

The Relationships Between Sulphate Reduction and COD/VFA Utilisation Using Grass Cellulose as Carbon and Energy Sources

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Abstract The release of mine effluents can have a damaging impact on receiving water bodies. Therefore, treatment of mine waters before discharge is imperative. A novel biological SO_4^{2-} removal technology has been developed whereby the degradation/fermentation products of grass cellulose, volatile fatty acids (VFA), function as the electron donors and SO_4^{2-} as the electron acceptor. The aim of the study presented here was to elucidate the interactions between the cellulose degradation rate, the chemical oxygen demand (COD), VFA production and its/utilisation rate as well as the sulphate reduction rate. To this end, two stirred batch reactors were operated: a test and a control reactor. The results showed that high COD and VFA concentrations were achieved after cellulose degradation, which resulted in a rapid decrease in the SO_4^{2-} concentration in the test reactor. The VFA results indicated that propionic and butyric acids were preferentially utilised, producing acetate. In the control reactor, the VFA and the COD production increased initially at the same rate, followed later by a decrease at a similar rate. These results suggest that the degradation products formed were utilised by the methanogenic bacteria to produce methane rather than by the sulphate-reducing bacteria, since the control reactor contained no sulphate (Visser 1995). Furthermore, these results showed a clear relationship between the COD/VFA production and the SO_4^{2-} reduction in the test reactor and between the COD and VFA pattern in the control reactor.

Keywords Biological sulphate removal · Batch reactors · COD/VFA production and utilisation · Relationship · Rumen microorganisms · Sulphate-reducing bacteria · Syntrophy

Introduction

South Africa is particularly rich in mineral resources and is one of the leading raw material exporters in the world. The wealth generation through mineral mining has been and still is

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substantial and has contributed significantly to the country's industrial development. However, mineral mining impacts negatively on the quality of South Africa's water resources. The mineral pyrite is associated with most mining activities. When the coal and other mineral deposits containing pyrite come into contact with air and water, acid mine drainage (AMD) is formed, containing high metal, salinity and acidity concentrations. AMD is a product of both operational and closed mines and often can be related back to a long mining history.

Mine water treatment and mine water management globally as well as in South Africa receive a great deal of attention, since the release of AMD in the environment has damaging impacts, due to its detrimental environmental characteristics [1]. Several methods of mine water treatment have been published, such as the chemical treatment using lime or the limestone–lime combination [2, 3], the physical treatment process, namely the reverse osmosis technology, presently in operation in South Africa [4], and the biological systems, mainly using ethanol as the carbon and energy source [5–8]. However, utilising ethanol as the energy source for the biological technology is not cost-effective since the price of ethanol is related to the oil price. Recently, Greben et al. [9] have described a biological sulphate removal technology using the degradation products of cellulose, originating from cut grass, as the carbon and energy sources for the sulphate-reducing bacteria (SRB). The grass cellulose is degraded by microorganisms, originating from rumen fluid of ruminants (RB), which hydrolyse and degrade the grass cellulose to polymers and monomers and eventually to volatile fatty acids (VFA) and hydrogen (H_2), which function as the electron donors for the SRB. These different groups of microorganisms operate symbiotically in one hybrid reactor, with the advantage that this syntrophic relationship of the different microbes makes the VFA- and H_2 -producing and utilising process very efficient. SRB keep the partial pressure for H_2 low [10, 11], thereby encouraging the continuous fermentation process for increased energy production. Studies have shown that the SRB outcompete the methanogenic and the homoacetogenic bacteria when sulphate is present as the electron acceptor [10–12].

Previously, the continuous sulphate-reducing process, operating a hybrid reactor system, was described, initially at 37 °C and later at 25 °C [13, 14]. In order to scale-up the process, batch studies were conducted to investigate the relationship between the sulphate reduction rates and the chemical oxygen demand (COD)/VFA production and utilisation rates.

Materials and Methods

Reactors

Two plastic stirred batch reactors, one test (T) and one control (C), each with a volume of 2 L, were operated at 25 °C. The fixed temperature was maintained by pumping water from a water bath (set at 25 °C) to water mantles surrounding the reactors. The pumps used are Watson-Marlow Bredel 323 peristaltic series. The contents of T and C were almost identical, except T contained sulphate-rich feed water, while C contained tap water. Nutrients (1 mL/L) were added to both T and C [9]. Table 1 depicts the experimental conditions of T and C. The pH of the reactor contents was controlled between pH of 6.6–6.9 to accommodate the RB [16].

Experimental

The reactors received 100 g of grass cuttings, 250 mL of RB and SRB each at the start of the experiment. SRB were added to both reactors, with the aim that similar conditions in

Table 1 The conditions in reactors T and C

Parameter	Reactors	
	T	C
Nutrient-rich tap water	2 L	2 L
Cut grass	100 g once off	100 g once off
Rumen fluid (RB) obtained from slaughterhouse	250 mL	250 mL
SRB, prepared according to Postgate [15]	250 mL	250 mL
Concentration SO_4^{2-}	2,500 mg/L	0 mg/L

both reactors were created (except no SO_4^{2-} was added to C). On day 9 (a.m.), the sulphate concentrations in T was 0 mg/L. Therefore, fresh SO_4^{2-} was added to T on day 9 (p.m.) so that the SO_4 reduction process in T could proceed. The same procedure was repeated on day 27, with the aim to stimulate further SO_4^{2-} reduction. The experiment was stopped when no further SO_4^{2-} reduction was observed in T, resulting in an experimental period of 49 days.

Sampling

Daily samples (200 mL) were taken from the reactors during the experimental period. Since batch-operated reactors were used, this sample volume was replenished daily with 200 mL nutrient-rich tap-water or nutrient-rich sulphate-containing water (for C and T, respectively). The sulphate concentration of the stock solution was 1,000 mg/L, which resulted in a daily addition of 100 mg/L SO_4^{2-} (200 mL stock added to a 2-L reactor).

Analytical

Determinations of sulphate, COD (soluble COD) and pH were carried out according to standard analytical procedures as described in *Standard Methods for the Examination of Water and Wastewater* [17]. All analyses were carried out on filtered samples (Whatman #1). The COD samples were pre-treated to eliminate the sulphide contribution to the COD concentration. The VFA analyses were done using a gas chromatograph (Agilent 7890) equipped with a flame ionisation detector (FID). The column used was a JW Scientific, DB-Wax, 30 m×0.32 mm×0.5 μm . The GC/FID programme can be summarised as follows: initial oven temperature 50 °C, for 2 min, temperature programmed to increase thereafter by 10 °C/min to 180 °C, with temperature held for 0 min at 180 °C, FID temperature 350 °C. The carrier gas (H_2) flow rate was set at 1.5 mL/min.

Results and Discussion

The Relationships Between SO_4^{2-} Reduction and the COD in T

The SO_4^{2-} reduction during the total experimental period of 49 days is presented in Fig. 1, showing that the sulphate concentration was reduced from 1,800 to 0 mg/L during the first 9 days of the experiment. Fresh sulphate was added to T on day 9 (p.m.) resulting in a SO_4^{2-} concentration of 2,150 mg/L in the reactor. This was rapidly reduced to 850 mg/L over a

period of 2 days, where after it was reduced to 350 mg/L over a period of 10 days. Although no further SO_4^{2-} reduction was observed during the following days, fresh sulphate was added to the reactor on day 26 to stimulate the SO_4^{2-} reduction. It was, however, observed that the SO_4^{2-} concentration was only reduced from 2,650 to about 2,000 mg/L over a period of 5 days. Thereafter, the SO_4^{2-} concentration remained at this concentration for the remaining 20 days of the experimental period. When performing discrete differentiation on the experimental data from the three sulphate reduction periods, namely from days 0 to 9, from days 10 to 20 and from days 26 to 31 (Fig. 1), the results indicated that the rate of SO_4^{2-} reduction, $d[\text{SO}_4^{2-}]/dt$, was -0.148 , -0.259 and -0.037 , respectively, indicating that the SO_4^{2-} reduction rate was the highest during the second period, which coincided with the high COD concentration on day 9. These results clearly showed the relationship between a high COD concentration and a fast SO_4^{2-} reduction, which are in agreement with the results of previous studies, when a SO_4^{2-} removal of $>80\%$ was achieved at a high residual COD concentration in the continuous operating reactors [9, 13].

Three experimental phases in T were recognised during the total duration of the experiment: days 0–9, 10–26 and 27–49. During these three periods, the COD utilisation rate and the SO_4^{2-} reduction rate were compared to investigate the relationship between these rates. It can be observed (Fig. 1) that, initially (days 0–9), the COD concentration increased, even though the SO_4^{2-} concentration decreased during that same period. These results indicate that the COD production rate from the grass cellulose was higher than the COD utilisation rate for the SO_4^{2-} reduction. The highest COD concentration in T was measured on day 9 at 10,590 mg/L. From day 9 onwards, the COD concentration decreased, which was ascribed to the utilisation due to the sharp decrease in the SO_4^{2-} concentration. The COD removal/utilisation rate is difficult to calculate, since the production and utilisation of COD occur concurrently. The SRB utilise the energy sources as soon as these are produced by the RB. Visser [16] reported that the SRB keep the partial pressure for H_2 low, resulting in a higher production of energy sources by the RB, as a consequence of a perfect syntrophy between the fermentation and energy utilising bacteria [11, 18].

When fresh SO_4^{2-} was added to the reactor on day 26, the COD concentration was still relatively high at 4,000 mg/L, decreasing to 2,000 mg/L from day 30 onwards. This decrease in COD concentration could, however, not primarily be linked to the SO_4^{2-} reduction during that period, since the SO_4^{2-} reduction rate was low at -0.037 during days 26–31, and thereafter, the SO_4^{2-} concentration remained stable at about 2,000 mg/L

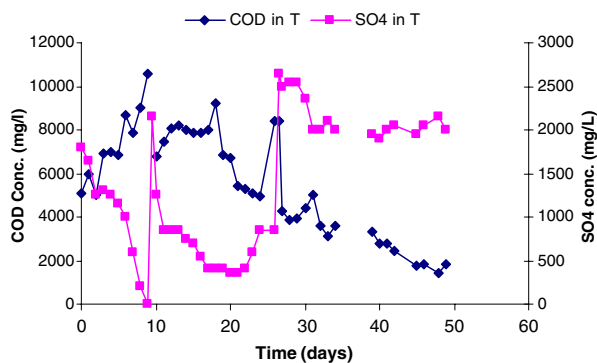


Fig. 1 The COD and SO_4 concentrations in T

during that period. Thus, although there was still a high residual COD concentration of 4,000 mg/L and a high SO_4^{2-} concentration in T, no further SO_4^{2-} reduction was observed, but at the same time the COD concentration did decrease. Logan reports that the anaerobic degradation of cellulosic material to simpler molecules by the fermenting microbes can limit the rate at which substrates become available to the SRB, indicating that the sulphate reduction seems controlled by the activity of the fermentation microbes (RB) [19]. It was interesting to note that a similar decrease occurred in the control reactor C shown in Fig. 2.

The COD Concentration in T

The biological sulphate reduction is dependent on a carbon and energy source, expressed as the COD concentration in the reactor. The theoretical COD/ SO_4 ratio needed for the sulphate reduction is 0.67; however, Rinzema and Lettinga [20] advised that this ratio should be approaching 1 in a sulphidogenic reactor, since the higher ratio assures that sufficient COD is available for both the SO_4^{2-} reduction and for the growth of the SRB. The addition of 100 g grass at the start of the experiment was to maintain a high COD concentration in the reactors, sufficient for prolonged SO_4 reduction. The organic carbon as supplied by the grass cellulose consists of (1) simple organic compounds, (2) polysaccharides, which are degraded more slowly, and (3) a recalcitrant fraction, which can take up to 180 days to ferment [21]. The carbon cycle is closed as a result of the cellulose-utilising microorganisms present in soil and the guts of animals [22]. Plant biomass is a sustainable source of energy when cellulose is utilised during anaerobic degradation, resulting in the production of VFA and other degradation products. This process involves many species of bacteria, such as the acetogenic bacteria and the methanogenic bacteria. The SRB also play a role in the degradation of the complex polymers in the presence of sulphate [11]. Greben and Baloyi [23] showed that the anaerobic degradation of plant biomass (grass) to VFA was enhanced when SRB were added to the fermentation process, even when no sulphate was present. This outcome indicated that the SRB participated in the degradation of the polymers and monomers to produce VFA. The utilisation of propionic acid in the absence of sulphate was shown by Harmsen [18].

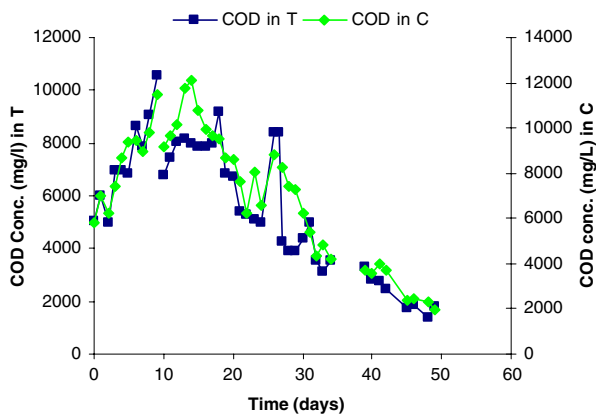


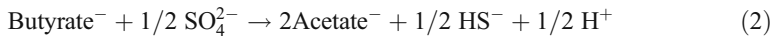
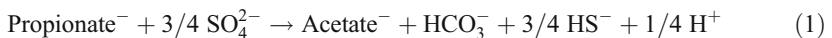
Fig. 2 The COD concentrations in C and T

Reactor C was operated to investigate the COD production when no sulphate forms part of the equation. Figure 2 shows the COD concentrations in reactors T and C.

Figure 2 shows that the COD concentration in T is lower than in C, due to the SO_4^{2-} reduction in T. Although COD utilisation for SO_4^{2-} reduction occurred in T, the trend of the COD concentrations in both reactors is similar. After day 10, the COD concentration decreased in T, which coincided with the SO_4^{2-} reduction after the second load of SO_4^{2-} was added to T on day 9 (Fig. 1). The average measured COD concentration in T was 5 494 mg/L, while this was 7,178 mg/L in C. The difference in COD concentration between both reactors increased initially (days 10–20) but decreased again when the SO_4^{2-} reduction was less remarkable in T (after day 20). The observed difference in the COD concentrations in T and C can, as discussed before, be ascribed to the COD needed for the SO_4^{2-} reduction in T. The start COD concentration in C and T was 5,780 and 5,060 mg/L, respectively, where after it steadily increased to 12,130 mg/L on day 14 in C and then decreased. This observation can also be made from the COD patterns in T, where the COD concentrations peaked on day 9 and then decrease thereafter. Seeing that the decrease in COD concentration occurred in both reactors, this decrease most likely should not only be ascribed to the SO_4^{2-} reduction, but to other factors, applicable to both reactors, such as methane production.

The VFA Concentration in T and C

The RB produce VFA mainly from the monomers in the grass cellulose fermentation process, which then are utilised by the SRB according to Eqs. 1 and 2:



The products of this sulphate reduction process, using VFA as the energy sources are sulphide, alkalinity and acetate. When 1 mol of propionic acid is utilised for the sulphate reduction process, 1 mol of acetate is formed, and when 1 mol of butyrate is used for the biological sulphate reduction process, 2 mol of acetate are produced. Thus, it can be expected that the acetate concentration in T is higher than in C, since SO_4^{2-} reduction in T occurred (Fig. 1). The acetate concentrations in C and T are presented in Fig. 3.

Figure 3 shows that overall high C2 acid concentrations were obtained in both T and C (higher than 2,500 and 2,000 mg/L, respectively). The acetate concentration in T is indeed higher than in C, indicating that acetate is produced after the biological SO_4^{2-} reduction in T according to Eqs. 1 and 2. The average acetate concentration in C was 1,070 mg/L, while this was 1,323 mg/L in T. The acetic acid concentration decreased in T from 153 to 33 mg/L after day 31, coinciding with the halt in the SO_4^{2-} reduction. The acetic acid concentration in C decreased from day 20, similar to the COD pattern, which also decreased after day 20, showing a relationship between the COD and VFA pattern in the control reactor.

The propionic and butyric acids concentrations are presented in Figs. 4 and 5, respectively. During the grass cellulose degradation process, VFA, H_2 and other intermediates (alcohols) are formed, which can be utilised by the SRB as energy sources for the biological sulphate-reducing technology. Visser indicated that the SRB prefer H_2 as the first choice, followed by propionic acid and butyric acid [10]. Generally, acetate is not favoured by the SRB as an energy source and often is the limiting factor in sulphate-

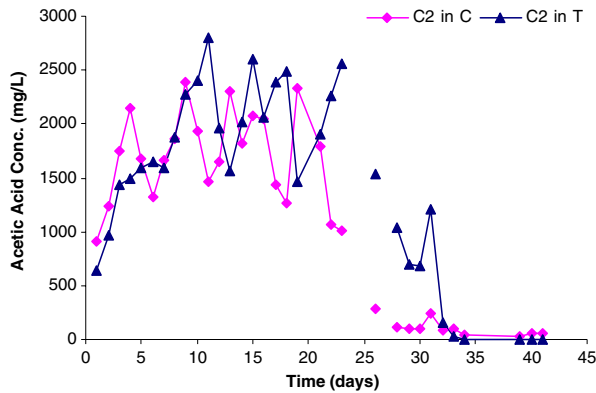


Fig. 3 The acetic acid concentrations in T and C

reducing reactors [6, 24, 25]. It has been reported by Reis et al. [26] that undissociated acetic acid can have an inhibitory effect on the SRB. However, Hulshoff-Pol et al. [5] indicated that when the SRB use H_2 as the energy source, they often use acetate as the carbon source. The acetogenic bacteria can produce acetate from H_2 and CO_2 in acetate limiting environments [5].

The results in T clearly indicate that the C3 acid was utilised for biological SO_4^{2-} reduction, compared to the C3 acid concentration in C, which was high at concentrations $>1,200$ mg/L (Fig. 4). After day 9, a decrease in the C3 acid concentration can be observed, which corresponded with the addition of a fresh SO_4^{2-} solution to T, on that day. Thus, the results of the SO_4^{2-} reduction, the COD utilisation and the C3 utilisation show a clear relationship, which reflects the symbiosis between the RB and the SRB. The SRB keep the propionic acid concentration low, thereby stimulating the fermenting bacteria to keep degrading the grass cellulose for more C3 acid production. The propionic acid concentration in C is high since there is no SO_4^{2-} reduction in C and, thus, no utilisation of the C3 acid. It can be seen from Fig. 5 that butyric acid was available in the reactor up to day 18, at higher concentrations than the C3 acid concentration. The butyric acid concentration increased till day 10, where after it decreased, which time coincided with the second addition of SO_4^{2-} . These results indicate the utilisation of the C4 acid for the

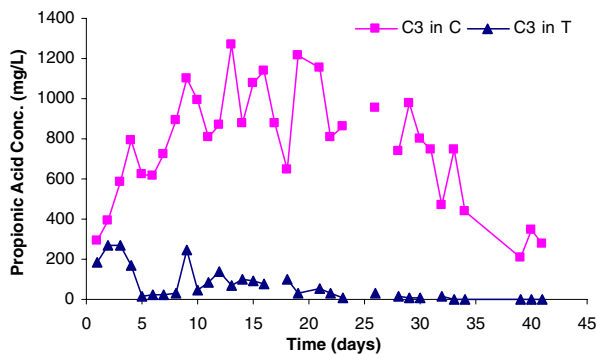


Fig. 4 The propionic acid concentrations in T and C

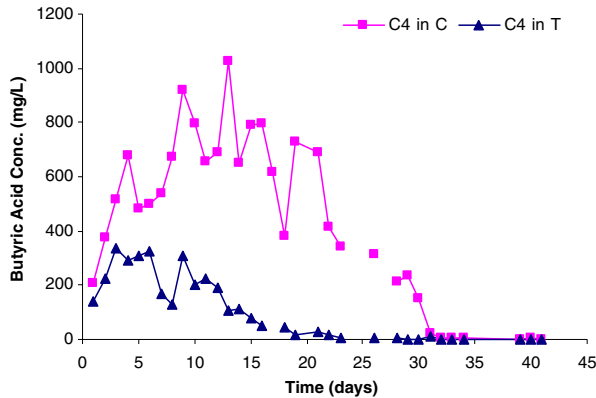


Fig. 5 The butyric acid concentrations in C and T

biological sulphate reduction. The findings shown in Figs. 4 and 5 show that the C3 acid concentration was lower than the C4 concentration, which indicated that the SRB preferred to utilise the C3 acid over the C4 acid [10].

The COD Concentration as a Function of the COD in T and C

The COD and the total VFA (expressed as COD in milligrams per litre) concentrations in T are presented in Fig. 6a. The results show that only a small part of the COD concentration comprises VFA, indicating that the greater part of the VFA concentration was utilised for biological SO_4^{2-} reduction and that the VFA in T mainly comprises acetic acid. It can also be observed that the acetic acid graph follows the pattern of the COD graph, showing that the VFA production and utilisation are related to the COD production and utilisation. This observation also indicates that part of the residual COD comprises acetate, which may be the rate-limiting factor in the continuous SO_4^{2-} reduction. When comparing the graphs in Fig. 6a, b, it can be seen that the VFA/COD contribution in C is the actual VFA produced, while in T it is the acetate produced after SO_4^{2-} reduction as the product of the C3 and C4 acids. Not all COD produced from grass cuttings can be utilised for the SO_4^{2-} reduction. Grass consists of 52% water, 14% cellulose and 28% hemicellulose, while the lignin content is 5% and the ash represents 0.5% [27]. Only cellulose and hemicellulose can be degraded to usable energy sources, while the lignin part of the grass cuttings is difficult to ferment and can therefore not be utilised for SO_4^{2-} reduction. The small ash fraction is inert and will not be used by the SRB or by the methanogenic bacteria (MB).

The COD and VFA concentrations as measured in C are the products of the grass cellulose degradation. The VFA/COD graphs for C are presented in Fig. 6b showing that the COD concentration and the VFA concentration (expressed as COD) follow the same pattern of the COD graph, indicating that the VFA production is integrally related to the COD production. The results in Fig. 6b indicate that the VFA production only forms a part of the COD production, indicating that a certain fraction of the COD comprises other organic material, such as monomers, alcohols and possibly some inert COD such as lignin and ash. It was observed that, initially, both the COD and the VFA concentrations increased but decreased from day 14. These results seem a clear indication that methane production occurred in C, since no SO_4^{2-} concentration is present in C, and thus, the produced H_2 is available for the production of methane (CH_4). Methane is formed by the MB from acetic

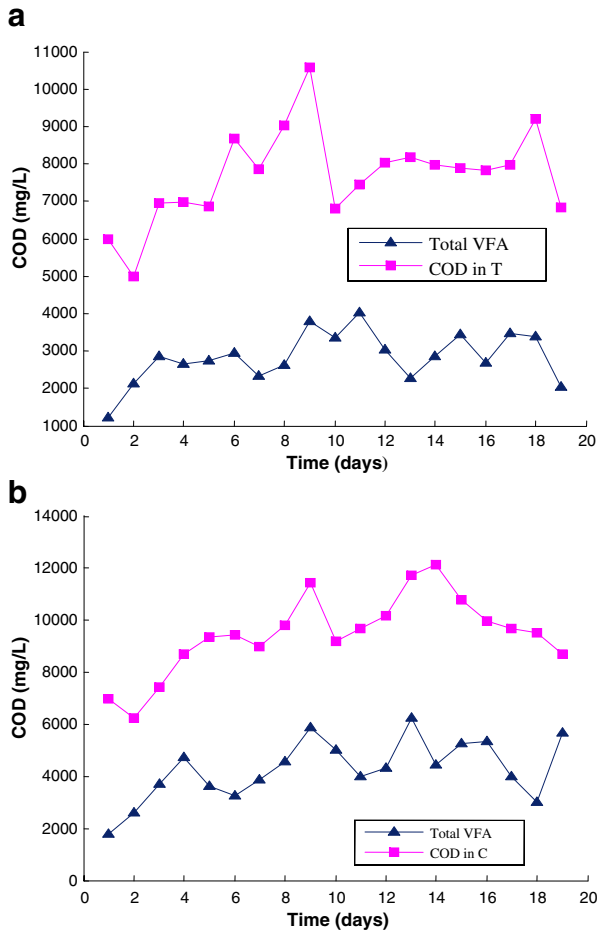


Fig. 6 The relationships between the VFA and COD concentration **a** in T and **b** in C

acid, H_2 and CO_2 produced after anaerobic degradation under non-sulphidogenic anaerobic conditions. No gas analyses were done in this study to confirm this hypothesis. The results after day 30 in T seem to indicate that both anaerobic processes occur in T: sulphate reduction and methane production, two fundamentally linked processes, which usually favour the sulphate reduction [10], depending on certain reactor conditions, such as the reactor pH and the COD and SO_4^{2-} concentrations in the reactor.

Conclusions

Results of this investigation showed a clear relationship between the grass cellulose degradation and a high COD concentration in the reactors. The highest COD concentration measured was 10,590 mg/L in the test reactor, which resulted in a fast SO_4^{2-} reduction, namely from 2,150 to 850 mg/L SO_4^{2-} over 2 days. The VFA pattern during this same period showed that almost all propionic acid as well as most of the butyric acid was utilised

in the test reactor for the SO_4^{2-} reduction, producing acetic acid in high concentrations. Thus, grass degradation resulted in the production of COD/VFA, which in turn was used for SO_4^{2-} reduction. The results of the control reactor showed that high concentrations of acetic acid were produced ($>2,000$ mg/L) and that the C3 and C4 acids were produced at concentrations $>1,000$ mg/L. The C3 production was slightly higher than the C4 acid production, which is favourable for the SO_4^{2-} reduction. When the VFA produced were combined (expressed as COD), it was observed that a fraction of COD was not accounted for in C, indicating that a fraction of the COD concentration consists of organics other than VFA. This part of the COD most likely comprises monomers, alcohols and undegradable COD, such as lignin. It was hypothesised that the COD degradation path in C followed the methane route, while the results in T seemed to indicate that not only the sulphidogenic reactions took place but also the methanogenic path, perhaps due to the COD being inert for further SO_4^{2-} reduction. The results of the study provided a good insight in the different degradation/production and utilisation rates as occurring in biological anaerobic reactors, with and without sulphate.

References

1. Younger, P. L., & Wolkersdorfer, C. (2004). Mining impact on the fresh water environment: Technical and managerial guidelines for catchment scale management. *Mine Water and the Environment*, 23, S2–S80.
2. Maree, J. P. (1994). Neutralization of acidic effluents with limestone. Water Research Commission Report No. 355/1/94.
3. Maree, J. P., Hagger, M. J., Strobos, G., Hlabela, P., Cronjé, H., van Niekerk, A., et al. (2003). Neutralization of acid leachate at a nickel mine with limestone. In *Proc. Sudbury 2003 Mining and the Environment, 28th CLRA Meeting*, Laurentian University, Sudbury, 25–28 May 2003.
4. Günther, P. (2006). Emalahleni mine water reclamation project. In *Proceedings of Mine Water Drainage (South African Perspective)*. Randfontein, 19–20 Oct 2006.
5. Hulshoff-Pol, L. W., Lens, P. N. L., Stams, A. J. M., & Lettinga, G. (1998). Anaerobic treatment of sulphate rich wastewaters. *Biodegradation*, 9, 213–224.
6. Lens, P. N. L., Visser, A., Janssen, A. J. H., Hulshoff Pol, L. W., & Lettinga, G. (1998). Biotechnological treatment of sulphate-rich wastewaters. *Critical Reviews in Environmental Science and Technology*, 28 (1), 41–88.
7. De Smul, A., Dries, J., Goethals, L., Grootaerd, H., & Verstraete, W. (1997). High rate of microbial sulphate reduction in a mesophile ethanol fed expanded-granular-sludge-blanket reactor. *Applied Microbiology and Biotechnology*, 48, 297–303.
8. Greben, H. A., Maree, J. P., Singmin, Y., & Mnqanqeni, S. (2000). Biological sulphate removal from acid mine effluent using ethanol as carbon and energy source. *Water Science and Technology*, 42(3–4), 339–344.
9. Greben, H. A., Baloyi, L. M., Sigama, S., & Venter, S. N. (2009). Bioremediation of sulphate rich mine effluents using grass cuttings and rumen fluid microorganisms. *Journal of Geochemical Exploration*, 100 (2–3), 163–168.
10. Visser, A. (1995). The anaerobic treatment of sulphate containing wastewater. *PhD Thesis*, Wageningen Agricultural University, Wageningen, The Netherlands.
11. Oude Elferink, S. J. W. H. (1998). Sulphate-reducing Bacteria in Anaerobic Bioreactors. *PhD Thesis*, Wageningen Agricultural University, Wageningen, The Netherlands.
12. Mizuno, O., Li, Y. Y., & Noike, T. (1998). The behaviour of sulphate-reducing bacteria I acidogenic phase of anerobic digestion. *Water Research*, 32, 1626–1634.
13. Greben, H. A., Baloyi, L. M., & Venter, S. N. (2007). Grass cellulose as cost effective energy source for biological sulphate removal. *Water SA*, 33(5), 729–735.
14. Greben, H. A., Sigama, J., Burke, L., & Venter, S. N. (2008). Cellulose fermentation products as energy sources for biological sulphate reduction. WRC report K5/1728.
15. Postgate, J. R. (1984). *The sulphate-reducing bacteria* (2nd ed., p. 208). Cambridge: Cambridge University Press.
16. Hungate, R. E. (1966). *The rumen and its microbes*. New York: Academic Press.

17. APHA. (1985). *Standard methods for the examination of water and wastewater* (16th ed.). Washington DC: American Public Health Association.
18. Harmsen, H. J. M. (1996). Detection, phylogeny and population dynamic of syntrophic propionate-oxidizing bacteria in anaerobic sludge. *PhD thesis*, Wageningen Agricultural University, Wageningen.
19. Logan, M. V., Reardon, K. F., Figueroa, L. A., McLain, J. E. T., & Ahmann. (2005). Microbial community activities during establishment, performance and decline of bench-scale passive treatment systems for mine drainage. *Water Research*, 39, 4537–4551.
20. Rinzema, A., & Lettinga, G. (1988). Anaerobic treatment of sulfate containing wastewater. In D. L. Wise (Ed.), *Biotreatment systems*, 3 (pp. 65–109). Boca Raton: CRC Press.
21. Marschner, B., & Kalbitz, K. (2003). Controls of bioavailability and biodegradability of dissolved organic matter in soils. *Geoderma*, 113, 211–235.
22. Lynd, L. R., Weimer, P. J., van Zyl, W., & Pretorius, I. S. (2002). Microbial cellulose utilisation: fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, 66(3), 506–577.
23. Greben, H. A., & Baloyi, J. (2004a). The beneficial use of a bio-waste product in the biological sulphate removal technology. In *Proceedings Wisa Biennial Conference and Exhibition*, Cape Town, South Africa, 2–6 May 2004.
24. Vallero, M. V. G., Lens, P. N. L., Bakker, C., & Lettinga, G. (2003). Sulfidogenic volatile fatty acid degradation in a baffled reactor. Sub-department of Environmental Technology, Wageningen University and Research Centre, Wageningen, The Netherlands.
25. Greben, H. A., Tjatji, M., & Maree, J. P. (2004b). Biological sulphate reduction at different feed COD/SO₄ ratios using propionate and acetate as the energy source. In *8th IMWA Conference Newcastle upon Tyne*, England.
26. Reis, M. A. M., Almeida, J. S., Lemos, P. C., & Carrondo, M. J. T. (1992). Effect of hydrogen sulphide on growth of sulphate-reducing bacteria. *Biotechnology and Bioengineering*, 40, 593–600.
27. Sonakya, V., Raizada, N., Dalhoff, R., & Wilderer, P. A. (2003). *Water Science and Technology*, 48(8), 255–259.