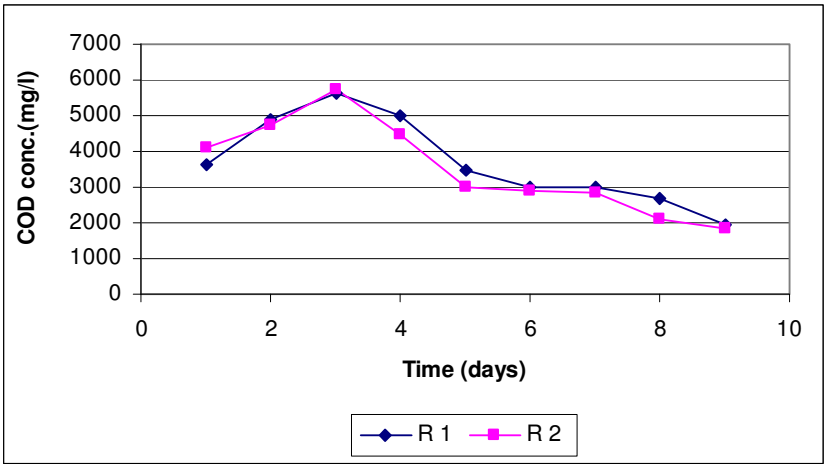

Figure 5.10. The COD concentration in R1 and R2
R1 received pretreated AMD
R2 received synthetic sulphate-rich water

5.3.4 Study 2b

5.3.4.1 Sulphate removal

The sulphate removal profile is illustrated in Figure 5.11. The graphs showed that the sulphate concentration of the treated water in both reactors was very similar at between 500 and 1500 mg/ℓ up to day 29. On day 29 the reactor received 1 kg grass and 1 ℓ rumen bacteria mixture (VSS of 10 g/ℓ) in order to improve the cellulose degradation and to generate a higher COD concentration to improve the biological sulphate removal in both reactors. The addition of fresh GC and the rumen inoculum resulted in a sulphate concentration of 400 mg/ℓ in the treated water on the next day, while it increased again to 1900 mg/ℓ on day 34 to decrease again to 350 mg/ℓ on day 35. From day 40-60, 20 g GC/d were added, which, after the feed rate was doubled on day 50, was increased to 40 g/d from day 61-78. The results indicated that after day 75, the sulphate reduction was <500 mg/ℓ for several consecutive days. On day 50, the feed-rate was increased to 30 ℓ/d, which resulted in an average sulphate load of 70.5 g/d. During this period, different amounts of GC were added. From d 50-62, it was 20 g/d and from d 62-78, it was doubled to 40 g/d, while on d 75, 500 g GC were added. This increased grass addition immediately resulted in improved sulphate reduction as seen in Figure 5.11, which again showed the relationship between GC addition and sulphate removal.

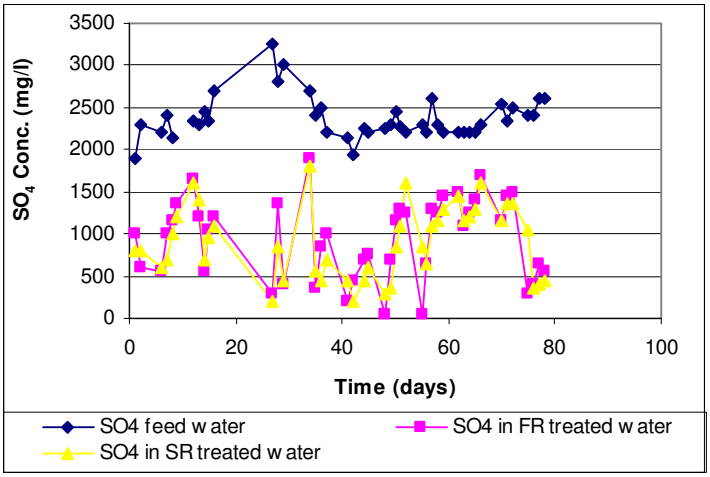

Figure 5.11. SO_4 concentration in feed and treated water in FR and SR reactors

The chemical composition of the feed and the treated water of FR and SR during three experimental periods is presented in Table 5.8. The data is based on the analyses of daily sample taking, which is averaged and presented in Table 5.8. During the first two periods, when the feed-rate was 15 ℓ/d, the highest sulphate reduction occurred in FR, followed by additional SO_4 reduction in SR. This resulted in an increased alkalinity and sulphide concentration and in a further decrease in the redox potential. These different results indicated preferred reactor conditions for sustained sulphate reduction and a further decrease in COD concentration. The highest sulphate reduction was obtained during Period 2, when the feed-rate was 15 ℓ/d and 20 g/d GC were added daily over 8 days. During this total experimental period 160 g GC were added while 205 g SO_4 was removed in FR and 215 g in FR and SR combined. These results indicated that 1 g GC resulted in a total of 1.34 g SO_4 removal. During the first period, the SO_4 reduction in FR was 1472 mg/ℓ, while this was 1708 mg/ℓ during the second period. For SR an additional SO_4 removal of 74 mg/ℓ (5.0%) and 83 mg/ℓ (4.9%), respectively, was noted. During period 3, the removal in FR was 1284 mg/ℓ, while no further reduction took place in SR. The sulphate removal during period 3 was less efficient, because the residual COD concentration at 480 mg/ℓ was too low for sustained SO_4 removal. The low COD was ascribed to the increased sulphate load on day 50, when the feed rate was doubled while initially the amount of GC stayed the same, but was doubled on day 62. From the sulphate reduction data in SR, it was concluded that an additional sulphate removal reactor is unwarranted as the additional capital costs are unjustifiable in order to increase the total sulphate reduction by only 5%. The higher sulphate reduction during Period 2 was ascribed to the daily GC addition of 20 g/d.

5.3.4.1.1 Grass added/sulphate removal ratio

From the batch tests results as described in Chapter 4 it was indicated that 8 g GC were needed to remove 1 g SO_4. From the studies using synthetic, sulphate-rich feed water, the sulphate removal was 3, 1.6, 1.5 and 1.3 g from 1 g GC, over 4 experimental periods, respectively. The decrease in sulphate removal over the four periods was ascribed to the lower COD concentration obtained from the 1000 g GC. From the results as obtained from the study feeding pre-treated AMD, it was shown that the sulphate removal using 1 g GC was 1.34 g in Period 2. This result compared favourably with that obtained using synthetic, sulphate-rich feed water. Most results obtained from the different studies demonstrated that regular addition of GC was essential in order to obtain a high COD concentration in the reactor and hence a sustained sulphate removal, as will be shown in the following paragraph.

5.3.4.2 COD concentration

Sulphate removal is dependant on the COD concentration in the reactor as was shown in the previous chapters. Fresh GC and 1 ℓ rumen fluid were added on d 27, which resulted in a rapid COD increase (Figure 5.12). However, the available COD concentration was utilised instantly and thereafter the COD concentrations in both FR and SR were too low for continued sulphate reduction. Only on d 62 (first day of adding 40 g/d GC) and on d 75 (adding 500 g GC, once off) were COD concentrations increases observed. This resulted in immediate sulphate reduction as shown in Figure 5.11. These results once again showed the relationship between a high COD and low SO_4 concentration in the bioreactor. The COD concentration in the reactor is dependant on the addition of GC and on the cellulose degraders (rumen microorganisms) providing the substrates for the SRB to carry out sulphate reduction. The COD concentration in FR during Period 3 was 480 mg/ℓ, (Table 5.8) while the SO_4 concentration was 1068 mg/ℓ, which resulted in a COD/SO_4 ratio of 0.45 in FR, which was too low to sustain sulphate reduction. The theoretical feed COD/SO_4 ratio is 0.67, in which case the available COD is used for the biological sulphate removal. Ideally the feed water COD/SO_4 ratio should be approximately 1, providing enough COD to sustain sulphate removal and cell growth (Rinzema & Lettinga, 1988).

5.3.4.3 $Sulphide_{produced}/Sulphate_{removed}$ ratio

The sulphide concentrations were 396, 504 and 355 mg/ℓ, respectively, which resulted in S^{2-}/SO_4 ratios of 0.27, 0.30 and 0.28. These ratios were similar to the theoretical ratio of 0.33. The somewhat lower values were ascribed to the metal-sulphide precipitation in the reactor, especially of FeS (Table 5.9).

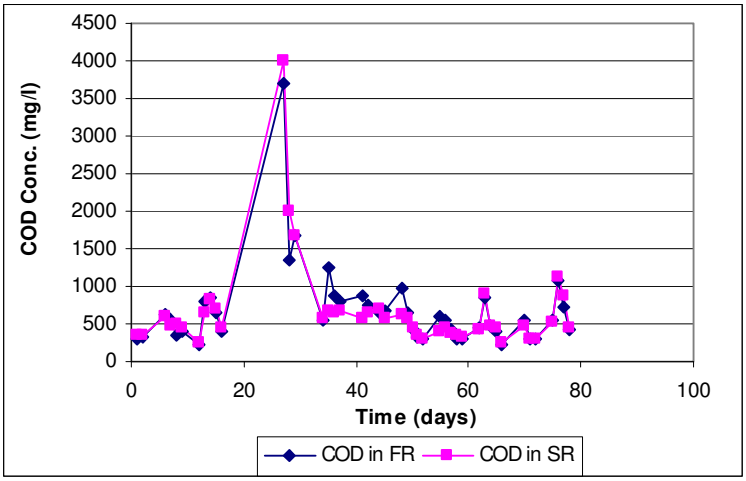

Figure 5.12. COD concentration in the FR and SR reactors

Table 5.8. The chemical composition of the feed and treated water in FR and SR

Parameter	Period 1 (d1-37)	Period 2 (d41-49)	Period 3 (d50-78)
Feed-rate (ℓ/d)	15	15	30
pH Feed (Value)	6.58	7.19	7.12
pH FR (Value)	7.68	7.65	7.42
pH SR (Value)	7.71	7.70	7.45
SO_4 Feed (mg/ℓ)	2489	2183	2352
SO_4 FR (mg/ℓ)	1017	475	1068
SO_4 SR(mg/ℓ)	943	392	1070
Alk Feed (mg/ℓ)	189	518	328
Alk FR (mg/ℓ)	1709	2208	1543
Alk SR (mg/ℓ)	1819	2482	1659
COD FR (mg/ℓ)	922	757	480
COD SR (mg/ℓ)	847	615	478
S^{2-} FR (mg/ℓ)	396	504	355
S^{2-} SR (mg/ℓ)	397	520	345
S^{2-}/ SO_4 ratio	0.27	0.30	0.28
Redox pot. FR (mV)	-175	-192	-183
Redox pot. SR (mV)	-176	-194	-183
VSS in effluent FR(mg/ℓ)	68	50	46

5.3.4.4 Metal removal

It was shown in Table 5.1 that the raw AMD from the mine in Witbank, South Africa, contained several metals. In order to remove the metals prior to feeding this AMD to the reactor, the AMD was pre-treated with the effluent from SR. This effluent contained high concentrations of sulphide: 397, 520 and 345 mg/ℓ, respectively in the three experimental periods (Table 5.8). The data in Table 5.9 show the metal concentrations in AMD, in pre-treated AMD and treated AMD, in both FR and SR. Most metals were removed to a large extent after the pre-treatment and those not completely removed were mostly precipitated during the biological sulphate removal process. All metal concentrations were < 0.10 mg/ℓ, except for iron and manganese. The precipitation of MnS is pH related and occurs at pH > 7.5. At the time of the metal analyses, the average pH in FR and SR were approximately 7.42 and 7.45, respectively (Period 3).

Table 5.9. Metal concentrations in AMD, in pre-treated AMD and in treated AMD

Metal (mg/ℓ)	AMD	Pre treated AMD	FR out	SR out
Aluminium	122	11	<0.07	<0.07
Copper	0.75	<0.04	<0.04	<0.04
Iron	76	2.2	0.22	0.14
Lead	0.25	<0.07	<0.07	<0.07
Manganese	9.3	6.8	3.8	3.0
Zinc	1.7	<0.07	<0.07	<0.07

5.4 CONCLUSIONS

The results of this study confirmed those presented in the previous chapters, namely that the fermentation products of grass cuttings can serve as carbon and energy sources for continuous, biological sulphate removal. Conducting the different studies using synthetic feed water led to the use of two and three stage continuous reactor systems, comprising a hybrid reactor (FR), followed by (two) packed bed reactor(s) SR and ASR, repectively. It was shown that the VFA produced, except acetic acid, were utilised for the biological sulphate removing process, although the sulphate removal rate was not dependant solely on the VFA concentration. Other intermediate products were utilised for sulphate reduction as higher sulphate removals were obtained than were expected from the available VFA concentrations. The addition of a large amount of grass (1000 g/week) resulted in a high COD_{used}/$Sulphate_{removed}$ ratio and in a high residual COD (acetate) concentration. When 150 g GC per two weeks were added, a continuous sulphate reduction was obtained. It was noted that

sustained sulphate reduction was dependant on a continuous COD production. The highest and stablest sulphate removal efficiency was achieved in FR in which the fermentation and sulphate reduction occurred simultaneously at a HRT of 4 days.

The results of the two stage reactor, to which 1000 g GC/week were added, showed a total SO_4 removal rate of 3.0 g SO_4 (ℓ.d). Under these conditions, it was found that the residual COD and acetate concentrations in the treated water were high at 1428 and 977 mg/ℓ, respectively. When operating a three stage reactor system, to which 150 g GC/2 weeks was added, the results showed a stable sulphate removal efficiency in the first reactor, during the four experimental periods (84, 91, 88 and 80%), respectively. Sulphate reduction was obtained in the second sulphate removal reactor, but the percentage removal decreased with time (36, 22, 12 and 9%, respectively over the four periods). This was most likely due to a shortage of readily available substrate (COD/VFA). The total sulphate removal in the third reactor was low and its use added no value to the sulphate removal process. It was shown that 1 g grass could remove 3, 1.6, 1.5 and 1.3 g sulphate over the four experimental periods, respectively.

When pre-treated AMD was used as feed water for the two stage reactor system at a feed rate of 15 and 30 ℓ/d, most of the sulphate was removed in the first reactor. The highest sulphate removal was obtained when GC (20 g) were added daily and when the feed rate was 15 ℓ/d. When the feed rate was doubled and the GC kept constant, a lower SO_4 removal resulted. Both sulphide and alkalinity were produced, the reactor pH increased and the redox potential in the reactor was at -194 mV, when the highest sulphate removal rate was obtained. Since most sulphate was removed in the first reactor with low additional concentrations of sulphate removed in the second and third reactor it was concluded to only operate a one stage (hybrid) reactor system. In order to take this technology to pilot plant scale, the fermentation and removal process in a one stage reactor needs to be well understood.

A process description based on practical results from these studies as well as on theoretical values, was developed to simulate the treatment of AMD. This is described in the following Chapter (6). Only the one stage reactor was used for the calculations in Chapter 6.

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