

CHAPTER 6

PROCESS DESCRIPTION OF THE GRASS-CELLULOSE FERMENTATION AND BIOLOGICAL SULPHATE REMOVAL TECHNOLOGY APPLYING MASS BALANCE EQUATIONS

6.1 INTRODUCTION

Mine effluents have to comply with the standards set by the Department of Water Affairs and Forestry (DWAF), when discharged into river systems. This governing body specifies that the sulphate concentration in AMD discharged should not exceed 500 mg/l in most areas of South Africa. In addition, metals and acidity should be removed before the water is released to public water bodies. The main components after biological treatment usually consist of sulphide, alkalinity as well as residual COD and SO₄. The pH of treated water should be 7 to 8.

In this chapter attention was given to the design of an anaerobic bioreactor for the removal of sulphate, (heavy) metals and the elevation of the pH in AMD. The description of the process was based on treating AMD of a specific quality (obtained from a closed coal mine located in the Witbank area, South Africa) and on the results obtained in Chapter 5 where pre-treated AMD was used as the feed water for the process. The volume of this to be treated AMD was 2 000 m³/d with a sulphate concentration of 2 kg/m³ and a pH of 2.5 (data provided by mine management).

The suite of mass balance equations incorporated all streams in and out of the reactor system and took the chemical and biochemical reactions into account that occurred in the system. The stoichiometric equations and growth kinetics for the rumen bacteria and the SRB were mainly based on theoretical values. All important parameters, such as sulphate concentration and flow-rates were taken into account for the process description. It was envisaged that such analysis of the process could provide the understanding of the biological processes of the fermentation and sulphate reduction in the one stage reactor system, when treating mine effluents.

6.2 PROCESS DESCRIPTION

For the reduction of sulphate to sulphide 8 electrons, equivalent to 0.67 g of COD per g of sulphate, are required (equation 6.1)



This reaction generates approximately 1 mol of ATP (Schlegel, 1993). SRB have a preference for hydrogen, propionate, butyrate and acetate in that order. The results of the previous chapter showed that sulphate reduction on hydrogen, propionate and butyrate proceeded well, while small concentrations of acetate was generally detected in the reactor effluent, which agreed with the findings of Visser (1995). Hydrogen produced by rumen bacteria was immediately used by SRB, thus the SRB kept the dissolved hydrogen concentration low and consequently, rumen bacteria were not inhibited by the production of hydrogen (Visser, 1995). The energy/carbon source is oxidized when sulphate is reduced to sulphide, which produces carbon dioxide and water. When higher carbon sources are oxidised acetate is produced. Some SRB can subsequently oxidise acetate to carbon dioxide and water. Considering substrate affinity and growth rates, the reduction reactions for hydrogen and the various VFA's are presented in equations 6.2 - 6.5:

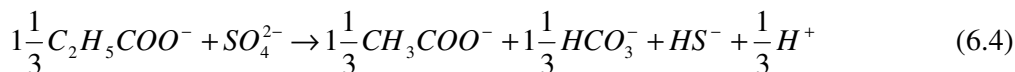
Hydrogen :



Butyrate :



Propionate :



Acetate :



The degradation of GC by rumen microorganisms and the subsequent sulphate reduction by SRB, using VFAs and hydrogen as carbon source are presented in Figure 6.1.

Like most other anaerobic bacteria, SRB have low growth rates, which are in the range of 0.55/day for the acetate oxidizing SRB up to about 2.5/day for hydrogen and propionate utilizing SRB (Visser, 1995). Other anaerobic bacteria, e.g. MB and AB have growth rates in the same range as SRB. These groups of bacteria are in constant competition for the available substrate when growing under anaerobic conditions. If a reactor is operated for a long period, consequently either SRB or MB/AB will dominate. This is dependent on kinetic parameters such as growth rate and substrate affinity of the bacteria groups. Visser (1995) showed that SRB can out-compete MB and AB for VFAs (except acetate) and hydrogen as substrates.

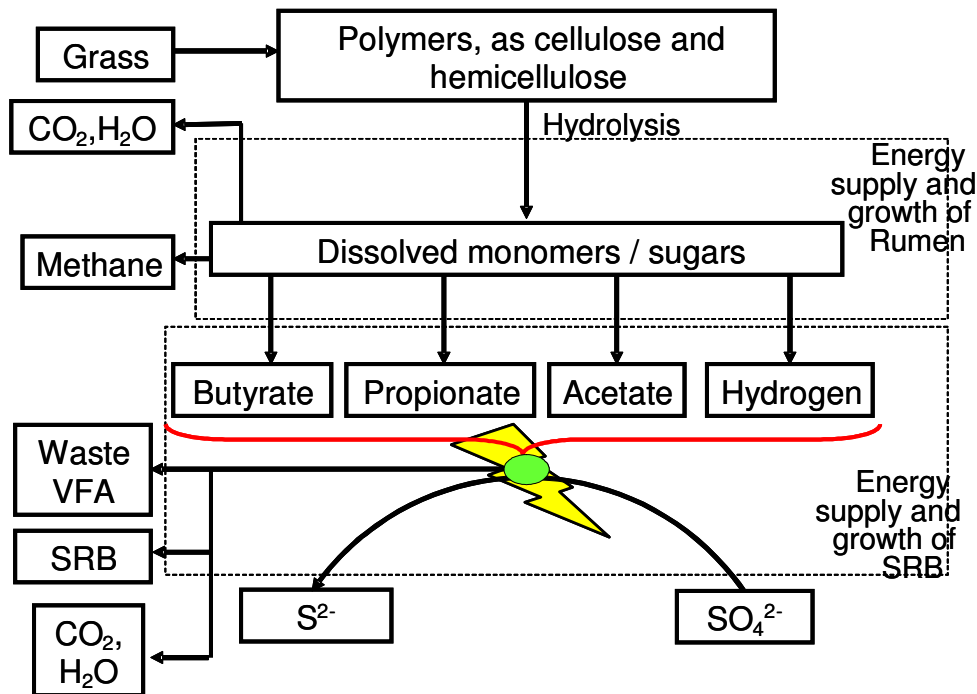


Figure 6.1. Grass (cellulose) degradation by rumen bacteria and subsequent biological sulphate reduction by SRB.

6.2.1 Metal Removal

The sulphide produced during biological sulphate reduction precipitated metals present in AMD to insoluble metal-sulphides, prior to feeding AMD to the reactor. Thus in full scale operation, part of the reactor effluent can be mixed with the incoming AMD. The metal-sulphides formed precipitated and were removed in a settler (Figure 6.2). The alkalinity concentration of the treated water neutralised the acidity in the AMD, thus the pH of the AMD increased, prior to its entering the reactor. The ratio, AMD: treated water was dependent on the acidity of the AMD and the alkalinity in the treated water. It was noted that the recycling stream also included the recycling of other substances such as the non-reduced sulphate in the treated water, which, however, was of no significance to the results presented.

6.2.2 Waste streams

6.2.2.1 Metal sulphides

Metals such as iron, copper, zinc and manganese precipitated with sulphide at the operating reactor pH (Janssen and Warmoeskerken, 1997). The precipitated metal-sulphides settled prior to the diluted AMD entering the reactor, thus preventing microbial toxicity. In full scale operation, the metal sulphides sludges can be stored in

the tailings dams, depending on the metal concentration. When these concentrations are relatively low, the metal sludge can be used as fertiliser on land.

6.2.2.2 Sludges

Bacterial sludges (rumen microorganisms and SRB) were produced in the bio-reactor and these need to be removed. Generally, waste sludge can be used as fertilizer.

6.2.2.3 Waste VFA or COD

The fermentation products generated by rumen bacteria, which were not used by SRB for the sulphate reduction, represented a certain residual COD concentration, which should comply with effluent standards on COD discharge. If the COD effluent concentration was higher than 100 mg/l, aerobic treatment needs to follow the anaerobic stage to digest the residual COD. The biomass produced after COD degradation can be separated in a clarifier and disposed of together with the other sludges.

6.2.2.4 Gases

When a reactor is optimized for sulphate reduction the amount of the methane produced will be very low. Furthermore, most of the sulphide produced did not escape as hydrogen sulphide gas, but remained dissolved as HS^- since the reactor pH was higher than 6.5.

6.2.2.5 Sulphide

The sulphide produced can be converted chemically to elemental sulphur using a Fe^{3+} solution (Maree *et al.* 2004). The elemental sulphur can be sold for the production of sulphuric acid. There is a market for sulphur in South Africa and in other African countries. At present, South Africa is a net importer of sulphur.

6.2.3 Process Flow diagram

The combined description of the biological processes in the reactor assisted to understand the degradation and the sulphate removal process and how these two processes were dependant on each other. In the previous chapters, it was observed that the degradation of grass supplied the SRB with a carbon and energy source to reduce sulphate to sulphide. The sulphide produced precipitated the metals, present in AMD, while the alkalinity produced was beneficial to increase the pH of the treated AMD.

The objective of this chapter was to construct a process flow diagram of the proposed biological sulphate removal process (Figure 6.2) to understand both the chemical and biological processes involved. As shown in Chapter 5, the incoming AMD stream was mixed with the treated effluent from the biological sulphate removing bio-reactor (1:1) in a mixing tank. Next the pre-treated AMD stream entered the biological sulphate removing bio-reactor. To this reactor, GC were added regularly with the aim to degrade the cellulosic component, using a rumen inocula as fermenters, to VFA and hydrogen. These degradation products were used by SRB as carbon and energy sources for biological sulphate removal to sulphide. During this syntrophic degradation and utilisation of fermentation products, rumen microorganisms and SRB used carbon for growth. The effluent stream of the reactor contained residual sulphate and COD concentrations, which consisted mainly of un-degraded grass (lignin). It also contained sulphide, alkalinity and some of the washed out biomass (dead cells and debris). It was predicted that the gas phase contained carbon dioxide and only small quantities of methane since most of the hydrogen produced was utilised by the SRB.

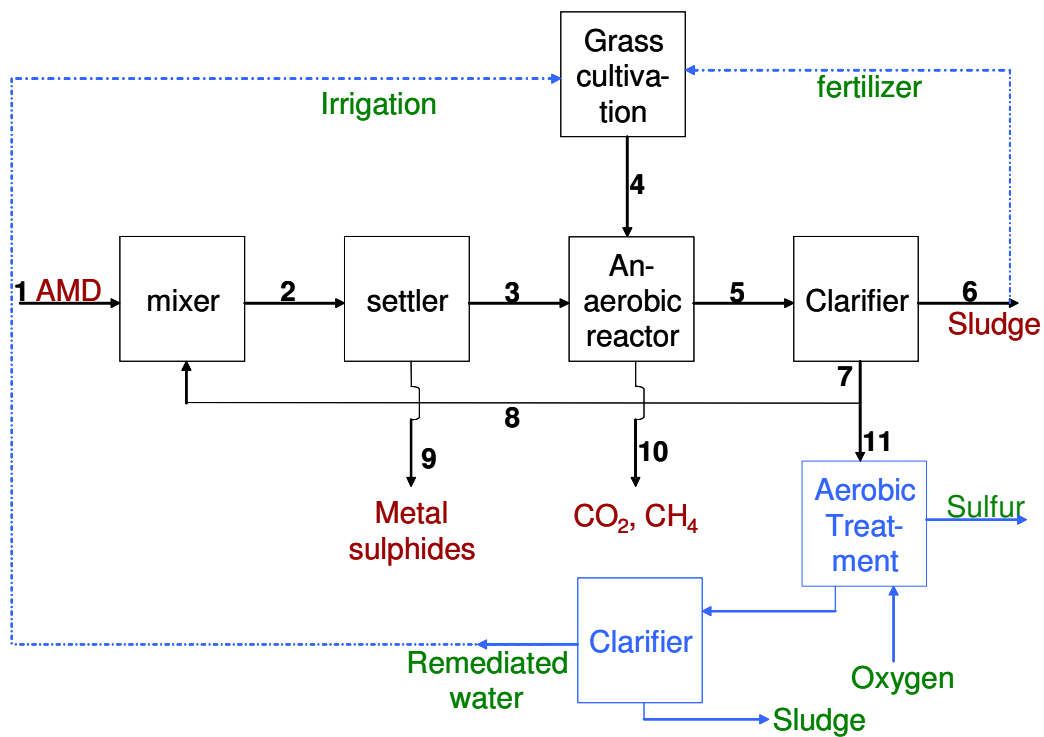


Figure 6.2. Process Flow- diagram of the proposed AMD treatment.

(Blue streams and reactors are outside the scope of this design).

In the proposed treatment system the grass would be cultivated on-site and irrigated with partly treated AMD and fertilized with waste sludge. Part of the effluent stream would be recycled to the front of the reactor system where it would be mixed with the feed stream. The other part of the effluent could be used for the irrigation of grass or could be discharged to rivers and dams, if the effluent standards of DWAF were adhered to. The predicted compositions of the separate streams in the process are presented in Table 6.1.

Table 6.1. Key components of each stream in the biological treatment

Stream*	Origin	Key components
1	AMD	Sulphate, Acidity and Metals
2	Mixed AMD+ effl (1:1)	Metal Sulphides, Sulphate and Sulphide
3	Supernatant of 2	Sulphate and Sulphide
4	Dry GC	Water, Cellulose, Hemicellulose, Lignin
5	Reactor effluent + sludge waste	Residual COD(acetate), Non-degraded grass, Biomass, Sulphate, Sulphide, Alkalinity
6	Sludge waste	Biomass
7	Overflow clarifier	Residual COD(acetate), Non-degraded grass, Sulphate, Sulphide, Alkalinity
8	Recycle stream	Non-used VFA, Non-degraded grass, Sulphate, Sulphide, Alkalinity
9	Metal waste	Metal Sulphides
10	Exhaust gas	Carbon Dioxide, Methane, Hydrogen-sulphide
11	Treated water	Residual COD(acetate), Non-degraded grass, Sulphate, Sulphide, Alkalinity

*Stream numbers are given in Figure 6.2.

6.2.4 Mass Balances

The mass balances were based on the assumption that the entire process was treated as in steady state. Firstly, the flow rates of the streams (1-11) in the process (Table 6.1) were taken into account. Secondly, the overall process balances for sulphur and sulphate were calculated and subsequently the mass balances over each process unit were reviewed. The grass cultivation was also analysed in the

mass balances and the surface area for grass cultivation was calculated. Thereafter, the stream tables of the entire process were presented.

6.2.4.1 Stream balance

The volume flow rates of each stream were determined. The following volume balance over the total system holds:

$$0 = \text{feed flowrate} + \text{flow entering with grass} - \text{effluent flowrate} - \text{flow in sludge} \quad (6.6)$$

The amount of water associated with the GC was negligible in comparison to the amount of water present in the feed flow. This is also true for the amount of water removed with the sludge, since very little sludge was produced. Consequently the feed flow rate is equal to the effluent flow rate. A ratio of 1:1 of the AMD and the sulphide/alkalinity rich effluent from the reactor was experimentally determined to give the required increase in pH value in the pre-treated AMD used as feed water. This complied with the amount of sulphide required for metal precipitation. The following balance applied for the mixing tank:

$$0 = \text{feed flow} + \text{recycle flow} - \text{flow to settler} \quad (6.7)$$

Since the feed flow was equal to the effluent flow, the sulphide/alkalinity rich recycle stream had the same volume as the feed flow. The flow to the settler is thus twice the feed flow rate. All water flow rates throughout the process were given in m^3/y (Table 6.2).

Table 6.2. Flow rates of the various process streams

Stream no*	Flowrate [m^3/y]	Stream no	Flowrate [m^3/y]	Stream no	Flowrate [m^3/y]
1	730 000	5	1 460 000	9	0
2	1 460 000	6	0	10	0
3	1 460 000	7	1 460 000	11	730 000
4	0	8	730 000		

* Stream numbers are presented in Figure 6.2.

6.2.4.2 Overall balances for sulphate and sulphide

Sulphate entered the biological reactor system with the feed stream and was used by the SRB as electron acceptor to reduce sulphate to sulphide. In addition, sulphate is assimilatively taken up by SRB, which however formed a negligible part of the total sulphate concentration. The following balance, considering sulphate, of the total system in steady state holds:

$$0 = \text{sulphate in} - \text{sulphate out} + \text{sulphate produced} - \text{sulphate consumed} \quad (6.8)$$

Sulphate is not produced in the process, thus the balance can be converted to:

$$0 = \phi_{v,in} \cdot C_{SO_4^{2-}} - \phi_{v,out} \cdot C_{SO_4^{2-},out} - \text{sulphate consumed} \quad (6.9)$$

ϕ is flow rate

C is concentration

The maximum allowed effluent sulphate concentration is 0.5 kg/m³ based on the DWAF requirements. The inflow and the outflow of the total system were equal and was 730 000 m³/y. From the balance it follows that 1 095 000 kg/y of sulphate is reduced to sulphide.

A similar balance for sulphide as for sulphate can be expressed:

$$0 = \text{sulphide in} - \text{sulphide out} + \text{sulphide produced} - \text{sulphide consumed} \quad (6.10)$$

Sulphide was produced through the conversion of sulphate. For every mole of sulphate reduced one mole of sulphide was produced. Sulphide was however also consumed for the precipitation of metals. The balance can be reduced to the following equation:

$$0 = -\phi_{v,out} \cdot C_{S^{2-},out} + \frac{32}{96} \cdot \text{sulphate produced} - \text{sulphide in metal precipitation} \quad (6.11)$$

For the reactor it meant that the total amount of sulphide produced equaled 365 000 kg/y. The amount of sulphide required for metal precipitation equaled 142 248 kg/y (calculated from the metal concentration, present in the AMD, which will be discussed later). As a consequence 222 753 kg/y sulphide will leave the system via stream 11 at a concentration of 0.3 kg/m³.

6.2.4.3 Mixer settler

The composition of stream 2 can be calculated. A balance of the mixing tank for sulphate is the following:

$$0 = \phi_{v,AMD} \cdot C_{SO_4^{2-},AMD} + \phi_{v,Rec} \cdot C_{SO_4^{2-},Rec} - \phi_{v,Mix} \cdot C_{SO_4^{2-},Mix} \quad (6.12)$$

It follows that the concentration of sulphate leaving the mixing tank is 1.25 kg/m³. The concentration of sulphide leaving the mixing tank and settler can be calculated from a similar equation as follows:

$$0 = \phi_{v,AMD} \cdot C_{S^{2-},AMD} + \phi_{v,Rec} \cdot C_{S^{2-},Rec} - \phi_{v,Mix} \cdot C_{S^{2-},Mix} - \text{sulphide in precipitation} \quad (6.13)$$

Since there is no sulphide in the AMD feed, the sulphide concentration after the mixer and settler becomes 0.05 kg/m³.

The metal concentrations in the incoming AMD are given in Table 6.3. The pH value of > 6.5 in the mixer will ensure the precipitation of all metals as well as manganese, except potassium and sodium. The precipitated metal sulphides will be removed in the settler. The metals precipitated as metal-sulphides and the concentrations thereof are presented in Table 6.3. The amount of sulphide needed to precipitate all metals can be calculated. This equals 142 248 kg/y. The amount of sulphide in the recycle stream equals 0.3 kg/m³ X 730 000 m³/y = 219 000 m³/y, the recycle ratio is thus sufficient to deliver enough sulphide to precipitate all metals present in the AMD.

The minimum recycle ratio, only to precipitate the metals, can be calculated from the recycled sulphide mass-flow according to:

$$\phi_{mass,S^{2-},Rec} = \phi_{v,Rec} \cdot C_{S^{2-},Rec} \quad (6.14)$$

The concentration of the sulphide (stream 11) equals 0.3 kg/m³. The mass flow rate is the minimal amount of sulphide required for the precipitation of all the metals. This gives a minimum required recycle flow rate of 474 160 m³/y, or a recycle ratio of 0.65:1.

Table 6.3. Amounts of removed metal sulphides

Metal sulphide	Metal sulfide effluent via stream 9 (kg/y)
CuS	44
Fe ₂ S ₃	103 165
MnS	10 743
ZnS	2 175

6.2.4.4. The reactor

For the reactor the following influent and effluent streams can be conceptualised and will be discussed in the following sections.

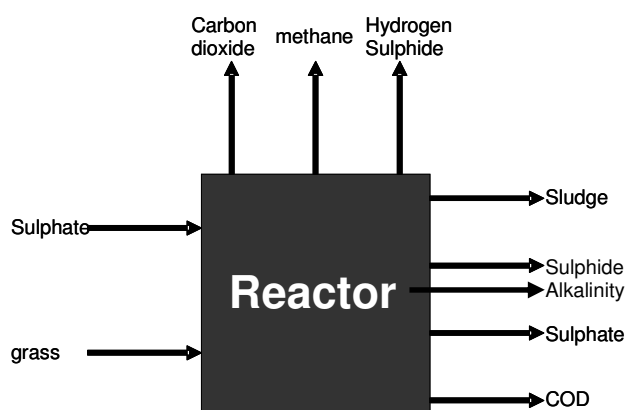


Figure 6.3. The reactor showing all in- and out-going streams.

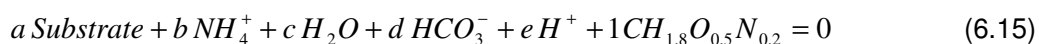
The sulphate concentrations entering the reactor as well as the sulphide concentrations leaving the reactor were discussed in the previous sections, when the total mass balances were considered. The sulphate concentration entering the reactor is 1.25 kg/m^3 while the sulphide concentration entering the reactor is 0.05 kg/m^3 . The effluent concentrations of sulphate and sulphide were 0.5 kg/m^3 and 0.3 kg/m^3 , respectively. It was assumed that the hydrogen sulphide formed was dissolved (HS^- form), due to the relatively high reactor pH ($\text{pH} > 6.5$). The mass balances in the reactor are derived by means of the following description:

- Firstly, the amount of waste was determined. This waste was presented in the form of COD of which the constituents were described in a later section

- Secondly, the growth of SRB was described, by determining the amount of COD (in the form of VFA) required for growth as well as for energy supply of SRB. The energy supply and growth are linked through microbial growth/yield relations i.e. the Yield ATP (Y_{ATP}) concept (ATP: Adenosine triphosphate).
- Thirdly, the VFA production (by rumen microorganisms) necessary for sulphate reduction was calculated. Subsequently the amount of grass/cellulose was calculated for growth, VFA production and for total sulphate reduction.

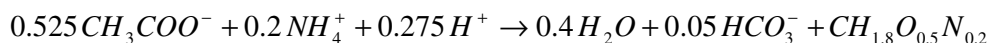
To estimate the amount of unutilised VFA, the theoretical values were compared with the experimental data and were used accordingly. In order to calculate biomass or sludge production, yields of bacteria growing on the substrates were required and thus theoretical ATP yields (for every mol of ATP produced 1 gram of biomass was produced) were used. The amount of ATP generated in SRB is 1 mol of ATP per mol sulphate reduced (Schlegel, 1993). Since 1 095 000 kg/y of sulphate was reduced an amount of 11 406 kmol of ATP was produced. Bauchop and Eldsen (1960) measured the amount of ATP required for the production of 1 g of biomass for several organisms. Their results showed an average yield of 10.5 g cells/mol ATP. This gives a biomass production of SRB of 120 000 kg/y. The SRB biomass is formed from COD, mainly consisting of acetate, butyrate and propionate obtained from the fermentation of cellulose by the rumen bacteria.

Biomass growth relations were calculated from the amount of each of the individual substrates (COD) needed for biomass production, namely 1 C-mol of biomass can on average be represented as $CH_{1.8}O_{0.5}N_{0.2}$ (Heijnen and Roels, 1979). Furthermore it was assumed that the N-source derived from grass can be represented as ammonium and that the nitrogen from grass is abundant. The following growth relations can then be derived:

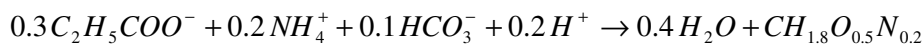


For each equation there are 5 unknowns and 5 balances, namely 4 elemental balances and one charge balance. Solving these balances gives the following growth relations for the production of 1 C-mol of biomass with butyrate, propionate and acetate as substrates:

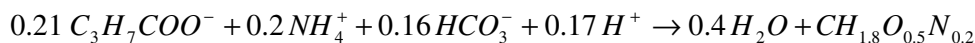
Acetate :



Propionate :



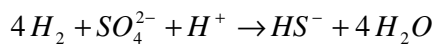
Butyrate :



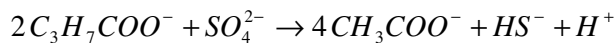
(6.16-6.18)

The energy for growth of the SRB was provided by the oxidation of COD and hydrogen, produced by the rumen bacteria, with the simultaneous reduction of sulphate to sulphide. For butyrate, propionate, acetate and hydrogen this occurred according to the following reactions:

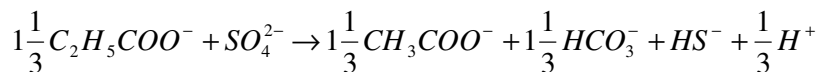
Hydrogen :



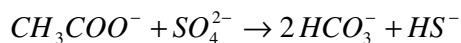
Butyrate :



Propionate :



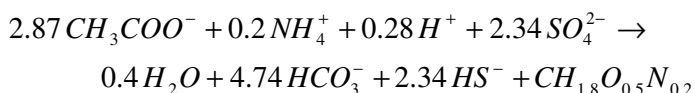
Acetate :



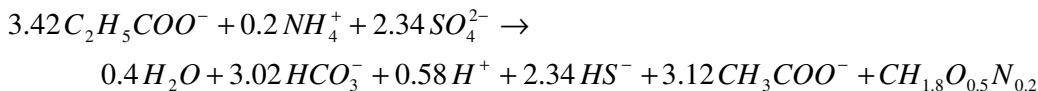
(6.19-6.22)

The growth relations and energy production reactions were linked through the ATP yield. Total growth relations can be derived which incorporated energy production and growth:

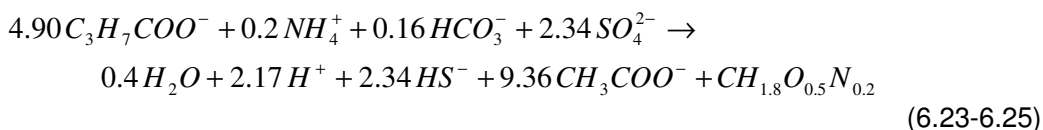
Acetate :



Propionate :

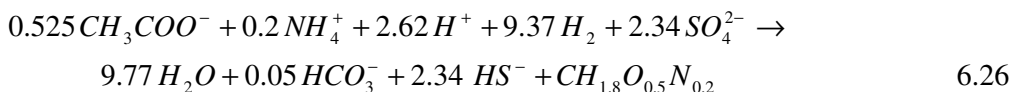


Butyrate :

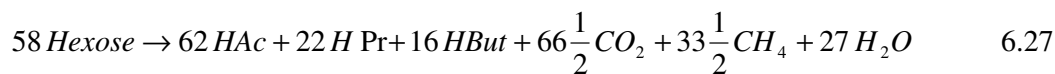


The SRB used hydrogen only as the energy source and needed carbon sources for cell growth, such as acetate or pyruvate, which were the main intermediates in biomass growth and energy reactions (Schlegel, 1993). Hydrogen was used by the acetogenic bacteria to produce acetate from H₂ and CO₂. The following growth relation with acetate and hydrogen were derived:

Hydrogen :



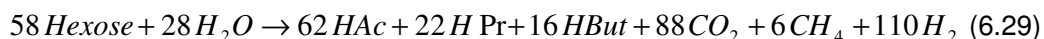
In order to establish the cell growth obtained from the utilization of the different VFA and hydrogen, it is necessary to know the distribution of the VFA and hydrogen produced by the rumen bacteria to calculate the total biomass yield on the total VFA. The hydrogen produced by the rumen bacteria inside the rumen is immediately used by MB, which produce methane from carbon dioxide and hydrogen. In the presence of SRB and sulphate, the MB were outcompeted by the SRB (Visser, 1995). In an optimized sulphate reducing reactor the hydrogen produced by rumen microbes was used by the SRB. The production of the various gases and VFAs by rumen bacteria occurred through the degradation of cellulose and hemicellulose in grass, which consisted mainly of hexoses (monomers, such as glucose). Hungate (1966) gives the following reaction for the degradation of hexoses by rumen bacteria:



This equation was derived from the stoichiometric relationships in microbial pathways and energy conservation laws and agreed fairly well with experimental results (Hungate, 1966). The formation of methane was ruled out since sulphate and SRB were present in the culture. Experimental results showed that the average amount of carbon dioxide produced in the experimental reactor was about 84 %. Methane is produced according to:



Due to the presence of SRB and sulphate in the bioreactor, 4 mols of hydrogen were gained since instead of methane production, the hydrogen was utilised by the SRB as the energy source. The overall reaction for the degradation of cellulose:



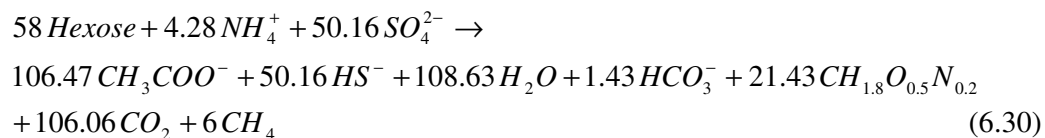
For each of the substrates (VFA and hydrogen) produced an amount of biomass was produced. On average, 30 % of the COD produced was not used in the reactor, based on experimental data. The COD values for 1 mol each of butyrate, propionate and acetate are 160, 112 and 64 g of oxygen, respectively. The VFA produced from cellulose is distributed in COD amounts as presented in Table 6.4 (Hungate, 1966).

Table 6.4. Distribution of the produced VFA represented as COD

VFA	Percentage COD
Butyrate	29
Propionate	27
Acetate	44

Visser (1995) reported that hydrogen was immediately used by the HSRB, which implied that rumen bacteria and SRB cultures lived in syntrophy: as soon as the hydrogen was produced, the SRB utilised it in the presence of SO_4 . Furthermore a preference for propionate and butyrate before acetate was determined experimentally as the preferred substrate for SRB. Considering the, on average highest growth rate of SRB on propionate, this will be assumed to be the preferred substrate (Visser, 1995). It was assumed that the SRB initially consumed the hydrogen produced for energy in combination with acetate as the carbon source. Secondly the SRB used propionic acid followed by butyric acid. During the consumption of the C3 and C4 acids, acetate was formed which was the substrate used last in the sequence. Part of the waste COD/VFA consisted almost entirely of acetate, which was experimentally confirmed (Chapters 3-5).

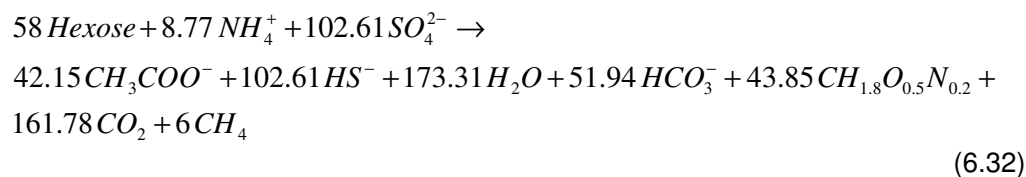
The total growth and the cellulose conversion equations provided the yield of biomass on cellulose (or hexose). The usage of hydrogen, propionate and butyrate will give the following equations:



Note, that in the reaction representing the degradation of cellulose, acetate, butyrate and propionate are represented as un-dissociated acids, while in the above equation they were dissociated. Furthermore, the acid produced reacted with the produced carbonate according to:



Only the acetate produced by the rumen bacteria and SRB had to be incorporated into the process description. The total VFA concentration, expressed as COD, was produced by the rumen bacteria in degrading 58 hexose units to 8992 g O₂. A COD waste of 30 % was equivalent to 2698 g O₂. Thus an amount of 4116 g COD (as acetate) was used for energy production and growth. This equaled 64 mol of acetate. Thus, the total equation for the growth of SRB on VFA produced from cellulose by rumen bacteria, with a 30 % waste of COD becomes:



According to Hungate (1966) the molar mass of hexoses in making up cellulose is reported to be 162 g/mol (dehydrated hexose). The yield from biomass on cellulose is thus 0.11 g biomass/g of cellulose, including loss of COD or acetate. Without loss the yield calculated would be 0.15 g biomass/g cellulose.

6.2.4.5 GC requirements based on the process description calculations

The cellulose or hexose was related to grass, in order to calculate the amount of grass required to sustain the total removal of sulphate. Sonakya (2003) reported the composition of fresh grass as presented in Table 6.5.

Table 6.5 Composition of fresh grass (Sonakya *et al.* 2003)

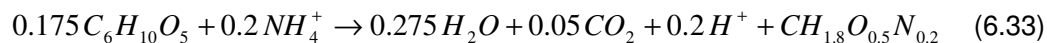
Compound	Percentage w/w
Water	51.84
Cellulose	14.00
Hemicellulose	28.30
Lignin	5.40
Ash	0.46

Only the cellulose and hemicellulose were digested by the rumen bacteria (Kalia *et al.* 2000). It is assumed that hemicellulose consisted of only hexoses as well. Thus 88 % m/w of the grass dry matter consisted of degradable hexoses. From the

equations derived above the amount of grass required for the reduction of sulphate and the growth of SRB was calculated:

For the reduction of 1 095 000 kg/y of sulphate and growth of SRB, 1.0 million kg of cellulose was needed, thus 2.5 million kg of fresh grass was required. Based on dry mass, an amount of 1.2 million kg of dry grass was needed for the reduction of sulphate and growth of SRB. The growth of rumen bacteria should however also be incorporated. This was also be done by means of ATP yield.

Bergen (1977) reported that rumen bacteria gain 2 mol of ATP for every mol of acetate produced and 3 mol of ATP for every mol of propionate or butyrate produced. The degradation of 1 mol of hexose (cellulose) thus provided 4.1 mols of ATP. The ATP yield of rumen bacteria was 16.5 g biomass/mol of ATP (Baldwin *et al.* 1970). The VFA and hydrogen produced for the reduction of 1.095 million kg of sulphate thus delivered 26 million mols of ATP to the rumen bacteria which resulted in 436 000 kg of rumen biomass produced per year. The hexoses in grass was represented as glucose minus water (hence the molecular weight of 162 g/mol). The following growth relation for rumen follows:



The yield of rumen-biomass on cellulose, without growth requirements was thus 0.87 g biomass/hexose. This implied that the amount of cellulose needed for the biomass growth of the rumen bacteria is 502 000 kg/y. The total amount of grass required for rumen bacterial growth is therefore 1.18 million kg/y of fresh grass or 572 000 kg/y, dry mass. The total amount of sludge produced is equivalent to the amounts of SRB, the amount of rumen bacteria and other debris produced. This provided a total sludge production of 556 000 kg/y.

The amount of grass required was for the reduction of sulphate and for the growth of the SRB and the rumen microbes. Thus the total amount of grass required was 3.65 million kg/y of fresh grass or 1.76 million kg/y dry mass (DM). This provided a removal efficiency of 0.62 g SO₄²⁻ removed per g of dry grass. The work of Mappledoram (1998) indicated that in an arid area of South Africa the yield of Kikuyu grass varied from 6-12 tonne DM per hectare, with a mean of 10.8 tonne DM/ha. In order to achieve this yield, the grass needed to be fertilised with 300 kg N/ha. In addition, phosphate and potassium were added on an annual basis to maintain a P

and K concentration in the soil of 20 and 120 mg/l, respectively. When grass was grown under irrigation, the yield was higher at 15.3 tonnes DM/ha (Harris, 1990). Since the Kikuyu grass grown at the mine will be irrigated regularly with the mine water, the yield of 15.3 tonne DM/ha will be used for further calculations. In order to grow enough grass to provide a yield of 15.3 DM/ha, a surface area of 131 ha of grass land is needed, which translated to an area of e.g. 1.14X1.14 km.

6.3 CONCLUSIONS

The calculations presented in this chapter provided a good understanding of the full technology description. It showed the implication of the various streams entering and leaving the reactor and the processes occurring in the reactor, e.g the COD utilization for the sulphate reduction as well as for the growth of the SRB and the rumen microbial population. From the technological process description it could be calculated that in order to treat AMD from a particular mine in the Witbank area, 15.3 tonnes DM/ha of grass cuttings was needed, which equated to a surface area of 1.14 x1.14 km of grassland. The presented calculations provided the basis for AMD treatment which contained 2000 mg/l sulphate and metals (mainly 76 mg/l iron, 10 mg/l manganese and 2 mg/l zinc) at a flow of 2 000 m³/d. In the process description it was assumed that the treated water will have a sulphate concentration of 500 mg/l and that all heavy metals will be removed. The residual metals such as calcium, magnesium and sodium can be removed with alternative treatment methods such as the desalination technology. Alternatively, the treated water can be used for irrigation or dust suppression at the mining site.

From the information in this and the previous chapters, it is evident that sulphate rich AMD can be treated biologically, using the degradation products of grass-cellulose as the carbon and energy sources. From the technology description, the required amount of grass was calculated for the operation of the process. For this technology to be economically feasible the costs associated with the cultivation and harvesting of the grass at the mining sites have to be taken into account. The advantage of the biological sulphate removal technology is that the sulphate in AMD can be reduced to concentrations of 500 (and lower) mg/l, that sulphide is produced to precipitate the metals in the mine effluent and that the alkalinity formed can increase the pH of the AMD.

From the above mentioned calculations, this system was compared to another biological sulphate removal process, which also operated on a waste product, e.g. primary sewage sludge as the carbon and energy source. This so called Rhodes BioSure plant, developed at Rhodes University (Rose, 2000), was commissioned recently at Grootvlei Gold Mine, with the aim to treat 10 M^l/day mine water, at a cost of R15 million. Grootvlei Mine is close to ERWAT's Ancor Waste water treatment works. The polluted mine water is piped by gravity to the Ancor sewage works into the BioSure plant. The treated water, after sulphide removal, is then directed to the Ancor sewage works for COD removal. Although the Rhodes Biosure Plant is an elegant biological sulphate removal technology, it has to be taken into account that not many mines are close enough to sewage plants to make this technology generally feasible. Another, similar plant is under consideration, but in that case the primary sewage sludge has to be transported by road to the mine, which is expensive and a potential health hazard. The BioSure Plant has taken 10 years to develop, by many scientists from different universities, mainly funded by the Water Research Commission (WRC) and Innovation Fund (IF).

At Anglo Coal's Navigation colliery, a biological pilot plant is currently in operation, which treats 3 M^l/d of AMD, with a sulphate concentration of 2.5 g/^l. This plant, constructed by Paques, The Netherlands, uses waste ethanol as the carbon and energy source, making it more cost effective than using technical grade ethanol. The sulphide produced is biologically oxidised to sulphur, using redox potential measurement to regulate the oxygen supply. The sulphur produced is contaminated with biomass, for which there is currently no application, except as soil improver. However, when this produced sulphur-sludge needs to be transported from the mining sites to areas where it is to be applied to the soil, additional costs are incurred for transportation, placing the technology at an economic disadvantage. Although this technology is full-proof, the main disadvantage is the rising price of ethanol coupled to the oil price, which also affects the price of waste ethanol.

When comparing the biological sulphate removal technologies to the chemical sulphate reduction process, in which the mine water is neutralised either with lime or limestone or a combination of the two, to also remove the metals such as iron and manganese, the sulphate concentration can only be reduced to ≈ 1500 mg/^l, the solubility of gypsum (CaSO₄.2H₂O). Thus, although the water is neutralised, the sulphate concentration in some areas may still be a concern depending on the waste load allocation requirements of DWAF. After the water is neutralised, the biological

sulphate removal technology can form a second stage. The overall process would then be considered an integrated mine water treatment system. Another chemical process with which to treat mine water is the barium process, in which BaS is added to the mine water, precipitating BaSO₄ and resulting in sulphate values lower than 200 mg/l. The BaSO₄ can be roasted at ≈ 1500 °C to regenerate BaS, for re-use in the process. The disadvantage of this technology is the heating of chemicals to a high temperature, which is costly in energy usage.

The neutralisation technology, which to recently employed lime, and which was replaced by limestone, took approximately 20 years to develop to the implementation stage. Since the early 2000s, several full-scale plants were constructed, of which the first one in Empangeni on the northern KZN coast. This plant is a combination of limestone and lime treatment. Two limestone plants have been erected in Witbank at the Navigation and Kromdraai Collieries. Further full scale implementation continues, both in South Africa (Northern Cape) as well as abroad (Botswana, Australia). Although this technology has found market acceptance, research is still continuing, aiming to improve on the challenges of sludge recycle and settlement, to render the technology even more cost effective. It was shown that when limestone is replaced by lime, the operational costs were cut by approximately 40-50%.

At present, a physical treatment plant is being built by Anglo Coal, where the mine effluent from two Anglo mines and one Ingwe mine will be treated with Reverse Osmosis (RO) membrane technology. This will result in potable water and in a brine, containing metals and other salt residuals. This plant will treat 120 Ml/day underground mine water at a cost of R 300-million. Part of the costs incurred will be recovered by the sale of drinking water to the Emalahleni (Witbank) Municipality, which has a shortage of potable water. This treatment of mine water is very attractive from a water management point of view, since useless polluted water can be rendered potable for a most urgent need, such as for human consumption.

The passive treatment system, as developed by PHD Consulting over the past 10-15 years in conjunction with the Innovation Fund (IF) and the Water research Committee (WRC), is receiving recognition. A demonstration-scale, passive treatment ponding system will be constructed at one of the BHPBilliton mines, treating 200 m³/day of AMD. The principle of this passive treatment system was explained in the literature review (Chapter 2).

Different treatment technologies are being implemented at different mines. Although the chemical compositions of most mine waters differ and mines are located in dissimilar areas, for most mine effluents, there is a treatment solution that has been developed in South Africa. This indicates that even though the AMD treatment research competition might initially seem to be superfluous, it has resulted in several, sound, treatment technologies, most of which have found applications.

The technology described in this thesis may, after further investigations, competes well with established technologies. To date (May 2007) only 3 years of research has been invested compared to 10-20 years with the above mentioned technologies. When comparing with other mentioned biological treatment methods, the advantage of using cellulose as the carbon source is that grass can in principle be grown anywhere, using sunlight as the energy source. Although primary sewage sludge will always be available, it may not always be available near a mining site, while plant biomass is sustainable, when partly treated mine water can be used for irrigation during the dry Souyh African winter months. The main observation of this study was the relationship between high COD concentration, and sulphate removal. Thus an increased sulphate load will result in an increased need for grass which may require a large reactor for the one stage operation. The other observation made from the study is that rumen microbes require increased temperatures for the fermentation of cellulose. Further research should be directed to investigating whether the more robust cellulose degraders from the rumen inoculum can adapt to ambient temperature.

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