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SIMULATION OF *IN SITU* BIOREMEDIATION OF CR(VI) IN GROUNDWATER AQUIFER ENVIRONMENTS USING A MICROBIAL CULTURE BARRIER

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Thesis submitted in fulfilment of the requirements of the degree of

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SYNOPSIS

The feasibility of *in situ* bioremediation of Cr(VI) in groundwater and aquifer media was investigated using microcosm and mesocosm reactors inoculated with indigenous species of bacteria from dry sludge. Microcosm cores were used to simulate contaminant movement in the vadose and aquifer zones of the aquifer system.

Cr(VI) breakthrough analysis through the experimental cores demonstrated successful Cr(VI) immobilisation in simulated barrier systems. Cr(VI) reduction was continuously monitored and microbial culture dynamics were evaluated using 16S rRNA genomic fingerprinting. A culture shift was observed in the microcosm cores with the emerging predominance of known Cr(VI) reducers — *Enterococci* from soil and *Lysinibacilli* from sludge — after operation for 45 days.

The Cr(VI) reduction process in the columns was determined to be enzyme mediated and non-competitively inhibited by Cr(VI). The microbial cultures under micro-aerobic conditions depicted a threshold Cr(VI) concentration (C_r) of approximately

100 mg/L which was much higher than the target operation concentration of 40 mg/L at the proposed remediation sites. Using the Computer Program for the Identification and Simulation of Aquatic Systems (Aquasim), it was possible to predict Cr(VI) removal efficiency and the impact of Cr(VI) toxicity on culture dynamics in the barrier. The study demonstrates the potential of applying selected Cr(VI) reducing bacteria in biological permeable reactive barrier systems in preventing the spread of the pollutant into adjacent water supply aquifers.

The impact of the presence of natural carbon sources was also evaluated by filtering the feed water through a saw dust bed. Reactors without added carbon source removed up to 70% Cr(VI), and no removal was observed in sterile controls. In the packed mesocosm reactor, the areas before the reactive barrier had no chromium reduction whereas most of the areas after the barrier achieved near 100% reduction.

The microbial dynamics were monitored by the 16S rRNA fingerprinting after exposure to Cr(VI). After operating the microcosm reactors under oxygen stressed conditions in the presence of other soil bacteria, a community shift was expected. The soil from inoculated reactors contained a wide range of soil dwelling species of bacteria as well as the newly introduced bacteria from the dried sludge. There was a noted presence of Cr(VI) reducing bacteria, *Microbacterium, Acinetobacter, Arthrobacter, Brevibacterium, Rumen bacteria,* and several *Enterococci* in the sludge culture and *Arthrobacter spp., Clostridium spp.*, and *Klebsella spp*. were amongst the evident among identified species.

A non-competitive inhibition model was used for the evaluation of aerobic performances in batch experimental studies, whereas the inhibition threshold term C_0-

C_r/C_0, was introduced for the anaerobic model performance for the reduction of chromium in batch studies. In sterile packed soil columns a model for saturated soil column with dispersion was adopted from AQUASIM 2.0. This model was used in combination with the chromium reduction rate adopted from the anaerobic batch modelling for most non sterile reactors in the microcosm performance. The study demonstrates the potential of applying selected Cr(VI) reducing bacteria in biological permeable reactive barrier systems in restraining the spread of the pollutant into adjacent water supply aquifers.

The outcome of this exercise could be useful in the formulation of biological permeable barriers for protection against the spread of the pollutant from hot spots in the area. This is serves as a significant step towards a pilot study.

DECLARATION

I Pulane E Molokwane, declare that the thesis which I hereby submit for a Doctor of Philosophy in Chemical Technology degree at the University of Pretoria is my own work and has not been previously submitted by me for any degree at this or other tertiary institution.

_____ _____

Pulane E Molokwane Date

DEDICATIONS

I dedicate this work to my mother and my siblings

My beloved Mother, Mmita, thanks for giving me life and instilling good principles in me. You did a great job Ma. Here's a "Red gown" you have been waiting for.

My siblings, Omphemetse, Rebaona and Kesaobaka, thanks for being my reason for living. Having you in my life kept me sane, I knew I had to remain abstemious for your sake. Thanks Ba-Mme.

Ausi Phemi, thanks for making me laugh at all times even when things were not rosy, life would not be the same without you in my life.

My step father, Keriri, thanks for being a support to my mother and the kids.

You all mean the world to me, ausi Pully loves you very much.
May god continue to bless you abundantly.

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My *Yahweh* has yet again demonstrated the plans He has for me, plans to prosper not to harm me, plans to give me hope and a future-Jeremiah 29:11.

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To my study leader Professor Evans Martin Nkhalambayausi-Chirwa, thank you for your guidance, patience and mammoth dedication to my training. Words cannot begin to express how treasured you are. Thank you so much**.**

To all my colleagues at the Environmental Engineering division, you had become family. Thank you for assisting in so many ways.

Professor Venter at the department of microbiology, Thank you for assisting with the microbiological work.

To all my dear friends I had in the past four years and still are around today, you are true friends. Thanks for being supportive when I was going through trials in my life. I cherish you.

May God Almighty bless you all.

"*An investment in knowledge pays the best interest."*
- Benjamin Franklin

"Obstacles are those frightful things you see when you take your eyes off your goal."
- Henry Ford

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LIST OF NOMENCLATURES

SYMBOLS

a	Surface area (L^2)
A	Effective cross sectional area (L^2)
a_i	surface area in the segment (L^2)
b	Dimensionless logistic pitch factor for the biomass
C	Cr(VI) concentration (state variable) (ML^{-3})
C	Cr(VI) concentration at a time of incubation t (ML^{-3})
C_{eq}	Equilibrium/saturation concentration (ML^{-3})
C_{eq}	equilibrium concentration at the surface for adsorptive process (ML^{-3})
C_{in}	Influent Cr(VI) concentration (ML^{-3})
C_o	initial Cr(VI) concentration (ML^{-3})
C_s	Cr(VI) concentration at the particle surface (ML^{-3})
D	Coefficient of molecular diffusion (L^2T^{-1})
ΔL	Grid section (L)
ΔL	change in reactor length (L)
ΔV	change in reactor volume (L^3)
F	Input Cr(VI) (MT^{-1})
j_c	mass transport rate ($ML^{-2}T^{-1}$)
k_{ad}	adsorption rate coefficient (T^{-1})
k_d	cell death rate coefficient (T^{-1})
k_L	mass transport rate coefficient (LT^{-1})
k_{ms}	specific substrate utilisation rate coefficient (T^{-1})
N	Grid number
Q	Flow rate (L^3T^{-1})
q_c	adsorption rate ($ML^{-3}T^{-1}$)

q_c	adsorption rate ($ML^{-3}T^{-1}$)
R_c	Cr(VI) reduction capacity (mg Cr(VI) removed /mg cells inactivated)
r_c	Cr(VI) reduction rate ($ML^{-3}T^{-1}$)
r_c	Cr(VI) reduction rate ($ML^{-3}T^{-1}$)
t	time (T)
t_0	logistic interval for biomass (T)
u	interstitial velocity (LT^{-1})
X	viable cell concentration (ML^{-3})
X_0	Initial viable cell concentration/density in the reactor (ML^{-3})
X_{max}	Maximum attainable viable cell concentration (ML^{-3})
X_o	initial viable cell concentration (ML^{-3})
Y	cell yield coefficient ($M \cdot M^{-1}$)

ABBREVIATIONS

AAS	Atomic Adsorption Spectrophotometer
BMM	Basal Mineral Medium
BPRB	Biological permeable Reactive Barriers
CFU	Colony Forming Unit
CRB	Chromium Reducing Bacteria
Cr(III)	Chromium 3
Cr(VI)	Chromium 6/Hexavalent Chromium
CT-PRB	Continuous Trench Permeable Reactive Barrier
DNA	Deoxyribonucleic Acid
EPS	Exo Polysaccharide
FGS	Funnel and Gate System
hrs	Hours
ICP-MS	Induction Coupled Plasma Mass Spectrophotometer
LB	Luria Bettani
MS-PRB	Multi Sequenced Permeable Reactive Barrier
NADH	Nicotinamide Adenine Dinucleotite Phosphate
PC	Plate Count
pH	Potential Hydrogen
PRB	Permeable Reactive Barrier
PVC	Poly Vinyl Chloride
rRNA	Ribosomal Ribonucleic Acid
Rpm	Rotation Per Minute
SA	South Africa
UK	United Kingdom

U.S.EPA	United State of America Environmental Protection Agency
WHO	World Health Organisation