CHAPTER 7

CONCLUDING REMARKS ON BIOLOGICAL MINE WATER TREATMENT TECHNOLOGY USING THE DEGRADATION PRODUCTS OF CELLULOSE

7.1 INTRODUCTION

To comply to sound water management, pollution prevention of effluents is the key parameter. Mining as well as other industries produce large volumes of polluted waste water, which should be kept separate from clean water to prevent pollution of these clean water sources. In the situation that good housekeeping of water management is adhered to, water treatment can be applied. The studies presented in the previous chapters showed that the biological sulphate removal technology can be used to remove the salinity and acidity of mine water.

In order to list the results of this study and how these results can possibly be used for future mine water treatment, a summary table is presented, reviewing the aims and results from Chapters 3-6 (Table 7.1). The information summarised in Table 7.1 shows that the investigations described in the different chapters all have one common objective: the production and utilisation of VFA for biological sulphate removal. The outcomes of each study was dicussed separately in the following sections

7.1.1 Mine effluents as feed water for the biological reactor

The characteristics of mine effluents are high acidity (low pH), high sulphate and high metal concentrations. The high sulphate concentration in AMD can be treated biologically using SRB, an electron donor and sulphate as the electron acceptor, operating a sulphidogenic bioreactor. The results of the studies in the different chapters showed that biological sulphate removal was obtained when using either artificial, sulphate-rich, feed water or pretreated AMD, obtained from a closed mine as feed water. In order to increase the pH of the AMD prior to feeding it to the reactor, one part of mine water was mixed with one part of the effluent of the biological reactor. This mixing had several advantages, namely the neutralisation of the AMD acidity by the alkalinity present in the reactor efluent, the metals in the AMD were precipitated as metal sulphides by the sulphide present and the sulphate concentration of the AMD was diluted.

Table 7.1. Summary of aims and results of this study

Chapter	Aim	Results
3	1. VFA production from GC using microorganisms from: - Natural grass degradation - Anaerobic digester consortium (ADC) - SO_4 adapted SRB consortium 2. Can VFA produced by cellulose degraders be used for SO_4 removal	1. VFAs were produced: Increased C3 and C4 acid production (30 and 22%, respectively), when more natural grass degraders were added to reactor ADC produced VFA, resulting in SO_4 removal Highest C2, followed by C3 and C4 acids 2. Highest SO_4 removal using VFA produced by cellulose degrading SRB
4	1. VFA production using SRB versus RB as fermentation organisms 2. Will highest concentration GC result in highest SO_4 removal?	1. SRB and RB both produced VFA: RB produced higher concentration of propionic acid, especially when adding tryptone to reactor 2. Highest conc. of GC resulted in highest SO_4 removal, using C3 and C4 acids, producing C2 acid.
5	Operation of 2 stage reactor system using • Artificial Feed water • AMD as feed water Operation of 3 stage reactor system using Artificial Feed water	An average 86% SO_4 removal was achieved in FR and 20% in SR, using artificial feed water at HRT of 4 d and ½ d, in FR and SR, respectively 78% SO_4 removal was obtained using pre-treated AMD as feed water, at HRT of 2 d in FR. No noticeable SO_4 removal in SR Av. 86% SO_4 removal in FR, 20% in SR and negliable SO_4 removal in ASR at 4 d, 1/2 d and 1/3 d HRT, resptively. The results of this chapter showed that the technology is feasible as long as grass is added in relation to sulphate load
6	Technological description of technology, based on obtained and theoretical data	Technological description was developed: Technological description provided overview of processes and was used to indicate amount of grass needed for SO_4 reduction from specific AMD, removing SO_4 to DWAF standards

7.1.2 Sulphate removal efficiency

The results from the initial feasibility (batch operated) studies, investigating whether cellulose degradation products could serve as carbon and energy sources for the biological sulphate removal process, indicated that the set objectives were attainable. The results obtained from the initial batch tests formed the basis for the subsequent studies.

7.1.3 Reactor System

The results described in Chapter 5 showed that a 2 stage reactor system was suitable for the purpose of the treatment of synthetic feed water, when high concentrations of grass-cellulose were added to the first reactor. However, it was observed that not only the GC were fermented in the first reactor, but that the degradation products were already utilized for biological sulphate removal in the first reactor. The highest sulphate removal efficiency was achieved in the first reactor at 86% while an additional 20% removal was obtained in SR. When a third reactor was added, the results indicated that hardly any additional sulphate was removed in the third stage, which was ascribed to a low residual COD concentration in that reactor. Most of the COD concentration was already utilised in the first and second reactors. When diluted AMD was used as feed water, operating the two stage reactor system, it was again observed that little additional sulphate was removed in the second stage and that actually the highest sulphate removal was again achieved in the first reactor. This finding was un-expected, since the fermentation of organic matter usually requires a low pH (4-6), while the biological SO_4 removal occurs at a preferred pH of 7.5. The first reactor contained GC, rumen consortia, SRB and packing material (ceramic rings) for SRB biofilm formation.

The VFA and other intermediates produced by the rumen microorganisms were thus mainly utilised by the SRB in the first reactor. It was postulated that the SRB kept the hydrogen partial pressure low, there by stimulating the degrading bacteria to produce more hydrogen. This microbial interaction showed potential syntrophic and symbiotic interactions between the different microorganisms in the reactor.

The three stage system added no value to the technology as no additional sulphate removal was observed in the third stage. Since the highest sulphate removal was observed in FR, containing the immobilised SRB, GC and rumen fluid in one reactor, future work will concentrate on a one stage reactor system.

7.1.4 The use of rumen inoculum for the fermentation of cellulose

The rumen associated bacteria degraded the grass-cellulose to VFA and other degradation products, e.g. hydrogen, for SRB to use as the electron donor for biological sulphate removal. It was observed that the rumen bacteria produced mainly C2, followed by C3 and C4 acids. The obtained results showed the interactions among the different groups of microorganisms. It can be expected that the symbiotic relationship between groups of bacteria and other microorganisms in the reactor is similar to processes occurring in natural environments. When a good understanding of the biological processes occurring during fermentation and sulphate removal is acquired, this knowledge can be applied to harness and enhance the activities in a bioreactor. Understanding part of the complex processes in the bioreactors can enable fine-tuning of the technology.

The information obtained during the studies described in this thesis showed that cellulose was degradable by rumen microorganisms outside the ruminant, which provided some understanding of natural occurring biological processes now taking place in a created environment, such as the bioreactors. Microbes change and mutate continuously due to environmental conditions (e.g. chemicals) and therefore it is difficult to predict the exact metabolisms and mechanisms taking place in the described reactors. A better understanding of the processes can be attained by applying molecular techniques to the microbial populations in the reactor, such as for instance the terminal restriction fragment length polymorphism (t-RFLP) procedure. This is a tool for a rapid fingerprinting method, studying diversity, structure and dynamics of microbial communities.

The use of molecular studies/tools would enable the researchers to investigate the changes in the composition of the rumen fluid microorganisms. It can be well inmagined that when the rumen fluid is extracted from the fistualted animal and placed in a container, the population in the rumen fluid will change, since certain microbes cannot live outside the rumen of the ruminant. When the rumen fluid is transported from Pretoria University to the CSIR (under the required anaerobic and temperature conditions), further changes in the microbial population are anticipated. This transformation process will proceed and thus the composition of the rumen fluid will undergo changes continuously. When, in this thesis was referred to rumen consortia, it must be understood, that different compositions of the rumen microorganisms were used in the reactors. Furthermore, when GC were added to the reactor other natural occurring microbes entered the reactor as well. The “un-known” consortium of rumen microorganisms was thus “contaminated” with other natural cellulose occurring microorganisms. Therefore, the rumen fluid microorganisms described for the different studies in the thesis most likely comprised a certain robust consortium of rumen microorganisms, mixed with anaerobic soil/grass microbes, responsible for cellulose degradation in the reactors. Although applying molecular techniques to the microbial populations in the reactors did not form part of this thesis, it is proposed that applying this tool will be incorporated in future research regarding the degradation of cellulose by rumen and grass obtained microorganisms.

7.1.5 The use of VFA and other fermentation products from biowaste product as energy sources for biological sulphate removal

SRB utilised the degradation products of cellulose as substrates for the biological sulphate removal. The preferred products were hydrogen, propionic acid and butyric acid in that order. Acetic acid is the product of propionate and butyrate degradation when the C3 and C4 acids were oxidized as electron donors for the biological sulphate reduction. The results showed, that in some instances, acetate was utilised in the sulphate removal process. It was speculated that other groups of bacteria (e.g. homoacetogenic bacteria) produced butyrate from 2 mols of acetate so that butyrate was available for the SRB. It was furthermore hypothesized that as soon as the RB produced VFA and hydrogen, these products were utilized in the sulphate removal process, which explained why the highest sulphate removal was always achieved in the first reactor.

7.1.6 Process description for the bioreactors

A process description was compiled on the basis of the chemical composition of the mine water, the volume of mine water to be treated and the results obtained from FR, when diluted mine water was used as feed water. In order to create a representative account of the biological sulphate removal technology using cellulose degradation products as the carbon and energy sources, several parameters were considered, such as the target sulphate concentration required in the treated water, the metal concentration in the AMD and the amount of sulphide used for the precipitation of the metals as well as the residual COD concentration in the treated water. Other factors included were the expected growth-rates of the sulphate reducing and rumen biomass, since part of the available COD in the reactor was used for sustained cell growth. The cellulose concentration needed had to be translated into the amount of

GC required. This necessitated an estimate of the cultivated areas to grow grass for the process to be sustainable. The technological process description was developed on the basis of treating a known mine water with a specific chemical composition.

7.1.7 The sociological and economic implications

In the previous section the amount of feed grass required by the technology was calculated on the basis of the outcomes of the continuous studies in Chpater 5. If the technology described in this thesis is feasible and be brought into operation at a coal mine, it would result in social and economic advantages. The reactor system would be erected near a dam containing mine effluent. Mining companies grow grass on the premesis surrounding the mining operations. The cultivation and harvesting of grass would provide employement for labourers, who would cut and mill the GC and make it available to the biological plant. The grass can be irrigated with mine water and fertilised with the waste sludge produced in the reactor(s), depending on the concentration of heavy metals. The provision of jobs is crucial for the social upliftment of communities and the project could develop in an SMME.

The technology described is likely to be a more economical option compared to other treatment technologies. An overview of the total process is presented in Figure 7.1. The principle of the technology presented in this thesis could also find application in the passive treatment of mine water, which would result in the treatment of smaller volumes. The process, however, is aimed at active treatment. The volume of mine water treated is dependant on the amount of grass that can be grown in the immediate vicinity of the mine.

7.1.8 Limitations of the presentated study

During the investigations described in the different chapters, limitations of the technology presented themselves. As already indicated the rumen fluid microbial population was mixed with natural occurring microorganisms attached to GC. This implied that the reactors comprised mixtures of microbes that most likely were never the same, although it can be hypothesized that a consortium of robust microorganisms populated the reactors. The main aim of this study concentrated on the possible use of a potential bio-waste product rather than adding that product to landfill or other disposal facilities. For this purpose GC were degraded and the degradation products were tested for a possible more cost effective mine water treatment option. Although the GC used for the investigations were collected from a stockpile of GC, stored in the cold room, differences in the composition of GC might have occurred. It is unavoidable that small parts of leaves sometimes entered the reactors. When this study will result in a pilot scale unit near a mining operation, it can be envisaged that the added GC will most likely also change in compostion.

Since the outcome of a study could not be predicted, the results of a certain investigation were often the reason from diverting from an original study plan to further explore the reason why promising results from the initial study were obtained. During all investigations, the practical implications of applying the technology to mine effluents had to be kept in mind. A one stage reactor system is a more cost effective way of operation than a two or three stage reactor system.

The presented study is a good example of the application of environmental microbiology to the treatment of polluted waste water.

7.1.9 Recommendations for future studies

Heating mine effluents (2Mℓ/d) to 37-39 ℃ is not feasible, due to high costs. Therefore, future studies will concentrate operating at decreasing temperatures and even at ambient temperature. It is enviseaged that certain microbes from the rumen fluid can adapt to ambient temperature, since certain researches obtain degradation of cellulosic material using mature cow manure. During future studies molecular tools will be applied to microbial populations in the reactors.

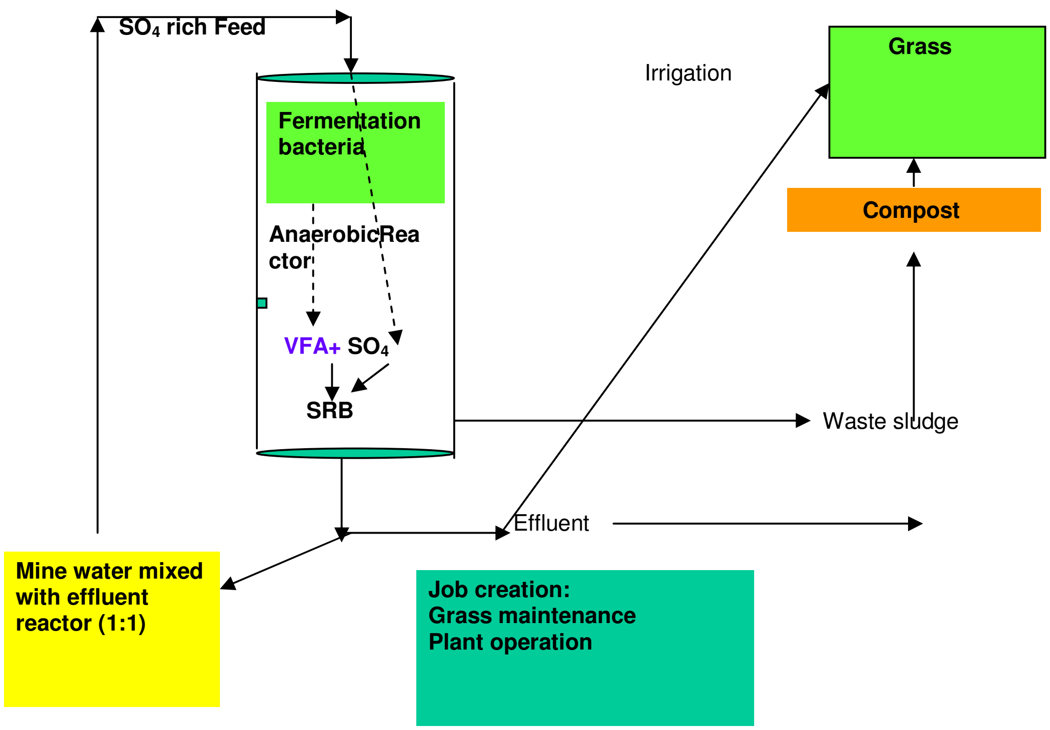

Figure 7.1. Grass cultivation and utilization for biological sulphate removal

7.2 CONCLUSIONS OF RESEARCH STUDY

The following conclusions were made from the studies as reported in this thesis:

- Naturally occurring microorganisms attached to grass produced acetic acid- (maximum of 600 mg/ℓ) propionic acid- (400 mg/ℓ) and butyric acid (160 mg/ℓ)
- Sludges, originating from an anaerobic digester (AD) and from a sulphidogenic pilot scale reactor (SRB) resulted in VFA production, mainly butyrate and acetate. SRB assisted in the degration of polymers and monomers.
- When the produced VFAs (by AD and SRB sludges) were used in a sulphate removal reactor, sulphate reduction was obtained
- The sulphate removal rate using the VFA produced by SRB in the biological sulphate removal process was slightly higher than when using sucrose as the carbon source in the control reactor.
- Grass-cellulose was fermented to VFAs, which subsequently were used in the biological sulphate removing reactor as electron donor.
- Rumen bacteria fermented grass-cellulose in higher propionic acid concentrations than SRB
- Adding tryptone to the reactor resulted in increased concentration of propionic acid compared to not adding tryptone. However adding tryptone to the process will add to the operational costs in full scale operation
- The propionic acid produced was used as the carbon and energy source for the biological sulphate removal.
- Part of the rumen microorganism consortia were sulphate reducers
- When 30, 60 and 90 g grass/ℓ were fermented by SRB, the fastest sulphate removal occurred in the reactor containing the highest grass concentration. This result showed a clear relationship between the cellulose concentration, the COD/VFA produced and the sulphate removal.
- Not all produced VFA was used for sulphate removal in the reactor with the highest grass concentration, thus grass and sulphate should be added proportionally to the reactor for the most efficient technology.
- Total sulphate removal was achieved in batch reactors, when using rumen fluid micro organisms as the fermentation bacteria and the SRB as the sulphate removers in one reactor,
- When 4x150 g grass cuttings were added to the fermentation/sulphate removing reactor (FR), an average of 86 % sulphate removal efficiency was

observed, during an experimental period of 77 days, using synthetic sulphate rich feed water.

- When pretreated AMD was used as feed water for the same reactor configuration the highest sulphate removal efficiency was 78%.
- The technological description, developed on the basis of the described process indicated that the amount of grass needed to remove 1.5 g SO_4/ℓ at a flow of 2000 m^3/day.
- Bacteria obtained from the rumen fluid from ruminants operated efficiently at 36-39 °C
- Applying the described technology will be expensive when mine effluents functioning as feed water need to be heated prior to biological treatment to 36-39 °C, to accommodate the rumen incocula.
- The described SO_4 removal technology can most likely compete with other South African developed SO_4 removal technologies, after showing that rumen microbes can adapt to lower operating temperatures degrading cellulose.

APPENDIX A

When the SRB use propionate and butyrate for the respiration to reduce sulphate to sulphide, reactions (1) and (2) can be applied:

Propionate$^-$ + ¾ SO_4^{2-} → Acetate$^-$ + HCO_3^- + ¾ HS^- + ¼ H^+ (1)

Butyrate$^-$ + ½ SO_4^{2-} → 2 Acetate$^-$ + ½ HS^- + ½ H^+ (2)

Table: The theoretical ratio between the VFA/$COD_{utilized}$/SO_4/$_{removed}$

Acetate

CH_3COOH + $2O_2$ ⟶ $2CO_2$+$2H_2O$

60 g acetate needs 64 g oxygen for total oxidation

60 g acetate provides 64 g COD

1 g acetate provides 64/60 = 1.07 g COD

1 mol acetate (60 g) to reduce 1 mol of sulphate (96 g)

per g reduced SO4 needed 60/96*1.07 = 0.67 g COD

Propionate CH_3CH_2COOH +1.75 SO_4

74 g prop needed to reduce 168 g SO_4

CH_3CH_2COOH +3 1/2 O_2 ⟶ 3 CO_2 + $3H_2O$

74 g prop provides (3.5*32)112 g COD

1 g prop provides 112/74 = 1.51 g COD

1 mol propionate(74g) to reduce 1.75(168 g) mol SO_4

per g reduced SO_4 needed 74/168*1.51= 0.67 g COD

Butyrate $CH_3CH_2CH_2COOH$ +2.5 SO_4

1 mol butyrate to reduce 2.5(240 g) mol SO_4

88 g butyric needed to reduce 240 g SO_4

CH_3CH_2COOH +5 O_2 ⟶ 4 CO_2 + $4H_2O$

88 g butyric provides (5*32)160 gr COD

1 g butyric provides 160/88 = 1.8 g COD

1 mol butyrate(88g) to reduce 2.5(240 g) mol SO_4

per g reduced SO4 needed 88/240*1.8= 0.66 g COD