CONCLUSION

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Isolation, Various Characterizations and Identification of Hexavalent Chromium Resistant Bacteria from Tannery Effluent

Successful isolation of six chromium-resistant bacterial strains was accomplished. The isolates S2 and T2 showed highest MIC values (4.20 g/L of Cr VI) comparable to other reported bacterial strains and hence could be used for bioremediation purpose. The sequencing analysis resulted in new high resistant strains having excellent removal characteristics. T2 was found to be *Enterobacter aerogenes*, having NCBI GenBank (USA) accession number of GU265554 and NII Culture Repository (India) accession number of NII 1111. The presence of cysteine (0.13 to 2.43 μg/g protein) in all the isolates indicated their chromium resistance. The study of cross-metal reactivity revealed that the strains were resistant to most of the heavy metals except mercury. Such resistance might be due to exclusion of metal species, production of low molecular weight binding proteins, transformations, bioaccumulation, etc. These bacterial strains could have a potential for bioremediation of contaminated water/soil containing the metal ions. Plasmid curing studies showed (all results being positive) that chromium resistance was manifested in genomic DNA and not in plasmid.

Bioremediation Potential of Isolates to Remediate Hexavalent Chromium

All the strains exhibited high rate of growth in LB media and the longest stationary phase (150 h) was achieved by S2. The best three bacterial strains (S2, T2, T4) showed high rate of growth with long stationary phases. The same strains gave very high removal

efficiencies (98.9% of Cr VI) in both LB and M9 culture media. From the studies of the isolated six bacteria it could be observed that of them namely *Enterobacter aerogenes, Aeromonas* sp., *Acinetobacter* sp. PD 12 could perform very well in removing Cr VI from a synthetic media. Since cross-metal activity results showed their resistance to many other metals, these bacteria might be exploited for bioremediation of contaminated soil/ wastewater streams. Chromium is toxic for plants at about 0.5 to 5.0 ppm concentration. *Enterobacter aerogenes,* when used to remediate Cr VI from contaminated soil in plant-pots, showed promising results. The result was highly encouraging since the plants could be saved from the severe effects of CrVI contamination by the application of such isolated bacteria. The optimum growth conditions with respect to pH and temperature of two highly efficient bacilli - *E. aerogenes* and *Acinetobacter* sp. PD 12 have been established to be: 35–37°C and pH 5–9 for *E. aerogenes* and 25–37°C and pH 7 for *Acinetobacter* sp. PD12. Since the optimum temperature for growth of both the strains is very near to room temperature and optimum pH is also within the range of pH of effluent water, bioremediation of Cr VI would have added advantage while addressing real application. The remediation study with various inoculums revealed that only moderate percentage of inoculum was sufficient to yield high rate of remediation by both bacteria. Also a high initial concentration of Cr VI (17.73 mg/L) could result in a high removal rate (0.31 and 0.27 mg per hour for *E. aerogenes* and *Acinetobacter* sp. PD 12, respectively). The chromium uptake by the strain *E. aerogenes* T2 was found to be 0.35 mg/L of chromium per hour and it could also remove 85.38% of Cr VI (1.3 mg/L of Cr VI) within 72 h of inoculation, from real samples, i.e. tannery effluents.

Analysis of microbe-metal interaction

Another very interesting observation was that all of the SEM, TEM, and AFM images and EDAX and XRD graphs of this isolate showed that the microbe could modify its physiology due to interactions with metal such as absorption, transformation or accumulation. A remarkable decrease in Cr VI in the effluent after remediation proved that the metal has been accumulated by the microbes in some complex form as evident from the TEM and EDAX results. The salient achievements could be concluded as (a) isolation of a new strain, (b) its superior activity compared to many similar strains reported in the literature, and (c) workability in broad pH range and normal parametric conditions of industrial effluent.

Bioremediation of Hexavalent Chromium by Microbially modified Calcium Alginate Beads

All characterizations of prepared calcium alginate beads (modified with lyophilized CFE of T2) showed that these could prove to be helpful in bioremediation (the bioremedial rate being 196.8 μg Cr VI L/g/of calcium alginate–T2 beads) of Cr VI detection in environmental samples, like tannery effluent. The same calcium alginate beads were used for bioremediation (via biosorption and biotransformation/bioaccumulation) of Cr VI from synthetic solution by a lab-level plug-flow reactor model. Equilibrium condition was reached at 100 h (where the amount of Cr VI present in input water was found to be same as that in output water, proving that probably the beads are saturated with biosorption). The same beads could be used repeatedly for 15 days for biosorption of Cr VI (at 37°C, pH 7). Various isotherm models, like Langmiur, Freundlich, Dubinin-Radushkevich etc. were studied with the adsorbents to find out that the adsorption was physical and proceeded through multilayers. This property of the

set-up could be utilized to remediate Cr VI from industrial effluent in the downstream processing units of the industries where Cr VI is spuriously used.

Development of Biosensor for Cr VI Estimation using Extracted Bacterial Biomass

A very simple technique has been developed for estimation of Cr VI in wastewater using three electrodes, via cyclic voltammetry. This was a sensitive electrode system and this could predict the chromium concentration in the concentration range (10-40 μg/L of Cr VI) which was below the threshold limit (50 μg/L of Cr VI) recommended by WHO. The sensor was low cost, reliable, and sensitive in *μg/L* level (6.568 μg/L of Cr VI) and possessed the advantage of working at environmental conditions, over other conventional methods. Thus the proposed sensing system could be a viable alternative to common/costly measurement procedures.