BIOLOGICAL SULPHATE REDUCTION USING LIGNOCELLLOSE HYDROLYSIS BY-PRODUCTS PRODUCED BY FUNGAL HYDROLYSIS OF *CENCHRUS CILIARIS* CV. MOLOPO (BUFFELSGRASS)

by

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"Try not to become a man of success
but rather a man of value."

Albert Einstein

I certify that the thesis hereby submitted, and the work presented therein, to the University of Pretoria for the degree of M.Sc. has not been previously submitted by myself in respect of a degree at any other University.

Signature:_____

Date:_____

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SUMMARY

The biological treatment of acid mine drainage using sulphate reducing bacteria (SRB) is an alternative to chemical treatment. However, substrate availability normally becomes the limiting factor for sustaining sulphate reduction. Biological pretreatment of *Cenchrus ciliaris* cv. Molopo (Buffelsgrass) by white rot fungi was investigated to enhance the biodegradability of this lignocellulose substrate. *In vitro* dry matter digestibility was used to measure the effectiveness of fungal delignification. None of the treatments increased the digestibility of the natural substrate. Only *Pleurotus ostreatus* was capable of improving the initial digestibility of steam pasteurized grass by 7% over 6 weeks. A brown leachate was produced in all treatments over the experimental period. Literature indicated that such a leachate could contain inhibitors or stimulants of bacterial growth. An antimicrobial activity assay indicated that the leachate did not inhibit aerobic Gram negative or Gram positive bacteria. The effect of the leachate on sulphate reduction by SRB was investigated at 3% and 6% concentrations, using 3% preconditioned inoculum. Overall sulphate reduction varied from 27.7% to 44.9% and long-term sulphate reduction rates of 9.4 mg/l/d to 15 mg/l/d were observed. Sulphate removal efficiencies (in terms of mg SO_4^{2-} reduced / mg COD consumed) of 27% to 52% were obtained. No biological sulphate reduction was observed in the control reactors. Reactors amended with 3% *P. ostreatus*, *S. commune* and *P. chrysosporium* leachate

performed better compared to other leachate treatments. Sulphate removal was comparable to literature values, but sulphate reduction rates were considerably lower in this study. Near-neutral pH was maintained in all leachate amended reactors, unlike the controls. Therefore, leachate buffering capacity could enhance SRB survival and consequently expedite biological sulphate reduction.

BIOLOGIESE SULFAATREDUKSIE DEUR GEBRUIK TE MAAK VAN LIGNOSELLULOSE AFBRAAK PRODUKTE GEPRODUSEER VANAF DIE AFBRAAK VAN *CENCHRUS CILIARIS* CV. MOLOPO (BUFFELSGRAS) DEUR SWAMME

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OPSOMMING

Die gebruik van sulfaatreduserende bakterieë vir die behandeling van suurmyn dreinering is 'n altenatief tot chemiese behandeling. Volgehoude sulfaatreduksie word normaalweg beperk deur die beskikbaarheid van die substraat. Wit-vrot swamme is gebruik om die bioafbreekbaarheid van *Cenchrus ciliaris* cv. Molopo (Buffelsgras) te verbeter. Die effektiwiteit van lignien afbraak deur die wit-vrot swamme is gemeet in terme van *in vitro* droë materiaal verteerbaarheid. Geeneen van die behandelings kon die verteerbaarheid van natuurlike Buffelsgras verbeter nie. Die verteerbaarheid van stoom-gepasteuriseerde Buffelsgras is deur *Pleurotus ostreatus* met 7% oor 6 weke verbeter. Tydens die eksperimentele tydperk is 'n bruin vloeistof in al die reaktors geproduseer. Volgens die literatuur kon hierdie uitloogmateriaal chemiese verbindings bevat wat bakteriële groei inhibeer of stimuleer. 'n Filtreerpapierskyfie tegniek het getoon dat beide Gram negatiewe en Gram positiewe aerobe bakterieë nie deur die uitloogmateriaal geinhibeer word nie. Die uitwerking van die uitloogmateriaal op biologiese sulfaatreduksie is ondersoek deur gebruik te maak van 3% of 6% van die uitloogmateriaal en 3% van 'n voorafbehandelde inokulum. Die totale sulfaatreduksie het gewissel tussen 27.7% en 44.9%, terwyl langtermyn sulfaatreduksie tempo's van 9.4 mg/l/d tot 15 mg/l/d aangeteken is. Die effektiwiteit van sulfaatverwydering (in terme van mg SO_4^{2-} gereduseer / mg chemiese suurstofbehoefte verbruik) het gewissel

tussen 27% en 52%. Geen biologiese sulfaatreduksie het in die kontrole reaktors plaasgevind nie. In vergelyking met die kontrole reaktors, was die sulfaatreduksie beter in die reaktors met 3% uitloogmateriaal van *P. ostreatus, S. commune* en *P. chrysosporium.* Alhoewel die sulfaatreduksietempo's baie laag was, vergelyk die totale sulfaatreduksie goed met dié wat in die literatuur beskryf is. 'n Neutrale pH is nie in die kontrole reaktors gehandhaaf nie, maar wel in al die reaktors wat uitloogmateriaal bevat het. Dus kan die bufferkapasiteit van die lignosellulose uitloogmateriaal sulfaatreduserende bakterieë se oorlewingsvermoë verbeter en gevolglik biologiese sulfaatreduksie voorthelp.

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Chapter 3

Biological pretreatment of *Cenchrus ciliaris* cv. Molopo for use in the passive treatment of mine water

LIST OF ABBREVIATIONS

α	alpha
β	beta
ADF	acid detergent fiber
AMD	acid mine drainage
APHA	American Public Health Association
APPL	acid-precipitable polymeric lignin
ATP	adenosine triphosphate
AWWA	American Water Works Association
BOD_5	5 day biochemical oxygen demand
cfu	colony forming units
cfu/ml	colony forming units per milliliter
cm	centimeter
COD	chemical oxygen demand
CoA	coenzyme A
CSL	corn steep liquor
cv.	cultivar
d	days
°C	degrees Celsius
dH_2O	distilled water
EMP	Embden-Meyerhof-Parnas
FNC	fine natural control
FNPc	fine natural *Phanerochaete chrysosporium*
FNPo	fine natural *Pleurotus ostreatus*
FNSc	fine natural *Schizophyllum commune*
g	gram
g/l	gram per liter
h	hour
HPLC	high performance liquid chromatography
INRA	Institut de la Recherche Agronomique
IR	infrared

IVD	*in vitro* digestibility
IVDMD	*in vitro* dry matter digestibility
IVRD	*in vitro* rumen digestibility
k	specific rate constant
LCCs	lignin-carbohydrate complexes
LiP	lignin peroxidase
l	liter
ME	malt extract
Mn	manganese
MnP	manganese-dependent peroxidase
$\mu g.g^{-1}$	microgram per gram
μm	micrometer
mg/l	milligram per liter
mg/l/d	milligram per liter per day
ml	milliliter
ml/l	milliliter per liter
mm	millimeter
min	minutes
M	molar
NDF	neutral detergent fiber
NMR	nuclear magnetic resonance
o	ortho
p	para
P. chryso	*Phanerochaete chrysosporium*
PCP	pentachlorophenol
%	percent
rpm	revolutions per minute
s	seconds
SEM	scanning electron microscopy
SF	submerged fermentation
spp.	species
SRB	sulphate reducing bacteria

SSF	solid-state fermentation
TAPC	total anaerobic plate count
TCA	tricarboxylic acid cycle
TEM	transmission electron microscopy
UV	ultraviolet
V	volt
WPCF	Water Pollution Control Federation

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