

BIOWASTE AS ENERGY SOURCE FOR BIOLOGICAL SULPHATE REMOVAL

By

HARMANNA ALIDA GREBEN-WIERSEMA

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

in the Faculty of Natural & Agricultural Sciences
University of Pretoria
Pretoria

Supervisor: Professor S N Venter

(May 2007)

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:

BIOWASTE AS ENERGY SOURCE FOR BIOLOGICAL SULPHATE REMOVAL

BY

HARMANNA ALIDA GREBEN-WIERSEMA

Supervisor: Professor S N Venter

Department: Microbiology and Plant Pathology

Degree: PhD (Water Resource Management)

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 SO₄ 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 m³ mine water per day, a total surface area of 1.1 km² 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.

ACKNOWLEDGEMENTS

"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 through the different versions of this thesis.

TABLE OF CONTENTS

DECLARATION	i
SUMMARY	ii
FOREWORD.....	v
ACKNOWLEDGEMENTS	vi
LIST OF ABBREVIATIONS	xv
CHAPTER 1 :	1
 1.1 INTRODUCTION	Error! Bookmark not defined.
1.1.1 Water demand	1
1.1.2 Origin of AMD.....	2
1.1.3 Environmental impact due to coal mining activities in South Africa .	2
1.1.4 Approaches for the treatment of AMD.....	3
1.1.5 Biological sulphate removal treatment technology.....	3
1.1.6 Bio Waste Products	4
1.2 STUDY OBJECTIVES	4
1.3 RESEARCH QUESTIONS	5
1.3.1 Cellulose degradation, VFA production, sustainable sulphate reduction.....	5
1.4 REFERENCES	6
2. CHAPTER 2 : LITERATURE REVIEW	8
2.1 SULPHUR CYCLE	8
2.2 IMPACT OF MINING AND MINE EFFLUENTS ON THE ENVIRONMENT	8
2.3 ACID MINE DRAINAGE (AMD)	10
2.4 ACID MINE DRAINAGE TREATMENT TECHNOLOGIES	11
2.4.1 Physical and Chemical Technologies	11
2.4.1.1 The Barium removal technology.....	11
2.4.1.2 The limestone neutralisation and precipitation technology	11
2.4.2 Biological treatment.....	11
2.4.2.1 Passive treatment	12
2.4.2.2 Active treatment.....	13
2.5 REACTOR DESIGN	14
2.6 MICROORGANISMS IN THE ANAEROBIC BIOREACTOR	15
2.6.1 Sulphate Reducing Bacteria (SRB).....	15
2.6.2 Acetogenic Bacteria (AB)	15
2.6.3 Methanogenic bacteria (MB)	16
2.6.4 Cellulose degrading microorganisms	16
2.6.4.1 <i>Acetivibrio cellulolyticus</i>	18
2.6.4.2 <i>Bacteroides cellulosolvens (Fibrobacter succinogenes)</i>	18

2.6.4.3	<i>Caldocellum saccharolyticum</i>	18
2.6.4.4	<i>Clostridium</i> species.....	18
2.6.4.5	<i>Erwinia</i> species	18
2.6.4.6	<i>Ruminococcus</i> species	19
2.7	PRODUCTS OF THE MICROBIAL ACTIVITY IN THE RUMEN	19
2.8	COMPETITION FOR SUBSTRATE IN THE ANAEROBIC REACTOR..	19
2.9	CARBON AND ENERGY SOURCES FOR BIOLOGICAL SULPHATE REMOVAL.....	21
2.9.1	Traditional carbon and energy sources	22
2.9.1.1	<i>Sucrose</i>	22
2.9.1.2	<i>Ethanol</i>	22
2.9.1.3	<i>Methanol</i>.....	23
2.9.1.4	<i>Hydrogen</i>.....	23
2.9.1.5	<i>Synthesis gas,</i>.....	25
2.9.2	Complex organic products as alternative carbon and energy sources.....	25
2.9.2.1	<i>Complex organic products.....</i>	25
2.9.2.2	<i>Production of Volatile Fatty Acids from complex organic material.</i> ..	26
2.9.2.3	<i>Anaerobic oxidation of Long-Chain Fatty Acids.....</i>	30
2.10	THE OXIDATION OF ORGANIC COMPOUNDS IN A SULPHIDOGENIC REACTOR.....	30
2.10.1	Fatty Acids in the sulphidogenic reactor	31
2.10.2	Competition for propionate and butyrate.....	32
2.10.3	Propionate utilisation treating sulphate rich effluent.....	33
2.10.4	Acetate degradation.....	33
2.11	BIOLOGICAL TREATMENT OF AMD: THE CHALLENGES	35
2.12	CONCLUSIONS	36
2.13	REFERENCES	37
3.	CHAPTER 3 : MICROBIAL CELLULOSE DEGRADATION FOR OPTIMAL VFA PRODUCTION AND BIOLOGICAL SULPHATE REDUCTION	45
3.1	INTRODUCTION	45
3.2	MATERIALS AND METHODS.....	46
3.2.1	Plant biomass	46
3.2.2	Microbial biomass.....	46
3.2.3	Experimental	46
3.2.3.1	<i>Hydrolysis study.....</i>	46
3.2.3.2	<i>Anaerobic degradation/ SO₄ removal study.....</i>	47
3.2.4	Analytical.....	48
3.3	RESULTS AND DISCUSSION	49

3.3.1	VFA production from grass-hydrolysis by natural occurring microorganism on grass	49
3.3.1.1	Acetic acid.....	49
3.3.1.2	Propionic acid.....	49
3.3.1.3	Butyric acid	50
3.3.2	Effect of cellulose degrading anaerobic microorganisms on the VFA production, followed by SO₄ reduction	51
3.3.2.1	VFA production.....	51
3.3.2.2	Sulphate reduction and sulphide production	53
3.3.2.3	VFA utilisation in reactors B1 to B4.	54
3.3.2.3.1	Reactor B1	54
3.3.2.3.2	Reactor B2	55
3.3.2.3.3.	Reactor B3	55
3.3.2.3.4	Reactor B4	55
3.4	CONCLUSIONS	57
3.5	REFERENCES	58
4.	CHAPTER 4 : THE EFFECT OF INCREASED GRASS CONCENTRATION ON THE VFA PRODUCTION AND SUBSEQUENT SULPHATE REDUCTION USING SRB AND RUMEN FLUID AS FERMENTATION INOCULA	60
4.1	INTRODUCTION	60
4.2	MATERIALS AND METHODS.....	61
4.2.1	Study 1	62
4.2.1.1	Experimental	62
4.2.2	Study 2.....	62
4.2.2.1	Experimental	62
4.2.3	Study 3.....	63
4.2.3.1	Experimental	63
4.3.1	Study 1. The use of SRB as fermentative and SO₄ removing bacteria.....	64
4.3.1.1	Sulphate reduction	64
4.3.1.2	VFA concentration	67
4.3.1.2.1	Acetic acid.....	67
4.3.1.2.2	Propionic acid	68
4.3.1.2.3	Butyric acid	69
4.3.2	Study 2. The use of RB and SRB as fermentative and SO₄ removing microorganisms	70

4.3.2.1	Sulphate reduction	70
4.3.2.2	VFA concentration.....	71
4.3.2.3	Butyric acid	74
4.3.2.4	Theoretical COD used/Sulphate reduced ratio	74
4.3.3	Study 3.....	75
4.3.3.1	Sulphate reduction	76
4.3.3.2	VFA production and utilisation.....	77
4.3.3.3	Sulphate removed/VFA utilised	79
4.4	CONCLUSIONS	79
4.5	REFERENCES	80
5.	CHAPTER 5 : VFA PRODUCTION AND CONTINUOUS BIOLOGICAL SULPHATE REMOVAL OPERATING A TWO AND THREE STAGE REACTOR SYSTEM FEEDING SYNTHETIC FEED WATER AND FROM ACID MINE WATER	83
5.1	INTRODUCTION	83
5.2	MATERIAL AND METHODS	84
5.2.1	Study 1a Two stage reactor system	84
5.2.1.1	Feed water	84
5.2.1.2	Carbon and energy source.....	85
5.2.1.3	Reactor system	85
5.2.1.3.1	Fermentation reactor (FR).....	85
5.2.1.3.2	Sulphate removal reactor (SR).....	85
5.2.1.4	Analytical.....	86
5.2.2	Study 1b. Three stage reactor system.....	86
5.2.2.1	Feed water.....	87
5.2.2.2	Reactor system	87
5.2.2.3	Biomass.....	87
5.2.2.4	Carbon and Energy Source	88
5.2.2.5	Analytical.....	88
5.2.3	Study 2a Effect of AMD on activity of rumen microorganisms	88
5.2.3.1	Feed water.....	88
5.2.3.2	Reactors	88
5.2.3.3	Biomass.....	88
5.2.3.4	Experimental	89
5.2.4	Study 2b. Operating the two stage reactor system using pre-treated AMD as feed water	90
5.2.4.1	Feed water.....	90
5.2.4.2	Carbon and energy source.....	90
5.2.4.3	Reactor system (FR and SR)	90
5.2.4.4	Analytical	90

5.2.4.5	Experimental	90
5.3	RESULTS AND DISCUSSION	91
5.3.1	Study 1a.....	91
5.3.1.1	Sulphate removal.....	91
5.3.1.2	VFA utilisation.....	93
5.3.1.3	Sulphide production	94
5.3.1.4	Alkalinity production	95
5.3.1.5	Reactor pH.....	95
5.3.2	Study 1b.....	96
5.3.2.1	Sulphate removal in FR, SR and ASR.....	96
5.3.2.2	COD profile in reactors FR, SR and ASR.....	102
5.3.2.3	VFA profile in FR, SR and ASR	102
5.3.3	Study 2a.....	104
5.3.3.1	Sulphate removal.....	104
5.3.3.2	COD/Sulphate.....	104
5.3.4	Study 2b.....	105
5.3.4.1	Sulphate removal.....	105
5.3.4.1.1	<i>Grass added/sulphate removal ratio.....</i>	107
5.3.4.2	COD concentration	107
5.3.4.3	Sulphide_{produced}/Sulphate_{removed} ratio	107
5.3.4.4	Metal removal.....	109
5.4	CONCLUSIONS	109
5.5	REFERENCES	111
6.	CHAPTER 6 : PROCESS DESCRIPTION OF THE GRASS-CELLULOSE FERMENTATION AND BIOLOGICAL SULPHATE REMOVAL TECHNOLOGY APPLYING MASS BALANCE EQUATIONS	113
6.1	INTRODUCTION	113
6.2	MICROBIAL PROCESSES DESCRIPTION	113
6.2.1	Metal Removal.....	115
6.2.2	Waste streams.....	115
6.2.2.1	Metal sulphides.....	115
6.2.2.2	Sludge.....	116
6.2.2.3	Waste VFA or COD.....	116
6.2.2.4	Gases.....	116
6.2.2.5	Sulphide.....	116
6.2.3	Process Flow diagram	116
6.2.4	Mass Balances	118

6.2.4.1	Stream balance	119
6.2.4.2	Overall balances for sulphate and sulphide	120
6.2.4.3	Mixer settler.....	120
6.2.4.4	The reactor	122
6.2.4.5	GC requirements based on the process description.....	128
6.3	CONCLUSIONS	130
6.4	REFERENCES	133
7.	CHAPTER 7 : CONCLUDING REMARKS ON BIOLOGICAL MINE WATER TREATMENT TECHNOLOGY USING THE DEGRADATION PRODUCTS OF CELLULOSE	135
7.1	INTRODUCTION	135
7.1.1	Mine effluents as feed water for the biological reactor	135
7.1.2	Sulphate removal efficiency.....	137
7.1.3	Reactor System.....	137
7.1.4	The use of rumen inoculum for the fermentation of cellulose.....	138
7.1.5	The use of VFA and other fermentation products from biowaste product as energy sources for the biological sulphate removal.....	139
7.1.6	Process description for the bioreactors.....	139
7.1.7	The sociological and economic implications.....	140
7.1.8	Limitations of the presented study.....	140
7.1.9	Recommendations for future studies	141
7.2	CONCLUSIONS OF RESEARCH STUDY.....	142
APPENDIX A		144

LIST OF TABLES

Table 2.1.	Organic substrates, mostly used for biological sulphate removal.....	14
Table 2.2.	Acetogenic and methanogenic reactions, and sulphate-reducing reactions involved in the degradation of organic matter in methanogenic bioreactors, and sulphate-reducing bioreactors, respectively.....	32
Table 3.1.	Overview of the three studies in this chapter	47
Table 3.2.	The experimental condition in F1, F2 and F3	47
Table 3.3.	The carbon sources used in the different batch reactors	48
Table 3.4.	The GC/FID programme for the detection of VFA.....	48
Table 4.1.	The experimental conditions in Study 1	62
Table 4.2.	The experimental conditions of study 2	63
Table 4.3.	The experimental conditions.....	63
Table 4.4.	The chemical composition of the nutrient solution	63
Table 5.1.	The chemical composition of AMD obtained from Witbank South.....	89
Table 5.2.	The metal concentration of AMD before and after dilution with sulphide rich effluent	89
Table 5.3.	The chemical composition of the treated water from FR and SR	91
Table 5.4.	The SO ₄ removal pattern in FR and SR.....	92

Table 5.5.	The chemical compositions of the feed and treated water during the four periods in FR, SR and ASR.....	100
Table 5.6.	The sulphate removing data in the three reactor system	101
Table 5.7.	The VFA profile in the three reactor system over the four experimental periods.....	103
Table 5.8.	The chemical composition of the feed and treated water in FR and SR.....	108
Table 5.9.	Metal concentrations in AMD, in pre-treated AMD and in treated AMD.....	109
Table 6.1	The key components of each stream in the biological treatment	118
Table 6.2.	Flow rates of the various process streams	119
Table 6.3.	Amounts of removed metal sulphides.....	122
Table 6.4.	Distribution of the produced VFA represented as COD	127
Table 6.5	Composition of fresh grass (Sonakya et al., 2003)	128
Table 7.1.	Summary of aims and results of this study	136

LIST OF FIGURES

Figure 2.1	The Biological Sulphur Cycle.....	8
Figure 2.2	Metabolic stages and products in the anaerobic digestion of complex organic matter.....	28
Figure 3.1	Acetic acid production in reactors G1 and G2.....	49
Figure 3.2	Propionic acid production in G1 and G2	50
Figure 3.3	Butyric acid production in G1 and G2.....	51
Figure 3.4	The VFA production in F2	53
Figure 3.5	Butyric acid production in F1, F2 and F3	53
Figure 3.6	Sulphate reduction in B1-B4.....	54
Figure 3.7	Sulphide production in.....	54
Figure 3.8	The VFA concentration in B1.....	55
Figure 3.9	The VFA concentration in B2.....	55
Figure 3.10	VFA concentrations in B3.....	57
Figure 3.11	VFA concentration in B4.....	57
Figure 4.1	The SO ₄ reduction and VFA pattern in T30	65
Figure 4.2	The SO ₄ reduction and VFA pattern in T60.....	65
Figure 4.3	The SO ₄ reduction and VFA pattern in T90	67
Figure 4.4	Acetic acid concentration in T30, T60 and T90.....	69
Figure 4.5	Propionic acid concentration in T30, T60 and T90.....	69
Figure 4.6	Butyric acid concentration in T30, T60 and T90.....	70
Figure 4.7	The sulphate removal in reactors R3 and R4.....	71
Figure 4.8	The propionic acid concentration in reactors R1-R4.....	72
Figure 4.9	The biological sulphate reduction in reactor L1.....	76
Figure 4.10	Propionic acid concentrations in reactors L1, L2 and L3.....	78
Figure 4.11	Acetic acid concentration in reactors L1, L2 and L3	78
Figure 4.12	The butyrate concentrations in reactors L1, L2 and L3.....	79
Figure 5.1	Schematical representation of the two stage reactor system.....	86
Figure 5.2	Schematic representation of the three stage reactor system	87
Figure 5.3	The SO ₄ and COD concentrations relating to operation FR.....	92
Figure 5.4	The SO ₄ and COD concentrations relating to SR	93
Figure 5.5	Sulphate removal and COD concentration in FR	96
Figure 5.6	Sulphate removal and COD concentration in SR.....	97
Figure 5.7	Sulphate removal and COD concentration in ASR	98
Figure 5.8	COD profile in the three reactor system	102

Figure 5.9	The SO ₄ removal pattern in R1 and R2	104
Figure 5.10	The COD concentration in R1and R2	105
Figure 5.11	SO ₄ concentration in feed and treated water in FR and SR reactors	106
Figure 5.12	COD concentration in the FR and SR reactors	108
Figure 6.1.	Grass (cellulose) degradation by rumen bacteria and subsequent biological sulphate reduction by SRB.	115
Figure 6.2.	Process Flow- diagram of the proposed AMD treatment.	117
Figure 6.3.	The reactor showing all in and out-going streams	122
Figure 7.1.	Grass cultivation and utilization for biological sulphate removal ..	Error! Bookmark not defined.

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