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BIOWASTE AS ENERGY SOURCE FOR BIOLOGICAL SULPHATE REMOVAL

By

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Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy (Water Resouce Management)

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously, in its entirety or in part, been submitted at any other university for a degree.

Signature:

Date:

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SUMMARY

Due to mining operations, polluted mine waters are continuously produced. The characteristics of these polluted waters, often referred to as Acid Mine Drainage (AMD), are high concentrations of acidity (low pH), salinity (mainly in the form of sulphate) and metals (e.g. iron, manganese, magnesium, calcium and sodium). From a water management perspective, the treatment of mine effluents is a necessity since water is a scarce commodity in South Africa, due to increasing demands on water resources. Globally, as well as in South Africa, studies are focussed on finding the best possible AMD treatment technologies. Neutralisation of AMD, using limestone and in some instances a combination of limestone and lime can not reduce the sulphate concentration to values < 1500 mg/ℓ, while the stipulation for the sulphate (SO_4^{2-}) concentration is set at 500 mg/ℓ by the Department of Water Affairs and Forestry (South African water quality guidelines).

The biological treatment technology can remove sulphate to concentrations of < 200 mg/ℓ. The disadvantage of the biological treatment process is the need for a carbon and energy source, which is most often not present in AMD and thus needs to be added, resulting in increased operational costs. In current systems ethanol is the preferred electron donor, however, its price is related to the oil price and thus has the tendency to increase. Investigations into identifying a cheaper carbon and energy source are therefore critical. Since grass is often grown around mining operations, it

was thought that further investigations in the use of the degradation products of grass-cellulose were feasible.
It was hypothesized that natural occurring cellulose degrading microorganisms from ruminants (cattle, sheep) could be utilised to hydrolyse and ferment grass cellulose to polymers, monomers, volatile fatty acids and other intermediates, which could be used by the sulphate reducing bacteria (SRB) as the carbon and energy sources for biological sulphate removal. The study presented here shows that the degradation products of cellulose could be used as the carbon and energy source for the biological sulphate removal in mine and other industrial effluents.

Initially, batch operated reactors were used, while later a two and three stage continuous reactor system for a combined fermentation and sulphate removal process were operated. It was shown that cellulose degrading microorganisms produced short chain volatile fatty acids (VFA), such as butyric-, propionic- and acetic acids and other intermediates from grass-cellulose. Sulphate reduction was obtained when these VFAs were subsequently used for biological sulphate removal. For all studies the grass cuttings were collected from the CSIR garden service and stored at 4 °C before use. No moisture was observed on the grass cuttings.

The sulphate removal rate, using the VFA produced as the carbon and energy source, was slightly higher than when using sugar as the control carbon source. When the amount of grass cuttings per litre feed water were increased as substrate to the reactors, a direct relationship between grass concentrations and sulphate removal was observed, since the fastest sulphate removal occurred in the reactor containing the highest amount of grass. A residual VFA concentration was observed for the highest concentration grass cuttings (90 gram gass per litre SO_4 rich feed water). This result indicated a positive correlation between grass addition and the subsequent sulphate reduction.

Using microbes from rumen fluid for cellulose fermentation and SRB as the sulphate removers in one reactor, sulphate removal was achieved, even after the addition of extra sulphate loads. In batch experiments it was observed that grass-cellulose was initially faster degraded by SRB, but the cellulose fermentation bacteria from the rumen produced higher propionic acid concentrations, a preferred carbon source for SRB.

After the different batch tests were conducted, the technology was tested in a continuous mode. A continuously fed biological sulphate removal reactor, containing grass cuttings, bacteria obtained from a bovine rumen and SRB was used. This reactor system was fed synthetically prepared sulphate rich water as well as mine water. Sulphate reduction (average of 86% removal efficiency), feeding synthetic sulphate rich water was observed during an experimental period of 77 days, adding fresh grass cuttings (150 g) four times to the reactor. When pre-treated mine water was used as feed water, the highest percentage sulphate removal was 78%. When the feed rate to the reactor was doubled (from 15 to 30 ℓ/d), without increasing the amount of grass cuttings added, the percentage sulphate removal decreased to 55%. These results showed a clear relationship between grass addition and sulphate reduction. When operating a two and three stage reactor system, the results showed that the highest sulphate removal occurred in the first reactor. Thus the fermentation process and sulphate removal was already achieved in a one stage reactor, which made the second and third stage superfluous.

A process description using mass balances was developed on the basis of the results obtained when the first reactor received diluted mine water as feed water. Factors, such as the COD concentration utilised for cell growth were based on theoretical based assumptions. The outcome of the calculations showed that in order to remove 1.5 g/ℓ/d sulphate treating 2000 m^3 mine water per day, a total surface area of 1.1 km^2 is needed to cultivate enough grass under irrigation, using (partly-treated) mine water, to sustain continuous sulphate reduction for one year. Although the described process offers promises for the biological sulphate removal process, it must be kept in mind that the the reactor was operated at 37 °C for optimal performance of the rumen associated bacteria. Heating of mine water to elevated temperatures is not cost-effective. Future research should focus on adapting the anaerobic fermentation consortium, originating from rumen fluid and grass cuttings to ambient temperatures in order to make the process competitive. It is envisaged that further development of the technology may result in a viable process, comparable to other South African developed sulphate removal treatment systems.

“ WATER”

“When I was a child, it was hard to get water. We walked for long distances to find water. We fetched water from a water hole. We had to wake up early in the morning to make sure that we were at least first or second at the water hole, otherwise the water hole would be empty. If we were too late and the water hole was already empty, we used to cook and drink run-off rainwater from the roof, although it was rusted. We used to catch run-off rain water from the roof in buckets and drums. If the water hole and the buckets and the drums were empty, we had to walk for even a longer distance to get water from another river and we had to ask permission from those people. We used to wash in the river, which was dangerous, especially for the boys, who loved swimming. We used to get very itchy from the river and it caused bilharzia”

Esther Ntombi Kaba. “*Water and when I was little*”. (translated from isiZulu). Hydropolitics in the Developing World – *A Southern African Perspective* (A. Turton and R. Henwood, eds.), AWIRU, Pretoria, 2002, 269 pp.

Quoted from p.113.

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"I can do what you can't do, and you can do what I can't do; together we can do great things."
- Mother Teresa of Calcutta -

In order to write a PhD thesis, extensive research has to be conducted, for which funding is required. I was in the fortunate position to obtain a BioPAD project, together with the University of Stellenbosch. BioPAD was initiated by the Department of Science and Technology to promote research in Biotechnology in South Africa. Our project received just under R 3 million in funding, while the total duration of the project was 2 years. The research focus was to investigate the production of degradation products from cellulosic material, to function as suitable carbon sources for the biological sulphate reduction. Mining is an important contributor to the economy, but results in vast volumes of polluted mine water, which needs to be treated, prior to discharge into rivers and dams. A great deal of research is focussed globally on the treatment of mine water.

The CSIR team concentrated on the operation of batch and continuous reactors, while at the University of Stellenbosch, Professor Alf Botha and Dr. Lydia Joubert and their teams, focussed on the microbiology of the degradation and fermentation of plant biomass. Without the input and support of this team, the CSIR team would not have been able to deliver the same kind of output. I learned the meaning of true collaboration through this BioPAD project. Together we managed to keep on brief, budget and time and delivered good science in the process, part of which is captured in this thesis.

I would like to acknowledge BioPAD, the CSIR, the Stellenbosch team as well as Thrip (for funding postgraduate students) for invaluable financial support. Special thanks go to Richard Eijsberg, who developed the technological description (Chapter 6) of the process and to Ellenore Steyn for analytical assistance. Thanks to the encouragement of my husband Dr. Jan Meint Greben, my colleague Dr. Jannie Maree and ex- Programme Manager, Dr. Johan de Beer, I felt encouraged to tackle this challenging task.

Last but certainly not least, thanks to my promoter, Professor Fanus Venter, who spent many hours going throught the different versions of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AMD	Acid Mine Drainage
AB	Acetogenic Bacteria
AF	Anaerobic Filter
AMB	Acetate Utilizing Methanogenic Bacteria
ASRB	Acetate Utilizing SRB
ATP	Adenosine Triphosphate
CH_4	Methane
CO_2	Carbon Dioxide
COD	Chemical Oxygen demand
DWAF	Department of Water Affairs and Forestry
EPS	Extracellular Polymeric Substances
FB	Fluidized Bed Reactor
GC	Grass cuttings
HSRB	Hydrogen Utilizing SRB
HMB	Hydrogen Utilizing Methanogenic Bacteria
HRT	Hydraulic Retention Time
IMPI	Integrated Managed Passive Treatment Process technology
MB	Methanogenic Bacteria
MPB	Methane Producing Bacteria
SRB	SRB
SO_4	Sulphate
RB	Rumen Bacteria
RR	Reduction Rate
SEM	Scanning Electron Microscopic
UASB	Upflow Anaerobic Sludge Bed
VSS	Volatile Suspended Solids
WSP	Waste Stabilisation Pond
WW	Waste water