BIOREMEDIATION AND BIOSENSING OF CHROMIUM BY ISOLATED CHROMIUM RESISTANT BACTERIA

ABSTRACT

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Hexavalent chromium (Cr VI) is highly toxic and carcinogenic; it enters the environment through several anthropogenic activities. Detection and remediation of chromium has been the subject of research of many scientists. In this dissertation, we have presented new powerful chromium resistant bacteria isolated from crude source and their utilization in bioremediation as well as biosensing of Cr VI. The thesis discusses i) isolation and identification of new chromium resistant bacteria, ii) detailed biochemical studies for characterization, iii) bioremediation capabilities of these bacteria, iv) utilization of the most powerful strain for lab scale bioremediation set-up, v) extraction of crude biomaterials from the bacteria followed by partial purification and finally vi) construction of biosensors using the crude extract.

Chromium resistant bacteria were isolated from tannery waste samples. These were characterized through biochemical tests and antibiotic assays. Cross metal reactivity, plasmid curing and cysteine quantifications were also performed. Gene sequencing (16SrRNA) was conducted for all the isolates and accession numbers received after submission to National Centre for Biotechnology Information (NCBI). At first the removal capacity was studied in broth cultures. Out of a total of six bacteria, three i.e. *Enterobacter aerogenes, Aeromonas* sp., *Acinetobacter* sp. PD 12 performed very well by removing about 99% (average) from initial 19.8 ppm of Cr VI from a synthetic culture media. *Enterobacter aerogenes*, being the most efficient strain, was later used successfully to remediate hexavalent chromium from soil of potted plants. *Enterobacter aerogenes* and *Acinetobacter* sp. PD12 were used to observe optimum bioconversion and bioaccumulation of Cr VI by varying parameters such as pH, concentration of hexavalent chromium or Cr VI, and inoculum volume. *E. aerogenes* was used to remediate chromium from tannery effluents in a laboratory level experiment. The bioremediations experiments were conducted by first immobilizing the cell free extract into calcium alginate beads and then observing the removal of Cr VI by batch and continuous (packed bed) modes of operation. Observation by scanning electron microscope and

chromium peak in Energy Dispersive X-ray Spectroscopic microanalysis revealed that *E. aerogenes* helped remediate a moderate amount of Cr VI (8–16 mg/L) over a wide range of pH values at 35–37°C (within 26.05 h).

Crude cell free extract (CFE) of *Enterobacter aerogenes* T2 was exploited to develop a stable biosensor for direct estimation of Cr VI in waste water, by using three-electrode assembly via cyclic voltammetry. The proposed sensor showed linear response in the range of 10-40 µg/L Cr VI and the limit of detection was found to be 6.568 µg/L Cr VI. No interference was observed in the presence of other metal ions such as lead, cadmium, arsenic, tin etc.