Abstract

The present thesis work explores the potential of isolated microbial strain(s) for bioremediation of co-contaminated system. In this study, phenol and Cr (VI) were chosen as model organic compound and heavy metal, respectively. Studies on a newly isolated bacterial strain harboring novel properties of tolerating and removing phenol and Cr (VI) individually as well as simultaneously from the wastewater makes the core of the thesis.

For removal of organic compound and heavy metals from co-contaminated system, there is a prerequisite of those microbial strain(s) that possess toxicants tolerance abilities. Thus, the work was started with isolation of such novel microorganisms and analyzing their tolerance properties towards individual as well as simultaneous presence of phenol and heavy metals.

A total of eleven phenol-tolerant bacterial strains (B1, B2, B3, B4, B5, B6, B7, B8, B9, C1, and C2) could successfully be isolated from wastewater and contaminated soil. These isolates were further screened for their higher phenol and heavy metals [Cr (VI), Cu (II), and Ni (II)] tolerance and co-tolerance using plate assay methods. All the eleven strains were able to grow in concurrent presence of lower phenol and heavy metals concentrations. Though growth of each of the microbial strains inhibited with increase in concentration of both phenol and heavy metals; strain B9 showed better visible growth in co-presence of higher concentrations of phenol and heavy metals compared to other strains.
All the aforementioned isolates were next studied for their growth and phenol utilizing efficiency in MSM (mineral salt medium) broth using acclimatization method. Among all of them, strain B9 was found to be the highest phenol utilizing bacterium. This strain was able to grow in the presence of up to 14 mM (1316 mg L\(^{-1}\)) phenol as sole carbon source and also resulted in its simultaneous degradation. *Hence, on the basis of growth and phenol degradation efficiency at higher phenol concentration (14 mM) and better co-tolerance to phenol and heavy metals (under plate study), strain B9 was selected for further detailed studies.* The strain B9 was identified as *Acinetobacter baumannii* by using 16S rRNA sequencing from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India and, deposited at its collection bank with accession No. MTCC 10506.

The optimum pH for phenol degradation by strain B9 was found to be pH 8.0, while the optimum temperature was observed to be in the range of 30-35°C. Transmission electron micrographs showed disorganized and convoluted cell membrane in case of phenol stressed cells, showing major effect of phenol on membrane. Enzymatic and spectrophotometric studies suggested the presence of ortho-cleavage pathway for phenol degradation which was further confirmed by using Gas Chromatography coupled with Mass Spectrometry (GC-MS) analysis.

The applicability of *Acinetobacter* sp. B9 in treatment of phenol contaminated industrial wastewater was checked by using it as pure culture and in the form of consortia with other potential isolates. Under laboratory setup, strain B9 showed 100% dephenolization of initial 47 mg L\(^{-1}\) phenol after 48 h wastewater treatment both as individual and in the form of consortia. However, dephenolization rate was higher in case of pure culture compared to mixed culture used.
After studying the phenol degradation aspect of *Acinetobacter* sp. B9, the isolate was subsequently studied for its Cr (VI) resistance and detoxification potential initially in synthetic media and later on in industrial wastewater. The strain tolerated high concentrations of Cr (VI) and could remove 67% of initial 7.0 mg L\(^{-1}\) of Cr (VI) with in 24 h of incubation. This Cr (VI) removal efficiency increased with addition of copper ions (7.0 mg L\(^{-1}\)) leading to 100% removal of initial 7.0 and 10 mg L\(^{-1}\) of Cr (VI) after 24 h of treatment. pH in the range of 6.0-7.0 and inoculum size of 2% (v/v) were determined to be optimum for dichromate removal.

Fourier Transform Infrared Spectroscopy (FT-IR) and Transmission Electron Microscopy (TEM) studies suggested sorption to be one of the major mechanisms behind the chromium resistance by strain B9. Prominent morphological changes were observed in chromium stressed cells during Scanning Electron Microscopy (SEM) analysis. When bacterial cells were inoculated in metal-finishing industrial wastewater, they resulted in 93, 55, and 69% removal of initial 30 mg L\(^{-1}\) Cr (VI), 246 mg L\(^{-1}\) total Cr, and 51 mg L\(^{-1}\) Ni, respectively after 144 h of wastewater treatment in a batch mode, confirming the efficacy of *Acinetobacter* sp. B9 for bioremediation of metals-rich industrial wastewater.

Co-contamination is a major hurdle in successful bioremediation of phenol and Cr (VI). Bioremediation of such type of mixed contaminants require suitable microbes that not only withstand the toxicity of both the toxicants but are also able to remediate them from the system. Thus, the feasibility of *Acinetobacter* sp. B9 for concurrent removal of phenol and Cr (VI) from co-contaminated system was evaluated next.

Initially, when mineral salt solution was used as culture medium, the strain was found to utilize phenol as sole carbon and energy source while no Cr (VI) removal was observed. However, addition of glucose as co-carbon source resulted in removal of both
the toxicants. This co-removal efficiency of the strain was further improved by using nutrient rich media (NB). Optimum co-removal was determined at 188 mg L\(^{-1}\) of phenol and 3.5 mg L\(^{-1}\) of Cr (VI) concentrations at pH 7.0. The strain was found to follow the same toxicants removal mechanisms in binary mixture as those were there in their single presence showing ortho-metabolic pathway for phenol degradation, while chromium’s reduction and sorption as one of the major mechanisms behind Cr (VI) removal.

*Acinetobacter* sp. B9 in the form of pure as well as in conjunction with other potential isolates (consortia) was later on checked for simultaneous bioremediation of phenol and Cr (VI) from tannery wastewater. With single *Acinetobacter* sp. B9 seeding, overall 100% removal of initial 47 mg L\(^{-1}\) phenol and 87% removal of initial 16 mg L\(^{-1}\) Cr (VI) could be achieved at 72 and 96 h, respectively. When same wastewater was treated with bacterial consortia, 100% phenol removal and 78% reduction of Cr (VI) was obtained after 96 h of treatment.

Overall, the entire work leads to isolation and identification of naturally occurring phenol and heavy metal tolerant microbe(s) especially *Acinetobacter baumannii* B9, which is able to decontaminate both phenol and hexavalent chromium in their single as well as simultaneous presence. Significant removal of three sets of contaminants viz. phenol, Cr (VI), and phenol+Cr (VI) from complex effluent systems by *Acinetobacter* sp. B9 strengthens the potentiality of the strain in industrial pollution control.