Development of Bacterial Consortium for Detoxification of Chromium from Tannery Effluent for Environmental Safety

SUMMARY

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Summary

Chromium contamination into the environment possesses a serious threat to all over the world. It is highly toxic, persistent and potent inhibitors of ecological and biological activities. It is one of the considerable environmental pollutants that is extensively used in leather tanning, steel manufacturing plants, mining and metal finishing, wood preservatives, textile, ceramics and chemical industries etc. Chromium exists in a number of valence states ranging from +2 to +6, but the most common and stable forms are trivalent Cr(III) as cation and hexavalent chromium Cr(VI) as divalent oxyanion chromate form. Cr(VI) is highly toxic in nature, causes itching and corrosion of respiratory tract, nasal and skin irritation, ulceration and lung carcinoma in humans, reduces soil fertility and soil microbial communities, retarded plant growth. As a metal Cr(VI) is non-biodegradable and can only be transformed from its highly toxic state of Cr(VI) to a non-toxic state of Cr(III) or removed by conventional treatment processes of adsorption/accumulation, ion exchange, and chemical precipitation. However, these methods are highly cost-intensive and generate enormous amount of toxic sludge and secondary pollutants. Thus, the study of metal resistant microorganisms for the detoxification/reduction of Cr(VI) has recently gained importance and offers a safe and eco-friendly option to achieve bioremediation of chromium contaminated environments. So, the aim of present investigation was to isolate and characterize Cr(VI) resistant bacterial strains from tannery wastewater and develop a potent bacterial consortium that was capable to reduce Cr(VI) at higher concencentrations to explore the reduction potentiality of bacterial consortium. Thus, the findings of the thesis present are summarized below:

1. The tannery wastewater sample was collected from the Common Effluent Treatment Plant (CETP) situated at Kanpur, Uttar Pradesh, India. The results of

physico-chemical analysis of TWW sample revealed that it was highly deficient in dissolved oxygen and highly alkaline in nature having high electric conductivity BOD, COD, suspended and dissolved solids values. The tannery wastewater also contained various heavy metals like Cr, Zn, Mn, Fe, and Ni among which the maximum concentration of Cr was present more than its recommended permissible limit and other pollutants such as sulphate phosphate and nitrate and chloride.

2. From the collected tannery wastewater a total of thirty bacterial isolates (SCRB 1-SCRB 30) were isolated and screened on the basis of their metal tolerance ability. On the basis of MIC, six bacterial strains were selected for reduction test and out these six, three bacterial strains (SCRB 10, SCRB 17, and SCRB 19) were showed maximum reduction 96.88%, 98.19% and 93.45% of Cr (VI) respectively, at 100 mg/L and maximum tolerance at 800 mg/L of Cr(VI) by agar diffusion method. These three bacterial strains also showed resistance and sensitivity against the tested antibiotics with a wide range of MIC values ranging from 250-800 mg/L for different heavy metals. Further, these bacterial strains i.e. SCRB 10, SCRB 17, and SCRB 19 were characterized and identified on the basis of cellular morphology, biochemical and 16S rDNA gene sequencing analysis as *Cellulosimicrobium* sp. *Kocuria flava,* and *Microbacterium paraoxydans* with their accession no. KX710177, KY883433, and KX710178, respectively. Moreover, the SEM-EDX and FTIR analysis characterized the reduced product of Cr(VI) and its demonstrate its effects on cellular morphology of bacterial cell.

3. During the chromium reduction experiment, all the three bacterial strains were found efficiently capable to reduce Cr(VI) individually. Further, these bacterial strains were checked for their compatibility to each other for the development of bacterial consortium. All the three isolated strains were compatible to each other and hence,

selected for development of bacterial consortium and used though-out the study. The reduction ability of bacterial consortium was found much higher than the individual bacterial strain not only at 100 mg/L but also at 500 mg/L in broth medium as well as in real tannery wastewater also. The AAS analysis of treated tannery wastewater clearly confirms that, the developed bacterial consortium is capable to remove total chromium from TWW. Thus, the use of bacterial consortium instead of single strain is highly effective and active approach for the remediation of Cr(VI) contaminated environment without any extra additional requirement.

4. The effect of various environmental (pH and temperature) and nutritional (carbon and nitrogen sources) parameters were also studied to achieve much maximum reduction percentage of Cr(VI) at higher metal concentrations by developed bacterial consortium. The developed bacterial consortium was showed marked increase in Cr(VI) rate in comparison to reduction with single individual strain. The optimization studies revealed that, pH 7.0 and 35 ºC temperature was the most optimum environmental conditions for reduction experiments whereas, glucose (0.5%) and yeast extract (0.01%) were found optimum carbon and nitrogen sources supplement to broth medium for effective reduction of Cr(VI) up-to by consortium culture. Moreover, the effect of resting and shaking condition, inoculum concentrations and different salt concentration was studies which affects Cr(VI) process. The results from such studies revealed that, maximum chromium reduction was achieved by bacterial consortium culture under shaking condition with increasing inoculum volume of 2 mL.

5. As consortium culture are more efficient and competent to survive under environmental stress condition. The investigation from present studies showed that optimization of environmental (i.e. pH and temperature) and nutritional parameter (carbon and nitrogen sources) is a pragmatic approach to enhance the reduction

capability of consortium cultures. But, when all the optimized conditions or parameter were applied at one time on the same culture medium of bacterial consortium the marked increase was achieved in at higher concentration of Cr(VI). At optimized condition of all parameter, bacterial consortium showed complete reduction of 100 and 200 mg/L of Cr(VI) and 72% and 41% reduction of 300 and 500 mg/L. Cr(VI). Thus, reduction/detoxification of Cr(VI) at all optimized conditions at the same time by developed bacterial consortium could be a more rapid and useful way to remediate highly chromium contaminated environments.

6. As the reduction of Cr(VI) to Cr(III) is an enzymatic process.Thus, the isolated Cr(VI) reducing bacterial strains were also studied for chromate reductase enzyme activity. Results obtained from the present investigations, showed that all three bacterial strains *Cellulosimicrobium sp. Kocuria flava, and Microbacterium paraoxydans* exhibits chromate reductase enzyme activity. The *Cellulosimicrobium* sp. and *Microbacterium paraoxydans* showed maximum enzyme activity in soluble cell fraction while, *Kocuria flava* showed membrane bound chromate reductase enzyme activity.

7. The toxicity assessment of tannery wastewater before and after bacterial treatment was also investigated by phytotoxicity on the seeds of two important agricultural pulses viz. *Phaseolus mungo* and *Cicer arietinum* to show toxic effects of untreated tannery wastewater. The results from phytotoxicity investigation showed that the germination was significantly reduced in untreated effluent. The seeds that were grown in untreated effluent had shown no or very little shoot and root growth compared to those seeds that grown in control and bacterially treated tannery effluent. This indicates that untreated tannery effluent had inhibitory effect on seed germination. But, the seeds exposed to bacterial treated tannery wastewater showed better development of both root and shoot.

Thus, treatment of tannery wastewater with bacterial consortium is an effective approach to reduce Cr(VI) concentration as well as phytotoxicity.

Thus, the whole work concludes that the developed bacterial consortium from three bacterial strains; *Cellulosimicrobium* sp., *Kocuria flava*, and *Microbacterium paraoxydans* was more effective and promising approach to reduce chromium and Cr(VI) at higher concentrations from tannery effluent. Hence, it can be used as a potential agent for the effective bioremediation of chromium contaminated environment.