

**STUDIES ON BIOREMEDIATION AND
NANOPARTICLE SYNTHESIS BY METAL
BIOACCUMULATING BACTERIA AND THEIR
ENZYMES**

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**DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY DELHI
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NANOPARTICLE SYNTHESIS BY METAL
BIOACCUMULATING BACTERIA AND THEIR
ENZYMES**

by

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DEPARTMENT OF CHEMISTRY

Submitted

in fulfillment of the requirements of the Degree of
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to the



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SEPTEMBER 2011

Dedicated to my Parents

CERTIFICATE

This is to certify that the thesis entitled “Studies on bioremediation and nanoparticle synthesis by metal bioaccumulating bacteria and their enzymes” being submitted by **Mr. Arvind Sinha** to the Indian Institute of Technology, Delhi for the award of the degree of *Doctor of Philosophy* in Chemistry is a record of bonafide research work carried out by him. Mr. Arvind Sinha has worked under my guidance and supervision, and has fulfilled the requirements for the submission of the thesis which, to my knowledge, has reached the requisite standard.

The results contained in this dissertation have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

September, 2011

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Abstract

The interactions between microbes and metals have been extensively exploited for bioremediation of heavy metals and biomineralization. The metallophiles possess endogenous ability to exquisitely regulate their physiology to overcome the toxic effect of external metal environment. During the process, different inorganic materials are synthesized or transformed. In combination with bioremediation these microbial processes can be harnessed to convert environmentally problematic metals into ‘high-end’ important metal nanoparticles. This very concept forms the core of the thesis. The work encompasses isolation of metallophiles, exploring mercury and manganese bioremediation by them with simultaneous nanobiosynthesis.

Mercury has unique property to enter into vapor stage at room temperature. About 90% of the conventionally remediated mercury is recycled back into the atmosphere. Thus, the ideal process for mercury remediation should be the one, wherein it can be trapped as Hg^{2+} or as Hg^0 (without letting it evaporate back to the environment). Manganese is less toxic than mercury. It has been prescribed to be within safe limit of 0.1 mg L^{-1} in drinking water. Its nanoparticles have wide spread applications. Both these issues are addressed in the thesis.

Five mercury tolerant bacteria belonging to genus *Enterobacter*, *Bacillus* and *Pseudomonas* have been isolated by mercury enrichment method from the soil samples. The isolates were characterized using biochemical tests and 16S rDNA sequencing. Out of these isolates, two metal bioaccumulating strains *Enterobacter* sp. EMB19 and *Bacillus* sp. EMB20 effectively remediated mercury and manganese with simultaneous synthesis of corresponding nanoparticles. These were investigated further.

The *Enterobacter* sp. strain was grown in presence of mercury in culture medium for studying the remediation profile. The lag phase was prolonged. Most of the mercury was remediated with the growth of the cells. Culture conditions viz. pH, inoculum size, carbon and nitrogen source were optimized for effective remediation. Under the optimized conditions, 5 mg L⁻¹ of mercury (Hg²⁺) was remediated in 72 h. TEM micrographs of the cells revealed that the remediated mercury got accumulated in the cytoplasm. The accumulation was further confirmed by EDAX.

The accumulated mercury particles were spherical and nanosized (average 3.75 ± 0.03 nm). Higher pH, longer incubation time and low concentration of mercury favored the synthesis. The characterization of mercury nanoparticles was done by Energy Dispersive X-ray analysis (EDAX), High Resolution Transmission Electron Microscopy (HRTEM), X-ray Photoelectron Spectroscopy (XPS), Powder X-ray Diffraction (PXRD) and Atomic Force Microscopy (AFM). These were found to be Hg₃(PO₄)₂ [JCPDS # 70-1798] in nature.

Elucidation of possible mechanism of mercury resistance and nanoparticle synthesis indicated involvement of mercury reductase activity. The remediation was plasmid mediated. The reductase was also characterized in terms of pH and temperature optimum and stability. The enzymes showed K_m and V_{max} 15.6 µM and 2.2 µmol min⁻¹ mL⁻¹ respectively. Its pH and temperature optimum were 7.2 and 55 °C respectively.

The *Enterobacter* cells were also used in immobilized form. These were entrapped in calcium alginate gel. The entrapped cells effectively removed 7.3 mg L⁻¹ of mercury from complex industrial effluent in 72 h. Cells could be reused for four consecutive cycles. The remediated mercury could be recovered by cell sonication.

Alginate entrapped *Bacillus* sp. cells were explored for mercury remediation in batch and continuous mode. The remediation potential was investigated by three different sorption isotherms, Langmuir, Freundlich and Dubinin-Radushkevich (D-R) models. The thermodynamics and kinetics of the remediation mechanisms were also evaluated. The study showed that the process followed pseudo-second-order kinetics and thermodynamically favored. Maximum biosorption capacity was found to be 104.1 mg g^{-1} at 30°C and pH 7.0. The continuous column experiment showed the process can remediate mercury upto 12 h from the synthetic effluent containing 10 mg L^{-1} mercury with a flow rate of 0.5 mL min^{-1} and 30°C at bed height of 10 cm.

The second isolate, *Bacillus* sp. cells were effectively used for the remediation of manganese with simultaneous synthesis of manganese oxide nanoparticles. Complete removal of 100 mg L^{-1} manganese was achieved in 96 h. The cells while remediating manganese synthesized intracellular monodispersed orthorhombic MnO_2 [JCPDS # 39-0375] nanoparticles with average size of $4.62 \pm 0.14 \text{ nm}$. The Ex-situ synthesis of manganese oxide nanoparticles was also explored using cell free extract. The ex-situ synthesized particles were more discrete and uniform. The effect of incubation time and manganese concentration on nanoparticle synthesis revealed that better accumulation is attained after 48 h and at manganese concentration lower than 200 mg L^{-1} . The nanoparticles were spherical and uniformly dispersed into the cytoplasm. These were recoverable by cell sonication.

Table of Contents

Certificate	i
Acknowledgements	ii
Abstract	v
Table of Contents	viii
List of Figures	xiv
List of Tables	xviii

CHAPTER 1 INTRODUCTION	1
1.1 Rationale of the thesis	1
1.2 Objectives	4
1.3 Scope of the thesis	5
1.4 Review of literature	8
1.4.1 Heavy metals	8
1.4.2 Heavy metals: As environmental pollutants	8
1.4.3 Heavy metals toxicity in living organisms	12
1.4.4 Mercury toxicity	15
1.4.5 Chemical nature and atmospheric deposition	15
1.4.6 Sources of mercury	16
1.4.7 Biogeochemical cycle of mercury	18
1.4.8 Effect of mercury on physiological processes	21
1.4.9 Mercury remediation	23
1.4.10 Microbial heavy metal interaction	26
1.4.11 Biosorption	32
1.4.12 Bioremediation	35
1.4.13 Mercury resistance in bacteria	35
1.4.14 Manganese as a pollutant	41
1.4.15 Manganese remediation	41
1.4.16 Manganese cycle/ speciation in the environment	43
1.4.17 Use of immobilized microbial cells in metal bioremediation	51

1.4.18 Interface with nanotechnology	56
1.4.19 Synthesis of nanoparticles	59
1.4.20 Microbial nanoparticle synthesis	61

CHAPTER 2 ISOLATION, CHARACTERIZATION OF HEAVY METAL/ MERCURY RESISTANT BACTERIA AND PRELIMINARY SCREENING FOR MERCURY BIOREMEDIATION

2.1 Introduction	70
2.2 Materials and methods	72
2.2.1 Materials	72
2.2.2 Isolation of heavy metal tolerant microbial strains	72
2.2.3 Biochemical characterization	73
2.2.4 16S rDNA analysis	73
2.2.5 Growth and heavy metal bioremediation by <i>Enterobacter</i> sp. EMB19	73
2.2.5.1 Culture and inoculum	73
2.2.5.2 Growth and mercury bioremediation	74
2.2.5.3 Transmission Electron Microscopy (TEM), Energy dispersive X-ray (EDAX)	75
2.2.5.4 Mercury reductase assay	75
2.2.6 Growth and heavy metal bioremediation by <i>Bacillus</i> sp. EMB20	76
2.2.6.1 Growth and manganese bioremediation	76
2.2.6.2 Transmission Electron Microscopy (TEM), Energy Dispersive X-ray (EDAX)	77
2.3 Results and discussion	77
2.3.1 Isolation and identification of heavy metal resistant microorganisms	77
2.3.2 Mercury bioremediation profile	81
2.3.3 Fate of remediated mercury, Transmission Electron Microscopic (TEM) and Energy Dispersive X-ray (EDAX) studies	83
2.3.4 Biochemical basis of mercury bioremediation	85

2.3.5	Manganese bioremediation by <i>Bacillus</i> sp. cells	86
2.4	Conclusions	90

CHAPTER 3 MERCURY BIOREMEDIATION BY *ENTEROBACTER* SP. CELLS

3.1	Introduction	91
3.2	Materials and methods	92
3.2.1	Materials	92
3.2.2	Culture and inoculum	92
3.2.3	Growth and mercury bioremediation	92
3.2.4	Transmission Electron Microscopy (TEM)	94
3.2.5	Energy Dispersive X-ray (EDAX) analysis of sonicated cells	94
3.2.6	Immobilization of <i>Enterobacter</i> sp. cells	94
3.2.7	Mercury bioremediation in synthetic effluent	94
3.2.8	Reusability of immobilized cells	95
3.2.9	Mercury bioremediation in effluent samples	95
3.3	Results and Discussion	96
3.3.1	Growth and mercury bioremediation	96
3.3.2	Mercury bioaccumulation its localization and characterization	101
3.3.3	Recovery of bioaccumulated mercury	103
3.3.4	Mercury bioremediation in synthetic effluent and reusability	106
3.3.5	Mercury bioremediation in effluent samples	108
3.4	Conclusions	110

CHAPTER 4 STUDIES ON MERCURY REDUCTASE, MECHANISM OF BIOREMEDIATION AND NANOPARTICLE SYNTHESIS BY *ENTEROBACTER* SP. CELLS

4.1	Introduction	111
4.2	Materials and methods	112
4.2.1	Bacterial strain	112
4.2.2	Inoculum and culture conditions	112

4.2.3	Growth, residual mercury and biosynthesis of nanoparticles	112
4.2.4	Characterization of mercury nanoparticle	113
4.2.5	Assay for mercury reductase activity	115
4.2.6	Determination of K_m and V_{max}	115
4.2.7	Determination of pH optimum and pH stability	115
4.2.8	Determination of temperature optimum and thermal stability	115
4.2.9	Preparation of competent bacterial cells	116
4.2.10	Isolation of <i>Enterobacter</i> sp. plasmid	116
4.2.11	Transformation of <i>E. coli</i> DH5 α cells	116
4.3	Results and discussion	117
4.3.1	Mercury bioaccumulation by <i>Enterobacter</i> sp.	117
4.3.2	Characterization of accumulated mercury nanoparticles	118
4.3.3	Effect of culture conditions on the nature of nanoparticles	121
4.3.4	Recovery of mercury nanoparticles by cell sonication	125
4.3.5	Biochemical basis of mercury bioremediation	126
4.3.5.1	Mercury reductase activity	126
4.3.5.2	Determination of K_m and V_{max}	127
4.3.5.3	Determination of pH optimum and stability	128
4.3.5.4	Determination of temperature optimum and stability	130
4.3.5.5	Plasmid isolation and transformation	131
4.4	Conclusions	133

CHAPTER 5 STUDIES ON MANGANESE BIOREMEDIATION AND MANGANESE OXIDE NANOPARTICLE SYNTHESIS BY *BACILLUS* SP. CELLS

5.1	Introduction	134
5.2	Materials and methods	136
5.2.1	Materials	136
5.2.2	Bacterial strain and culture conditions	136
5.2.2.1	Inoculum	137

5.2.2.2 Culture conditions	137
5.2.3 Biosynthesis of manganese oxide nanoparticles	137
5.2.4 Effect of varying culture conditions on nanoparticle synthesis	138
5.2.5 Analytical methods used for identification and characterizations of synthesized nanoparticles	138
5.2.5.1 Transmission Electron Microscopy (TEM)	138
5.2.5.2 X-ray Photoelectron Spectroscopy (XPS)	138
5.2.5.3 Powder X-ray Diffraction (PXRD)	139
5.2.5.4 Fourier Transform Infrared (FT-IR) characterization	139
5.2.5.5 Possible recovery of manganese oxide and High Resolution Transmission Electron Microscopy (HRTEM) of cell lysate	139
5.2.5.6 Atomic Force Microscopy (AFM)	140
5.2.6 Ex-situ synthesis of manganese oxide nanoparticle by cell extract of <i>Bacillus</i> sp. and their TEM, UV-Visible characterization	140
5.3 Results and discussion	141
5.3.1 Manganese remediation and localization of nanoparticles	141
5.3.2 Effect of manganese concentration on the nature of nanoparticles	144
5.3.3 Characterization and identification of accumulated nanoparticles	147
5.3.4 Ex-situ synthesis and recovery of manganese oxide nanoparticles by cell sonication	152
5.4 Conclusions	155

CHAPTER 6 MERCURY BIOREMEDIATION BY IMMOBILIZED *BACILLUS* SP. CELLS: KINETICS AND SCALE UP

6.1 Introduction	156
6.2 Materials and methods	157
6.2.1 Materials	157

6.2.2	Culture and inoculum	157
6.2.3	Composition of synthetic mercury effluent	157
6.2.4	Immobilization of <i>Bacillus</i> sp. cells	158
6.2.5	Batch bioremediation process	158
6.2.6	Column experiment	158
6.3	Results and discussion	159
6.3.1	Effect of pH	159
6.3.2	Effect of initial mercury concentration	160
6.3.3	Biosorption isotherm models for mercury remediation	161
6.3.4	Biosorption kinetics	169
6.3.5	Column breakthrough curve	173
6.4	Conclusions	177
Overall Conclusions		178
Future Perspectives		180
References		181
List of Publications		241
Brief Bio-Data		244