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

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by

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Submitted

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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.

Dr. S.K. Khare
Professor of Biochemistry
Department of Chemistry
Indian Institute of Technology, Delhi

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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 (Hg^{2+}) 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 $Hg_3(PO_4)_2$ [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 ± 0.14 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.

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