Executive Summary

Chromium has long emerged as an essential component in the manufacturing processes of several products. With the advancement of technology, the uses of chromium have further proliferated into the fabric of various industries like steel, chemicals, plating, dyes and pigments, leather etc. The importance of chromium cannot be overstated. However, this heavy metal does leave a footprint in terms of its toxicity to the environment.

With globalization, as the consumption of products have spread across the world, so have the manufacturing industries - they have migrated to low cost, developing countries. Many of these countries do not have the required infrastructure to treat industrial waste effectively and such wastes are either discharged directly into the ecosystem or with nominal treatment – as a result, hazard of toxic chemicals like chromium has reached a significant scale. Thus, reducing chromium concentration down to acceptable limit is deemed to be enormously significant from sustainability standpoint.

Since, the most stable forms of chromium in environment are trivalent and hexavalent, mineralization of chromium waste is however not possible hence transformation is the solution to the problem of chromium related toxicity. Transformation of highly soluble Cr(VI) to less soluble Cr(III) restrict the toxicological effect of Cr(VI).

Studies on chromium toxicity and its remediation have also witnessed a lot of advances. Various processes and technologies have been introduced and adopted by industries. Most of the conventional methods for Cr (VI) remediation involve high energy inputs as well as chemicals which inevitably lead to the problem of secondary waste and are also costly to implement. Hence, it is necessary to develop new technologies which will be viable as well as scalable. This has led to the paradigm on bio-transformation, where biological species are used to remediate industrial wastes contaminated with chromium.

Reports of several researchers have been studied in detail during the review of literatures and the recurring observation was on the efficiency of bacterial strains in reducing chromium, or high chromium resistance of bacterial species over other microbes. A wide range of microbes, isolated either from contaminated or from chromium free soils, have revealed efficient reduction of Cr(VI) to Cr(III). Microbial tolerance limit of Cr(VI) concentration may play an important role in effective remediation of Cr(VI) from polluted environments. Underlying mechanism of chromium resistance by both aerobes and anaerobes were demonstrated by various researchers and the reduced product was further characterized to determine its immobility. Moreover, it was noted during the review of literatures that very few studies had been conducted for application-oriented studies on removal of chromium along with organic substrate, from real life wastewater.

Researchers in the recent years critically evaluated that pollution from leather processing industries has been reckoned to be one of the largest polluters in the world. Tannery

effluent is enriched with both organic and inorganic components. Since Cr(III) is used for processing of hides and skin, Cr(III) in the effluent is the most expected form. But due to redox reaction taking place in the sludge an increase in the formation of Cr(VI) may occur. This needs to be addressed for the sake of sustainable environment.

The present research investigation has been carried out with the objective to identify a potent biological species which can remove chromium; standardizing the optimum conditions for its growth and performance and investigate its mechanistic approach against chromium resistance. Further characterization of reduced product obtained from the biotransformation of chromium has also been carried out to specify the immobilization of the final product. The application of the same biological species in real life tannery wastewater has also been studied in a batch reactor for evaluating its performance under real life condition as well as its effectiveness for removal of chromium in situ environment.

In search for the chromium resistant bacteria from the bank soil sample contaminated with chromium (37085.82 ± 1124.183 mg/kg), twenty-four (24) bacteria were isolated with different types of colonies. Minimum inhibitory concentration of the isolates was observed within a range of 250 to 450 mg/L of Cr(VI) as $K_2Cr_2O_7$. Seven distinct microbial strains out of the twenty-four were selected based on the chromium removal efficiency and characterized morphologically. While further studying the growth pattern of the isolates under the stress of Cr(VI) one gram-positive chromium resistant bacteria CRB 1 showed least changes in doubling time on growth of biomass with a high removal efficiency and thus it was selected for further detailed studies.

The selected chromium resistant bacterium CRB 1 was identified as ***Bacillus mycoids*** based on biochemical and 16S rRNA gene sequencing. The optimum growth condition was standardized as pH 7.5, temperature 37 ˚C, salinity 1% and shaking velocity at 140 rpm. The total protein content and protein profile on SDS PAGE were investigated. Total protein content of the cell free extract of the bacterium was not changed so far, with or without treatment of Cr(VI). This observation referred that protein synthesis by the bacterium was least affected by the presence of Cr(VI) in cell metabolism. SDS PAGE profile of cytosolic proteins showed significant bands of two proteins of molecular weight around 72 kDa and 26 kDa for both samples treated with and without chromium.

The reduced product was characterized further to study the immobility of the final product. AAS analysis revealed that Cr(VI) is immobilized within the cell either its native oxidation state (Cr(VI)) or in reduced state. SEM-EDX study manifested the presence of chromium in the treated sample. In FT IR study, substantial changes in peak intensity and peak shift were observed for the bacterial sample treated with Cr(VI) and compared to the sample grown without Cr(VI).

Since, the properties of chromate resistance and reduction are not necessarily interrelated, before using a selected microorganism or an indigenous microbial mixed-culture for devising bioremediation strategies for Cr(VI) contaminated soils or wastewater, there is a need to understand how the Cr(VI) resistance mechanism takes place within the microbial system. When Cr(VI) is reduced to Cr(III) by a soluble

chromium reductase enzyme it produces intermediates viz. Cr(V) and/ or Cr(IV). These intermediates Cr(V) or Cr(IV) induce the formation of reactive oxygen species (ROS) due to its high reactivity and produce ROS generated oxidative stress inside the cell. It has been noted that microbes are able to activate several defense mechanisms such as releasing oxidative stress combating enzymes like superoxide dis-mutase (SOD), catalase and other enzymes which scavenge the ROS species inside the cell. Activity of chromium reductase enzyme was monitored in presence of electron donors like NADP and NADPH. The chromate reductase assay supported that the Cr(VI) get reduced by reductase enzyme and NADPH acted as electron donor for the aerobic direct Cr(VI) reduction. While on addition of NADPH to CFE the activity increased by 9.17 folds and 13.07 folds when Cr(VI) concentration was 0.32 mM and 0.67 mM respectively. It was observed that the chromate reductase activity in CFE was similar for the cells grown in medium in presence or absence of Cr(VI). This observation indicates that the chromate reductase property was not induced by Cr(VI). This was further substantiated by the protein profile obtained for the bacteria. From, Lineweaver–Burk double reciprocal plot k_m and V_{max} values were calculated to be 65.42 µg/mL and 9.514 µg/mL/h (R^2=0.989), respectively for removal of Cr(VI) from synthetic growth medium at 37 ˚C. The reduction of Cr(VI) to Cr(III) has been observed to follow first order reaction kinetics and rate constant was calculated as k = -1.09 X 10^{-4} / sec. The half-life (t_{half}) was calculated as 1.77 h. Activity of stress markers like SOD, Catalase, Glutathione reductase and peroxidase were studied. Notable increase in SOD activity, catalase activity and reduced glutathione reductase were found. These observations supported that Cr(VI) entered inside the cell and reduced to Cr(III) as ROS is induced by the intermediates like Cr(V) and/ or Cr(IV). Another stress marker peroxidase activity showed declining nature and it is indicative that presence of Cr(VI) may suppress the activity of peroxidase. *B. mycoids* also produced EPS and it may help to remove Cr(VI) in medium.

The performance of the bacterial species in a batch reactor using real life tannery waste water was studied. The average BOD_5:COD ratio obtained was 0.323 for primary treated composite tannery effluent. At the time of treatment of primary treated tannery effluent, the SCOD removal was found to vary 68-95% for a fixed Cr(VI) concentration and for Cr(VI) removal it was 45 -75 % for a fixed COD value over the retention period of 96 hours. Bio-kinetic coefficients for the pure culture have established satisfactory biodegradation of soluble organics present in real-life primary treated tannery effluent and their values are reasonably comparable with those observed for municipal wastewater. The marginal inhibitory effect of chromium uptake on substrate utilization is envisaged from decreasing values of Y and μ_{max} and increasing values of k_d with the increase in initial Cr(VI) concentration of 10 to 50 mg/L. The inhibitory effect of chromium uptake is also obvious from the increasing values of Chromium Inhibition Constants (K_i), which varied from 0.182 to 0.518 mg of Cr(VI)/L for the initial Cr(VI) concentration of 10 to 50 mg/L.

The present research work was conducted to develop an efficient strategy on chromium bioremediation using bacterial strain. This detailed understanding is necessary to enable

any lab scale biotechnological technique to evolve into large-scale reactor engineering, which can be a highly effective and proficient solution in this regard.