

**BIOREMEDIATION OF METALLIC FISSION PRODUCTS IN
NUCLEAR WASTE: BIOSORPTION AND BIORECOVERY**

NONHLANHLA NGWENYA

A thesis submitted in fulfilment of requirements for the degree of

PHILOSOPHIAE DOCTOR (CHEMICAL TECHNOLOGY)

in the

**FACULTY OF ENGINEERING, BUILT ENVIRONMENT AND
INFORMATION TECHNOLOGY**

UNIVERSITY OF PRETORIA

2011

ABSTRACT

BIOREMEDIATION OF METALLIC FISSION PRODUCTS IN NUCLEAR WASTE: BIOSORPTION AND BIORECOVERY

By

Nonhlanhla Ngwenya

Supervisor: Professor EMN Chirwa
Department: Chemical Engineering
Degree: Philosophiae Doctor (Chemical Technology)

The performance of a growing sulphate reducing bacteria consortium for Sr^{2+} , Co^{2+} and Cs^+ removal from solution in a batch sulphidogenic bioreactor was investigated. Metal removal by the growing bacterial consortium, and microbial culture growth and metabolic activities (biological sulphate removal) were continuously monitored in the bioreactors over the duration of the treatment period. On the other hand, diversity changes within the bacterial consortium before and after bioreactor operation (28 days) were performed using the partial 16S rRNA fingerprinting method.

In the original bacterial consortium, *Enterococcus* and *Staphylococcus* sp. were the dominant bacterial species. However, the presence of Sr^{2+} , Co^{2+} and Cs^+ in the growth media, resulted in the emergence of new bacterial species belonging to the *Citrobacter*, *Paenibacillus*, and *Enterococcus* and *Stenotrophomonas* genera, respectively. The *Citrobacter* and *Paenibacillus* sp. demonstrated high tolerance towards the presence of the divalent cations, Sr^{2+} and Co^{2+} , respectively, while the *Enterococcus* and *Stenotrophomonas* sp., demonstrated Cs^+ high tolerance. The bacterial growth and sulphate removal rate were significantly decreased at initial metal ion concentrations ≥ 100 mg/L. The toxicity and inhibitory effects of the metals on the present SRB consortium was observed in the order $\text{Sr} > \text{Co} > \text{Cs}$.

The metal uptake capacity (q_t) of the bacterial consortium decreased with increasing initial metal concentration, and complete Sr^{2+} , Co^{2+} and Cs^+ removal was observed at initial metal concentrations ≤ 75 mg/L. Overall, the present SRB consortium demonstrated a superior Sr^{2+} removal capacity ($q_{max} = 405$ mg/g), and the least for Cs^+ , where $q_{max} = 192$ mg/g. The present SRB culture exhibited a superior Sr^{2+} and Cs^+ binding capacity, compared to other studies in literature. Results from Sr^{2+} , Co^{2+} and Cs^+ biosorption kinetics indicate that initial concentration and solution pH played a vital role in determining the rate of metal removal kinetics. The experimental data was successfully analysed by the pseudo-second-order rate model, demonstrating that chemisorption is the main rate limiting step for the removal of Sr^{2+} , Co^{2+} and Cs^+ from solution. In this study, the adsorption behaviour of protons and of Sr^{2+} , Co^{2+} and Cs^+ onto the bacterial consortium cell surfaces was evaluated under anaerobic conditions as a function of pH (4-10), ionic strength (0.01, 0.05, 0.1M) and temperature (25, 50 and 75°C). Acid-base titrations of the bacterial suspension indicated that the titration data could be adequately described by a four site nonelectrostatic model, with pK_a values of 4.41, 6.69, 8.10 and 10.

The Sr^{2+} , Co^{2+} and Cs^+ adsorption data could be fitted with a two site nonelectrostatic model, involving the type 1 and 2 sites (carboxylic and phosphoryl sites). Increasing the ionic strength had a negative effect on the adsorption of metal ions from solution. There was no observed temperature dependence on the adsorption of Co^{2+} and Cs^+ from solution. In summary, results obtained in this study have shown that the processes involved in microbial Sr^{2+} , Co^{2+} and Cs^+ removal from contaminated sources is a direct function of the microbial characteristics and efficiency, mass transfer and surface complexation effects under varying environmental conditions. One important goal to be achieved in future studies will be the determination of the intrinsic stability constants and the structure of the formed metal-complexes species. These constants can be used directly in risk assessment programs.

DECLARATION

I Nonhlanhla Ngwenya, 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 institutions.

Nonhlanhla Ngwenya

Date

ACKNOWLEDGEMENTS

A major research project like this is never the work of anyone alone. First and above all, I thank God for the wisdom and perseverance that he bestowed upon me and for granting me the capability to succeed.

I would also like to extend my sincere gratitude to:

- The study leader, Prof. Evans Chirwa, for accepting me into his research group, and for his generous time and support throughout the duration of this study.
- Dr Chris Daughney for his generosity by sharing with me the program FITMOD and also his time and assistance with surface complexation modeling studies.
- The South African Nuclear Human Asset Research Programme (SANHARP) team and the National Research Foundation (NRF) of South Africa, for financial assistance to whom I am indebted to.
- Colleagues and friends from the Water Utilisation Research Group for their support and friendship

I would like to pass my heartfelt gratitude to my family for their unconditional support. To my Father, who bid this life an early farewell, I wish you would have lived longer to see me graduate, rest in peace Mabuya. To George, my true friend and partner, thank you for encouraging me to enter the doctoral program, and for giving up your PhD degree so I can have mine; for having more faith in me than I had in myself; you have always built my confidence and sustained faith in my abilities, thank you. During my time as a student I had the opportunity to be a mother to an energetic little boy, who also contributed immensely in shaping me as a person.

‘I Owe You All More Than Words Can Tell’

TABLE OF CONTENTS

	PAGE
Title page.....	i
Abstract.....	ii
Declaration.....	iv
Acknowledgements.....	v
List of Figures.....	x
List of Tables.....	xiii
Nomenclature.....	xvi
1 INTRODUCTION.....	1
1.1 Introduction.....	1
1.2 Aim and Objectives.....	2
1.3 Scope of the Study.....	2
1.4 Methodology.....	3
1.5 Significance of the Study.....	4
2 LITERATURE REVIEW.....	5
2.1 BACKGROUND.....	5
2.2 SPENT NUCLEAR FUEL MANAGEMENT.....	6
2.2.1 Structure and Composition of Spent Nuclear Fuel.....	6
2.2.2 Spent Nuclear Fuel Reprocessing.....	7
2.2.3 Spent Nuclear Fuel Disposal.....	8
2.3 ENVIRONMENTAL FISSION PRODUCT CONTAMINATION.....	9
2.3.1 Speciation and Chemistry of Fission Products.....	9
2.3.2 Mechanisms of Release into the Environment.....	10
2.3.3 Biological Hazards of Fission Products.....	11
2.3.4 Fission Product Treatment Options.....	11
2.4 METAL IONS UPTAKE BY BACTERIA.....	13
2.4.1 Microbial Mechanisms of Metal Uptake.....	13
2.4.2 Genetic Diversity of Bacterial Biosorbents.....	15
2.5 METAL ADSORPTION BY BACTERIA.....	17
2.5.1 Factors Influencing Metal Adsorption by Bacteria.....	17

2.5.2	Modelling the Kinetics of Metal Adsorption by Bacteria.....	20
2.5.3	Modelling Equilibrium Batch Biosorption: Isotherm models.....	22
2.5.4	Surface Complexation Modelling.....	25
2.5.5	Metal Desorption.....	28
2.6	THE APPLICATION OF MICROBIAL TECHNOLOGY IN NUCLEAR WASTE MANAGEMENT.....	29
2.6.1	Scientific Framework of Technology.....	29
2.6.2	Prospects of Radionuclide Bioremediation in Passive Microbial Bioreactors.....	31
2.6.3	Challenges and Future Perspectives.....	32
3	MATERIALS AND METHODS.....	33
3.1	CHEMICALS AND REAGENTS.....	33
3.2	MICROORGANISM.....	33
3.3	MEDIA.....	34
3.4	BATCH SRB BIOREACTOR EXPERIMENTS.....	35
3.4.1	Bioreactor Configuration.....	35
3.4.2	SRB Screening for Sr, Co and Cs Removal and Tolerance.....	36
3.4.3	Kinetics of Sr ²⁺ , Co ²⁺ and Cs ⁺ Removal in the SRB Bioreactor.....	36
3.4.4	Model Formulations for SRB Bioreactor Processes.....	37
3.4.5	Modelling Software.....	38
3.5	KINETICS OF Sr ²⁺ , Co ²⁺ AND Cs ⁺ BIOSORPTION FROM AQUEOUS SOLUTION.....	39
3.5.1	Kinetic experiments.....	39
3.5.2	Kinetic modelling.....	40
3.6	MAXIMUM BIOSORPTION CAPACITY OF Sr ²⁺ , Co ²⁺ AND Cs ⁺	42
3.6.1	Equilibrium Sorption Experiments.....	42
3.6.2	Equilibrium Isotherm Modelling.....	42
3.7	ADSORPTION OF PROTONS AND Sr ²⁺ , Co ²⁺ and Cs ⁺ ONTO SRB CELLS.....	43
3.7.1	Preparation of Bacterial Biosorbent.....	43
3.7.2	Metal Adsorption Experiments.....	44
3.7.3	Surface Complexation Modelling.....	44
3.8	ANALYTICAL PROCEDURES.....	46
3.8.1	Bacterial Culture Characterisation.....	46

3.8.2	Total Organic Carbon Analysis.....	48
3.8.3	Solid Phase Sr, Co and Cs Species Analysis.....	49
3.8.4	Metal Concentration.....	50
3.8.5	Sulphate Concentration.....	51
3.8.6	Biomass Concentration.....	51
4	Sr²⁺, Co²⁺ AND Cs⁺ REMOVAL IN A BATCH SULPHIDOGENIC BIOREACTOR.....	53
4.1	PROSPECTS OF RADIONUCLIDE REMEDIATION IN AN SRB BIOREACTOR.....	53
4.2	SRB CHARACTERISATION AND SCREENING.....	54
4.2.1	Partial Characterisation of the Initial SRB Consortium.....	54
4.2.2	Microbial Diversity Analysis in the Sr, Co and Cs Bioreactors.....	55
4.2.3	Mechanisms of Sr ²⁺ , Co ²⁺ and Cs ⁺ Removal in the Bioreactors.....	61
4.3	SIMULATION OF SULPHIDOGENIC BIOREACTOR PROCESSES.....	64
4.3.1	Modelling Approach.....	64
4.3.2	Model Calibration and Parameter Estimation.....	65
4.3.3	Simulations of SRB Bioreactor Processes in the Presence of Strontium.....	66
4.3.4	Simulations of SRB Bioreactor Processes in the Presence of Cobalt.....	71
4.3.5	Simulations of SRB Bioreactor Processes in the Presence of Cesium.....	77
4.3.6	Sensitivity Analysis.....	83
4.4	SUMMARY.....	84
5	KINETIC AND EQUILIBRIUM STUDIES FOR Sr²⁺, Co²⁺ AND Cs⁺ UPTAKE ONTO BACTERIAL CELLS.....	86
5.1	BACKGROUND.....	86
5.2	KINETIC STUDIES OF Sr ²⁺ , Co ²⁺ and Cs ⁺ BIOSORPTION.....	87
5.2.1	Effect of Initial Concentration.....	87
5.2.2	Effect of pH.....	91
5.2.3	Effect of Sorbent Dose.....	94
5.2.4	Effect of Metabolic State.....	96
5.3	EQUILIBRIUM SR ²⁺ , CO ²⁺ AND CS ⁺ BIOSORPTION.....	98
5.3.1	Sr ²⁺ , Co ²⁺ and Cs ⁺ Biosorption from Single Metal Solutions.....	98



5.3.2	Competitive Binding of Sr^{2+} , Co^{2+} and Cs^+ in Binary Metal Solutions.....	100
5.4	ADSORPTION OF PROTONS AND Sr^{2+} , Co^{2+} AND Cs^+ ONTO SRB CELL SURFACES.....	101
5.4.1	Surface Complexation Modelling Approach.....	101
5.4.2	Modelling the Acid-Base Properties of SRB.....	101
5.4.3	Determination of Apparent Stability Constants for Metal-Bacteria Complexes.....	104
5.5	SUMMARY.....	110
6	CONCLUSIONS AND RECOMMENDATIONS.....	112
	BIBLIOGRAPHY.....	116
	APPENDICES.....	140

LIST OF FIGURES

	PAGE
2.1 Background Global nuclear power generating capacity (%) per country (IAEA, 2009)....	6
2.2 Schematic representation of spent fuel structure and composition after reactor operations. (Adapted from Chirwa, 2010).....	7
2.3 Common nuclear waste management practices. LLW= low level waste, ILW=intermediate level waste and HLW=high level waste (Adapted from Raj <i>et al.</i> 2006).....	8
2.4 Elevated view of a permeable reactive barrier configuration for groundwater treatment. (Adapted from Gavaskar, 1999).....	30
3.1 Schematic diagram of the laboratory-scale anaerobic SRB batch bioreactor. (1= anaerobic bioreactor, 2= 10% zinc acetate trap solution, 3= magnetic stirrer, 4= sampling syringe, 5= rubber stopper, 6= N ₂ gas inlet and 7= H ₂ S gas outlet).....	35
4.1 Phylogenetic tree of <i>Enterococcus</i> and <i>Staphylococcus</i> sp. related clones obtained from the original SRB culture (control). The cloned genes are named according to the isolate number.....	55
4.2 Phylogenetic tree of <i>Citrobacter</i> sp. related clones obtained from cultures grown in Sr ²⁺ contaminated medium. The cloned genes are named according to the source/contaminating metal and isolate number.....	57
4.3 Phylogenetic tree of <i>Paenibacillus</i> sp. related clones obtained from cultures grown in Co ²⁺ contaminated medium. The cloned genes are named according to the source/contaminating metal and isolate number.....	58
4.4 Phylogenetic tree of <i>Enterococcus</i> sp. related clones obtained from cultures grown in Cs ⁺ contaminated medium. The cloned genes are named according to the source/contaminating metal and isolate number.....	59
4.5 Phylogenetic tree of <i>Stenotrophomonas</i> sp. related clones obtained from cultures grown in Cs ⁺ contaminated medium. The cloned genes are named according to the source/contaminating metal and isolate number.....	60
4.6 Partitioning of Sr ²⁺ species in the SRB biomass after exposure to a metal solution. F1= exchangeable fraction, F2= bound to carbonates, F3= bound to oxides, F4= bound to sulphide/organics, and F5= residual fraction.....	62

4.7	Experimental and model predicted growth of an SRB biomass in a batch bioreactor in the presence of different initial Sr^{2+} concentrations.....	66
4.8	Experimental and model simulations of sulphate reduction in a batch SRB bioreactor in the presence of different initial Sr^{2+} concentrations.....	68
4.9	Experimental and second-order model plot showing the removal of different Sr^{2+} initial concentrations by a growing SRB consortium in a batch bioreactor.....	70
4.10	Experimental and model predicted growth of an SRB biomass in a batch bioreactor in the presence of different initial Co^{2+} concentrations.....	72
4.11	Experimental and model simulations of sulphate reduction in a batch SRB bioreactor in the presence of different initial Co^{2+} concentrations.....	74
4.12	Experimental and second-order model plot showing the removal of different Co^{2+} initial concentrations by a growing SRB consortium in a batch bioreactor.....	76
4.13	Experimental and model predicted growth of an SRB biomass in a batch bioreactor in the presence of different initial Cs^+ concentrations.....	78
4.14	Experimental and model simulations of sulphate reduction in a batch SRB bioreactor in the presence of different initial Cs^+ concentrations.....	80
4.15	Experimental and second-order model plot showing the removal of different Cs^+ initial concentrations by a growing SRB consortium in a batch bioreactor.....	82
4.16	Time course of the sensitivity functions of SRB biomass concentration with respect to other kinetic parameters.....	84
5.1	Pseudo-second-order model plot for the effect of different initial metal concentrations on the removal of Sr^{2+} , Cs^+ and Co^{2+} from solution by SRB.....	89
5.2	Pseudo-second-order model plot for the effect of pH on the removal of Sr^{2+} , Cs^+ and Co^{2+} from solution by SRB.....	93
5.3	Pseudo-second-order model plot for the effect of sorbent dose on the removal of Sr^{2+} , Cs^+ and Co^{2+} from solution by SRB.	95
5.4	Pseudo-second-order model plot for the effect of metabolic state on the removal of Sr^{2+} , Cs^+ and Co^{2+} from solution by SRB.	97
5.5	Langmuir (A) and Freundlich (B) isotherm plots for Sr^{2+} , Cs^+ and Co^{2+} sorption by SRB at a biomass density of 0.5g/L.....	99
5.6	Potentiometric titration data of a viable SRB consortium reported as H^+ added per gram (wet weight) in 0.01M, 0.1M and 0.5M NaNO_3 and 25°C. Data shown are representatives of each of the three replicates performed at the different ionic strengths.	102

5.7	Ionic strength dependence on the adsorption behaviour of Sr^{2+} , Co^{2+} and Cs^+ onto a viable SRB consortium at 25°C	106
5.8	Temperature dependence on the adsorption behaviour of Sr^{2+} , Co^{2+} and Cs^+ onto a viable SRB consortium at 25°C	109

LIST OF TABLES

	PAGE
2.1 Genetic diversity of bacterial biosorbents.....	16
2.2 Expressions for the most frequently used reaction rate models.....	21
2.3 Expressions of the diffusion-based kinetic biosorption models.....	22
2.4 Some of the frequently used single component isotherm models.....	23
2.5 Examples of multi-component adsorption models.....	24
3.1 Composition of media for SRB enrichment and growth.....	34
4.1 Phylogeny of the SRB isolates from Sr^{2+} , Co^{2+} and Cs^+ contaminated bioreactors.....	56
4.2 Optimised Monod parameters for SRB population growth in the presence of Sr^{2+}	65
4.3 Optimised Monod parameters for biological sulphate reduction in the presence of Sr^{2+} ...	67
4.4 Optimised kinetic parameters for Sr^{2+} removal by growing SRB cells in a bioreactor.....	69
4.5 Optimised Monod parameters for SRB population growth in the presence of Co^{2+}	71
4.6 Optimised Monod parameters for biological sulphate reduction in the presence of Co^{2+} ...	73
4.7 Optimised kinetic parameters for Co^{2+} removal by growing SRB cells in a bioreactor.....	75
4.8 Optimised Monod parameters for SRB population growth in the presence of Cs^+	77
4.9 Optimised Monod parameters for biological sulphate reduction in the presence of Cs^+	79
4.10 Optimised kinetic parameters for Cs^+ removal by growing SRB cells in a bioreactor.....	81
5.1 Pseudo-first order model parameters for the effect of initial concentration on the kinetics of Sr^{2+} , Cs^+ and Co^{2+} removal in single metal solutions.....	88
5.2 Pseudo-second order model parameters for the effect of initial concentration on the kinetics of Sr^{2+} , Cs^+ and Co^{2+} removal in single metal solutions.....	88
5.3 External diffusion parameters for the effect of initial concentration on the kinetics of Sr^{2+} , Cs^+ and Co^{2+} removal in single metal solutions.....	90
5.4 Pseudo-second order model parameters for the effect of pH on the kinetics of Sr^{2+} , Cs^+ and Co^{2+} removal in single metal solutions.....	92
5.5 Pseudo-second order model parameters for the effect of sorbent dose on the kinetics of Sr^{2+} , Cs^+ and Co^{2+} removal in single metal solutions.....	94
5.6 Pseudo-second order model parameters for the effect of metabolic state on the kinetics of Sr^{2+} , Cs^+ and Co^{2+} removal in single metal solutions.....	96

5.7	Langmuir and Freundlich model parameters for the removal of Sr^{2+} , Cs^+ and Co^{2+} in single metal solutions.....	98
5.8	Equilibrium sorption performance of various sorbents for Sr^{2+} , Co^{2+} and Cs^+ uptake from aqueous solution.....	99
5.9	Equilibrium sorption model parameters for Sr^{2+} , Cs^+ and Co^{2+} uptake from binary systems.....	100
5.10	Compilation of SRB deprotonation constants, surface site densities and variance at 0.01M, 0.1M and 0.5M NaNO_3 and 25°C as calculated by FITMOD.....	103
5.11	Compilation of Sr^{2+} , Co^{2+} and Cs^+ stability constants and variance at different ionic strengths (0.01, 0.1 and 0.5M) and 25°C as calculated by FITMOD using the nonelectrostatic model.....	107
5.12	Compilation of Sr^{2+} , Co^{2+} and Cs^+ stability constants and variance at different temperatures and 0.1M and 25°C as calculated by FITMOD using the nonelectrostatic model.....	108

NOMENCLATURE

A	External sorption area (m^2/g)
a_{RP}	Redlich–Peterson adsorption constant ($(\text{l mg}^{-1})^\beta$)
β	Redlich–Peterson adsorption constant
b	Langmuir adsorption constant (l mg^{-1})
C	Metal concentration at time t (mg/L).
C_{eq}	Residual metal ion concentration at equilibrium (mg l^{-1})
C_{ini}	Initial concentration of metal in solution (mg/L),
C_t	Metal concentration at time t (mg/L)
C_0	Initial metal ion concentration (mg l^{-1})
k_1	First-order adsorption rate constant (min^{-1})
k_2	Second-order adsorption rate constant ($\text{g mg}^{-1} \text{min}^{-1}$)
k_C	Second-order rate coefficient (L/mg/h)
k_f	External diffusion coefficient (cm/s).
k_i	Intraparticle diffusion rate ($\text{mg.g.min}^{0.5}$).
k	Freundlich adsorption constant
K_s	Half saturation constant (mg/L)
n	Freundlich adsorption constant
n_p	Number of data points
n_{II}	Number of chemical components for which both total and free concentration are known
n_u	Number of adjustable parameters.
q	Adsorbed metal ion quantity per gram of biomass at any time (mg g^{-1})
q_{eq}	Adsorbed metal ion quantity per gram of alga at equilibrium (mg g^{-1})

- Q^0 Langmuir adsorption constant (mg g^{-1})
- q_{max} Maximum sorption capacity (mg.g^{-1})
- R Gas constant ($=8.314 \text{ J mol}^{-1} \text{ K}^{-1}$)
- R^2 Correlation coefficient
- S Sulphate concentration (mg/L)
- $S_{,mod}$ Calculated (model) concentration at time t (mg/L),
- $S_{,exp}$ Experimental concentration at time t (mg/L)
- S_Y Default experimental error calculated by FITMOD
- t Time (minutes)
- T Solution temperature ($^{\circ}\text{C}$, K)
- μ Specific growth rate ($1/\text{h}$)
- μ_{max} Maximum specific growth rate ($1/\text{h}$)
- Y Error in the mass balance calculations
- X Bacterial concentration (mg l^{-1})
- $Y_{x/s}$ Bacterial yield coefficient ($\text{mg of biomass produced per mg substrate utilized}$)