

**CADMIUM AND COPPER BIOSORPTION BY A BACTERIAL STRAIN ISOLATED  
FROM SOUTH AFRICAN ANTIMONY MINE**

by

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RESEARCH DISSERTATION

Submitted in fulfillment of the requirements for the degree of

**DOCTOR OF PHILOSOPHY**

in

**BIOCHEMISTRY**

in the

**FACULTY OF SCIENCE AND AGRICULTURE  
(SCHOOL OF MOLECULAR AND LIFE SCIENCES)**

at the

**UNIVERSITY OF LIMPOPO**

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**2010**

## **Declaration**

I declare that the dissertation hereby submitted to the University of Limpopo for the degree Doctor of Philosophy has not previously been submitted by me for a degree at this or any other University; that this is my own work in design and in execution, and that all material contained therein has been duly acknowledged.

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## **Dedication**

This work is fully dedicated to five individuals with different roles in my life: my brother, Joseph, my mom, Maria, to the family of my former high school principal, Mr. and Mrs. P.S. Mohale for their support and finally, to my daughter, Lesedi, young as you are, you are my inspiration.

## **Acknowledgements**

I would like to thank the National Research Foundation (NRF) for their financial support. I feel privileged to have been part of Research Exchange for Undergraduate students (REU) between the University of the Free State and The University of Tennessee where I have been able to experience in part, the environment in the mine, interaction with international students and scientists and also, the experience in laboratory research exercises. This developed so much interest in me that I finally became part of a research group, Life in Extreme Environment (LEExEn) group, which was a collaborative work between the University of the Free State and the then University of the North, now the University of Limpopo. This offered me a great opportunity to pursue research in the field of bioremediation. I would like to acknowledge everyone who was involved directly or indirectly and to also extend my gratitude to Prof. D Litthauer, Prof. E van Heerden and Prof. EK Abotsi for extending the interaction by affording me an opportunity to learn more about some molecular biology aspects in science. My visit in the University of the Free State would not have been easier if it had not been for the bright faces in the department and my assistant/mentor, Dr. E Botes who has been on my side right from the start till the end, thank you.

I have been part of the Department of Biochemistry, Microbiology and Biotechnology (BMBT) of the University of Limpopo for some years and for all the years, it has been rewarding. The exercises in seminars, progress reports in research and conferences organized, all developed me in a wide variety of skills; personal and interpersonal, communication, information management and perspective skills. To single out, Dr. KLM Moganedi and Dr. KC Lucas, thank you for all your ideas and support. To the Department of Chemistry, I would like to thank Mr. C Maswanganyi for technical support.

To Mr. and Mrs. Mohale, you have been my family right from the start. May God of all human kind bless you and be with you forever. I lack words to express how I feel for laying a strong foundation and being my pillars throughout. If it had not been for you, I would not have been here. Thank you.

To my mom, brothers and sisters, your patience was all I needed, thank you for understanding.

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## List of Abbreviations

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- AIX - Ampicilin, isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactoside (X-gal)
- API - Application programming interface
- Biolog - Biological
- DMSO - Dimethyl sulfoxide
- DNA - Deoxyribose nucleic acid
- dNTP - Deoxynucleotide triphosphate
- EDTA - Ethylene diamine tetraacetic acid
- EEA - European Environment Agency
- EPS - Extracellular polysaccharides
- GM isolate - Gravelote Mine samples
- IPTG - Isopropyl B-D-galactoside
- LB - Luria Bertani medium
- MCL - Maximum contaminant level
- MH - Mueller-Hinton medium
- MIC - Minimal inhibitory concentration
- PCR - Polymerase chain reaction
- rDNA - Ribosomal DNA
- RNA - Ribonucleic acid
- rpm - Revolutions per minute

- SDS - Sodium dodecyl sulphate
- TRIS - Trisaminomethane
- TYG - Tryptone, yeast extract and glucose
- US-EPA - United States Environmental Protection Agency
- USGS - United States Geological Survey
- v/v - Volume per volume
- w/v - Weight per volume
- X-gal - 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactoside



## List of symbols

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• %	-	Percentage
• <	-	Less than
• =	-	Equal to
• >	-	Greater than
• $\Delta G^{\circ}$	-	Gibbs free energy of biosorption ( $\text{kJ mol}^{-1}$ )
• $\Delta H^{\circ}$	-	Enthalpy change of biosorption ( $\text{kJ mol}^{-1}$ )
• $\Delta S^{\circ}$	-	Entropy change of biosorption ( $\text{J mol}^{-1} \text{K}^{-1}$ )
• $\mu\text{g L}^{-1}$	-	Microgram per liter
• $\mu\text{m}$	-	Micro-meter
• $\mu\text{M}$	-	Micromolar
• $a$	-	Temkin isotherm constant ( $\text{L g}^{-1}$ )
• As(III)	-	Arsenite
• $\beta$	-	Constant related to energy of adsorption
• $\text{Ca}^{2+}$	-	Calcium ion
• $\text{CaCl}_2$	-	Calcium chloride
• $C_{\text{ad}}$	-	Adsorbed metal ion concentration at equilibrium ( $\text{mg L}^{-1}$ )
• Cd	-	Cadmium
• $\text{Cd}^{2+}/\text{Cd(II)}$	-	Cadmium ion
• $\text{CdCl}_2 \cdot \text{H}_2\text{O}$	-	Cadmium chloride monohydrate
• $\text{CdHPO}_4$	-	Cadmium hydrogen phosphate

- $C_e$  - Equilibrium metal ion concentration
- $C_f$  - Final (residual) metal ion concentration ( $\text{mg L}^{-1}$ )
- $C_i$  - Initial metal ion concentration ( $\text{mg L}^{-1}$ )
- Co - Cobalt
- $\text{Co}^{2+}/\text{Co(II)}$  - Cobalt ion
- $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  - Cobalt chloride
- $\text{COO}^-$  - Carboxylic group
- Cr(VI) - Hexavalent chromate ion
- $C_s$  - Adsorbate solubility at a given temperature
- $C_t$  - Metal concentration ( $\text{mg L}^{-1}$ ) at time  $t$
- Cu - Copper
- $\text{Cu}^{2+}/\text{Cu(II)}$  - Copper ion
- $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  - Copper chloride
- $\varepsilon$  - The column porosity, Polanyi's adsorption potential
- g - Gram
- h - Hour
- $\text{H}^+$  - Hydrogen ion
- Hg - Mercury
- Hg(II) - Mercuric ion
- $\text{HNO}_3$  - Nitric acid
- $\text{HPO}_4^-$  - Phosphate ion
- $\text{J mol}^{-1} \text{K}^{-1}$  - Joules per mole per Kelvin

- K - Kelvin
- K<sup>+</sup> - Potassium ion
- $k_0$  - Mixed order rate constant
- $k_1$  - Pseudo-first order rate constant (min<sup>-1</sup>)
- $k_2$  - Pseudo-second order rate constant (g mg<sup>-1</sup> min<sup>-1</sup>)
- $k_3$  - Second order rate constant (g mg<sup>-1</sup> min<sup>-1</sup>)
- $K_4$  and  $K_0$  - Rate constants of saturation type biosorption  
(L g<sup>-1</sup> min<sup>-1</sup> and L mg<sup>-1</sup> respectively)
- $K_c$  - Equilibrium constant
- KCl - Potassium chloride
- $K_F$  - Freundlich adsorption constant
- kJ mol<sup>-1</sup> K<sup>-1</sup> - Kilo Joule per mole per Kelvin
- $K_L$  - Langmuir adsorption constant (L mg<sup>-1</sup>)
- $K_R$  - Equilibrium parameter
- L - Liter
- $M$  - Mass of dry weight/biomass (g)
- M - Molar concentration
- mg/g - Milligram per gram
- mg/L - Milligram per liter
- Mg<sup>2+</sup> - Magnesium ion
- MgCl - Magnesium chloride
- mM - Milli Molar
- Mn<sup>2+</sup> - Manganese ion

- $n$  - Freundlich adsorption constant
- $\text{NaAsO}_2$  - Sodium arsenite
- $\text{NaCl}$  - Sodium chloride
- $\text{NH}_2/\text{NH}_3^+$  - Amino group
- $\text{Ni}$  - Nickel
- $\text{Ni}^{2+}/\text{Ni(II)}$  - Nickel ion
- $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  - Nickel chloride
- $^\circ\text{C}$  - Degrees Celsius
- $\text{OH}^-$  - Hydroxyl group
- $\text{Pb}$  - Lead
- $\text{PO}_4^{2-}$  - Phosphate
- $q$  - Metal sorption capacity/adsorbed metal ion quantity per gram of dry weight of biomass at any time ( $\text{mg g}^{-1}$ )
- $q_e$  - Adsorbed metal ion quantity per gram of biomass at equilibrium ( $\text{mg g}^{-1}$ )
- $q_{\max}$  - Maximum metal sorption capacity ( $\text{mg g}^{-1}$ )
- $Q^0$  - Langmuir adsorption constant ( $\text{mg g}^{-1}$ )
- $q_t$  - metal uptake per gram of biomass at time  $t$
- $R$  - Universal gas constant ( $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ )
- $r^2$  - Regression correlation coefficient
- $\text{R}_2\text{NH}/\text{RNH}_2$  - Amino group of generic protein
- $\text{RCOOH}$  - Carboxyl group of generic protein

- $R-OSO_3$  - Sulphate group of generic protein
- rpm - Revolution per minute
- SH - Sulfhydryl group
- $SO_3H$  - Sulphonic acid
- $T$  - Temperature ( $^{\circ}C$  or K)
- $t$  - Time
- $V$  - Volume
- $w_o, w$  - The initial and final volumes (L), respectively
- Zn - Zinc
- $Zn^{2+}/Zn(II)$  - Zinc ion
- $ZnCl_2$  - Zinc chloride

## Abstract

A heavy-metal resistant bacterium (GM 16) was isolated from a South African antimony mine, and the non-viable cells of the isolate were used to investigate its biosorption capacity for Cd(II) and Cu(II) from aqueous solution in a batch process. The biosorption of both metals were found to be influenced by factors such as pH of the metal solution, initial metal ion and biomass concentrations, rate of agitation, presence of other metal ions, contact time of the metal solution with the biomass and temperature. The initial biosorption of both Cd(II) and Cu(II) was rapid and equilibrium was reached within 1 hour of biomass contact with the metal solutions. The sorption of both metal ions was higher in weak acid than in strong acid conditions and the optimum pH values for Cd(II) and Cu(II) biosorption were 7 and 6, respectively. The presence of the other metal ions in the metal adsorption media influenced the biosorption of both Cd(II) and Cu(II).  $Mg^{2+}$  ions decreased the uptake of Cu(II) and Cd(II) by 4.7 and 6.5 %, respectively. Whereas  $K^+$ ,  $Na^+$  and  $Ca^{2+}$  ions increased the uptake of Cd(II) by 12.3, 8.7, and 3.2 %, respectively, they slightly decreased the sorption Cu(II) (2-6.4 %). Increases in initial metal ion (40-120 mg L<sup>-1</sup>) and biomass (0.8-4.8 g L<sup>-1</sup>) concentrations enhanced the sorption of Cd(II) and Cu(II) by GM16 biomass. When the biomass concentration was increased from 0.8 to 4.8 g L<sup>-1</sup>, the biosorption capacity of Cd(II) increased from 5.5 to 14.5 mg g<sup>-1</sup> while that of Cu(II) increased from 2.8 to 14.7 mg g<sup>-1</sup> at optimum pHs and a temperature of 40 °C. Maximum adsorption of both metals occurred at an agitation rate of 100 rpm. In addition, increase in initial metal ion concentration from 40-120 mg L<sup>-1</sup> increased the initial adsorption rates ( $h$ ) and the equilibrium metal sorption capacity ( $q_e$ ) of the GM 16 biomass from 6.07 to 16.51 mg g<sup>-1</sup> for Cu(II) and 8.9 to 17.9 mg g g<sup>-1</sup> for Cd(II). Adsorption equilibrium data for both metal ions fitted well to the Langmuir adsorption model with high correlation coefficients ( $r^2 > 0.90$ ) but the data for Cu(II) could also be described by the Freundlich adsorption model. Increase in temperature from 25-40 °C only caused marginal increases in maximum metal sorption capacities ( $q_{max}$ ). The results on kinetic analysis showed that the biosorption processes of Cd(II) and Cu(II) ions by the non-viable GM 16 cells followed pseudo-second order kinetic model better

than the pseudo-first order model, although the calculated metal sorption capacities obtained with the model were overestimated. The calculated thermodynamic parameters showed that the biosorption of Cd(II) and Cu(II) ions was feasible, spontaneous and slightly endothermic for Cd(II) but slightly exothermic for Cu(II) under examined conditions. Based on 16S ribosomal DNA sequencing, the bacterial isolate (GM 16) was identified as a *Bacillus* sp. and is closely related to *Bacillus thuringiensis* and *Bacillus cereus* strains.

The biosorption capacity of the non-viable GM 16 biomass was higher than the biosorption capacity reported for the viable GM 16 cells, 65 % of Cd(II) was removed by non-viable biomass whereas 48 % was removed by the viable biomass. For the biosorption of Cu(II), the % metal ion adsorbed for the non-viable GM 16 cells was slightly higher than the % adsorbed for the viable cells although not statistically significant. Only 67 % of Cu(II) was removed by the non-viable cells whereas 65 % was removed by the viable cells.