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# Bioremediation of Cr (VI) from Tannery effluent using *Bacillus* spp and *Staphylococcus* spp

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**Abstract:** In the present study, biosorption of Chromium by microorganisms from tannery effluent were investigated. The efficient bacteria isolated from tannery effluent were identified as *Bacillus* spp and *Staphylococcus* spp. The effect of pH and temperature on the biosorption capacity was investigated. The optimum pH and temperature was found to be pH 7 and 37ºC for *Bacillus* spp followed by *Staphylococcus* spp, the optimum pH was 8 and temperature 37ºC respectively. The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. Both the isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals ranging from 0.05 mg/L to 5 mg/L. Minimum inhibitory concentration of Cr (VI) resistant *Bacillus* spp 3.8 mg/L and *Staphylococcus* spp were 2.5 mg/L respectively.

**Keywords:** Bioremediation, Cr(VI) reduction, Chromate-resistant bacteria, *Bacillus* spp and *Staphylococcus* spp

## INTRODUCTION

Water is one of the most important natural resources, essential for all forms of life. This natural resource is being contaminated every day by various anthropogenic activities such as rapid growth of populations, urbanization and industrialization that ultimately make the environment polluted. Since recent years, sewage waters have been used for irrigation purposes (Vasseur *et al*., 2000). There are greater concerns about heavy metal contamination in the receiving water system and land. The occurrence of toxic heavy metals in the soil is of geogenic or anthropogenic origin. Heavy metals from the point of origin and other sources can be transported to distant environments. High levels of heavy metals can damage soil fertility and may affect productivity (Chang *et al*., 1992). Heavy metals in the environment may also change plant diversity and affect aquatic life.

Chromium in trace concentration is an essential element in the diet, because it regulates the glucose metabolism in the human body. Excess amounts of chromium uptake are very dangerous due to its carcinogenic effect. Chromium in soils affects the plant growth (Shanker *et al*., 2003), it is non-essential for microorganisms and other life forms and when in excess amounts it exerts toxic effect on them after cellular uptake Cr (VI) is more toxic than Cr (III). The movement of chromium and its bioavailability poses a potential threat to the environment. In this context, it is important to note that, large numbers of leather industries are engaged in chrome tanning processes in South India. There is a possibility of chromium contamination in soils and waters around industrial sites. Cleaning up of the chromium contaminated sites is a challenging task because removal of Cr (VI) in aqueous solution is very difficult. There are a number of methods employed (Boddu *et al*., 2003) for removal of hexavalent chromium from industrial wastewater such as the use of various types of adsorbents.

The toxicity of Cr in various industrial effluents is well documented. Hexavalent Cr compounds pose health risks to humans, plants, animals and fishes. Due to its carcinogenicity and mutagenicity, the United States Environment Protection Agency (USEPA) has designated Cr as a "Priority pollutant" or Class A" pollutant (Srinath *et al*., 2002; Lee and Jones, 1998). Hence, proper treatment of industrial wastewater is essential before releasing into the recipient environment.

Tanneries are a major source of chromium pollution and release Cr (VI) ranging from 40 – 25,000 mg/L of wastewater. The maximum tolerance of total Cr for public water supply has been fixed at 0.05 mg/L as per Indian standards. The environmental protection agency has formulated the maximum permissible levels of Cr (VI) into water bodies at 50 $\mu g/dm^3$ and in drinking water as 3 $\mu g/dm^3$ and that of Cr (III) as 100 $\mu g/dm^3$ (Palmer and Puis, 1994).

To check the chromium coming into environment there are various treatment options, however, they are energy expensive and not successful due to their high running cost. Besides, conventional methods for treatment of toxic chromate (Ohtake and Silver, 1994) required large amount of chemicals, energy and are unsuitable for small scale leather, dye and electroplating units. In this context, biotransformation of $Cr^{6+}$ to non toxic $Cr^{3+}$ by bacteria offers a viable, economically safe and sustainable alternative. However, development of a feasible chromate bioremediation process requires isolation of efficient chromate reducing bacterial strains, evaluation of their ability to survive, multiply and simultaneously reduce chromate in industrial wastewaters. Despite the potential of microbial $Cr^{6+}$ reduction envisaged by several authors for a long time, Cr bioaccumulation has gained attention during the recent past (Gadd and White, 1993). The present study is focused on bioremediation of chromium (VI) from tannery effluent using *Bacillus* spp and *Staphylococcus* spp under laboratory condition.

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## MATERIALS AND METHODS

### Sample collection

The tannery effluent sample was collected in sterile plastic bottles from leather industry at Vaniyambadi in vellore district. Some physicochemical parameters of effluent *viz*., Temperature (ºC), pH, Biological oxygen demand (BOD mg/L) and Chromium (mg/L) were measured (APHA, 1989).

### Isolation and identification of Cr-resistant bacteria:

For isolation of Cr-resistant bacteria, samples were serially diluted in sterile phosphate buffer (pH 7.2) and spread inoculated onto nutrient agar amended with 50 µg/mL of Cr(VI). A filter- sterilized solution of $K_2Cr_2O_7$ was used as the source of Cr(VI), which was added to the sterile molten nutrient agar to prevent problems associated with autoclaving chromate containing solutions (Babich *et al*., 1982). The inoculated plates were incubated at 37ºC for 48 hrs. After the incubation period, the plates were observed for the growth of microorganisms. The best two isolates were randomly selected for further studies. Microscopic and biochemical tests were applied to this isolate according to Bergey's manual of systematic bacteriology. The genus to which the isolates belong were determined.

### Determination of Minimum Inhibitory Concentration (MIC) of Cr(VI):

The MIC of chromium at which no colony growth occurred was determined by the Agar dilution method (Luli *et al*., 1983). Nutrient agar plates supplemented with different concentrations of Cr(VI) were inoculated aseptically with a culture of bacterial isolates in exponential growth phase. The plates were incubated for 36-48 hrs at 37ºC. Minimum concentration of Cr(VI) allowing growth of the isolates was an indicator of positive tolerance.

### Tolerance to other heavy metals:

Chromate tolerant isolates were also studied for tolerance to other heavy metals. The fresh overnight peptone water broth culture of the isolates was inoculated aseptically on nutrient agar plates supplemented individually with other heavy metals. The metal salts used were $CuSO_4$, $ZnSO_4$, $NiCl_2$, $PbSO_4$ and $CdCl_2$. The cross metal resistance was checked by increasing the concentration of respective metal in a stepwise manner with 0.05 mg/L of metal checked in nutrient agar plate. The isolates exhibiting growth after overnight incubation at 37ºC were considered tolerant to the metal.

### Growth of microorganisms and Biosorption:

*Bacillus* spp and *Staphylococcus* spp was incubated at 37ºC and at 150 rpm for 24 hrs in nutrient broth. At the end of incubation, biomass was separated from medium by centrifuging at 5000 rpm and it was kept in the oven at 50ºC to remove the free water as much as possible. Then it was suspended in deionized water separately in order to use it in the biosorption. 100 mL solutions containing 100 mg/L $Cr^{6+}$ was prepared from stock solution containing 1g/L $Cr^{6+}$ ($K_2Cr_2O_7$). Then 4.0 g microorganism (gmo/L) was added to the medium (20 gmo/L) and adsorptions of metals were investigated for different pH values adjusted by using HCl and NaOH at 37ºC. The solution containing the biomass was agitated in a shaker at 150 rpm during the adsoption. Samples taken at predetermined intervals were centrifuged and supernatants were analyzed. The analyses of $Cr^{6+}$ ion was carried out by Atomic adsorption spectrophotometer (Perkin-Elmer) at 0.01 ppm sensitivity level after dilution of the samples (Semra Ilhan *et al.,* 2004)

By taking the determined optimum conditions into consideration, the capacity of microorganism to remove the mentioned metal from the tannery effluent was searched with the same method.

## RESULTS

### Physicochemical characteristics of effluent:

Some physico-chemical characteristics of tannery effluent were ascertained, from where chromium tolerant bacteria were isolated. The temperature of the effluent sample was 38ºC, pH is 8.7, Biological oxygen demand was 1010 mg/L and the Cr (VI) level present was 1.18 mg/L.

### Isolation and MIC determination:

Chromium resistant isolates were identified as *Bacillus* spp and *Staphylococcus* spp according to Bergey's manual Systematic Bacteriology. Both isolates exhibited high resistant to Cr (VI). Minimum inhibitory concentration of Cr (VI) resistant *Bacillus* spp and *Staphylococcus* spp grown on chromium incorporated media was determined as 3.8 mg/L and 2.5 mg/L respectively.

### Tolerance to other metals:

The chromate resistant isolates were also tested for tolerance to different heavy metals. *Bacillus* spp was able to resist $Cd^{2+}$ (0.05 mg/L), $Cu^{2+}$ (0.2 mg/L), $Pb^{2+}$ (0.5 mg/L), $Ni^{2+}$ (0.3 mg/L), and $Zn^{2+}$ (0.05 mg/L). *Staphylococcus* spp also resisted $Cd^{2+}$ (0.05 mg/L), $Cu^{2+}$ (0.2 mg/L), $Pb^{2+}$ (0.5 mg/L), $Ni^{2+}$ (3 mg/L), and $Zn^{2+}$ (0.05 mg/L).

### Growth of microorganisms and Biosorption:

From the optimization study it was noted that the *Bacillus* spp and *Staphylococcus* spp showed (68.9/57.3 mg/L) biosorption activity at pH 5. Whereas at pH 6 the results noted was (82.0/60.4 mg/L), followed by pH 7 (94.5/69.2 mg/L) and at pH 89.7/72.3 mg/L). Similarly at temperature 28ºC the biosorption level noted was (54.6/61.2 mg/L) at 32ºC (70.5/68.4 mg/L), 37ºC (92.4/72.6 mg/L) and 40ºC (76.1/59.7 mg/L). The results showed that pH 7 and temperature 37ºC were found to be optimum for *Bacillus* spp followed by *Staphylococcus* spp, the optimum pH was 8 and temperature 37ºC for the biosorption study (Table 1&2).

From the optimization study the effective pH 7 and temperature 37ºC was maintained in tannery effluent for *Bacillus* spp and the results noted was (86.4 mg/L), whereas for *Staphylococcus* spp pH 8 and 37ºC was maintained in tannery effluent the results noted was (70.6 mg/L).

Table 1: Effect of pH on biosorption of Cr (VI) mg/L by *Bacillus* and *Staphylococcus* spp.

| pH | Cr(VI) adsorbed 100 mg/L | |
|---|---|---|
| | *Bacillus* spp | *Staphylococcus* spp |
| 5.0 | 68.9 | 57.3 |
| 6.0 | 82.0 | 60.4 |
| 7.0 | 94.5 | 69.2 |
| 8.0 | 89.7 | 72.3 |

Table 2: Effect of temperature on biosorption of Cr (VI) mg/L by *Bacillus* and *Staphylococcus* spp.

| Temperature ºC | Cr(VI) adsorbed (100 mg/L) | |
|---|---|---|
| | *Bacillus* spp | *Staphylococcus* spp |
| 28 | 54.6 | 61.2 |
| 32 | 70.5 | 68.4 |
| 37 | 92.9 | 72.6 |
| 40 | 76.1 | 59.7 |

## DISCUSSION

In general, potential microorganisms especially bacterial species can remove heavy metals from solutions by biosorption or bioaccumulation or both. A variety of mechanisms exist for the removal of heavy metals from aqueous solution by bacteria, fungi, ciliates, algae, mosses, macrophytes and higher plants (Pattanapipitpaisal *et al*., 2002; Rehman *et al*., 2008).

Biosorption largely involves physical adsorption followed by chemical bondage and does not require energy. Once, the metal ions are diffused on the cell surface, they bind to sites, which exhibit chemical affinity for the metal. It is a passive accumulation process, which may include adsoption, ion-exchange, complexation, chelation, and microprecipitation.

Chromium resistant bacteria have been isolated from tannery effluents by several groups (Sultan and Hasnain, 2007). During the present investigation *Bacillus* spp and *Staphylococcus* spp both were found to be highly resistant to chromium at a concentration of 3.8 and 2.5 mg/l, respectively. *Bacillus* spp showed maximum resistance against $Ni^{2+}$ at 3 mg/l and the order of resistance regarding the metal concentration was $Ni^{2+} > Pb^{2+} > Cu^{2+} > Zn^{2+} > Cd^{2+}$. *Staphylococcus* spp also showed maximum resistance against $Ni^{2+}$ and the order of resistance regarding the metal concentration was $Ni^{2+} > Pb^{2+} > Cu^{2+} > Zn^{2+} > Cd^{2+}$.

Under the optimum conditions (Table 1& 2) the highest chromium uptake was 94.5 mg/L (pH 7) and 92.9 mg/L (temp 37ºC) for *Bacillus* spp. The chromium uptake by *Staphylococcus* spp was 72.3 mg/L and 72.6 mg/L at pH 8.0 and temperature 37ºC**.** Hence both bacteria not only exhibited the ability to survive in contaminated wastewater but also demonstrated a marked increased in remediation of toxic Cr (VI) in their presence. Several researchers have also reported the direct reduction of Cr (VI) in contaminated effluents of the metal finishing industry (Ganguli and Tripathi, 2002).

One potential method is microbially catalyzed reduction of Cr (III), which was first reported with *Pseudomonas* spp. (Romanenko and Koren'Ken, 1977). Since then, significant progress has been made towards understanding the processes controlling enzymatic reduction of Cr (VI) in Gram- negative bacteria, especially those belonging to the genera *Pseudomonas*, *Desulfovibrios* and *Shewanella* (Ackerley *et al*., 2004). Several Gram-positive bacteria are also known to reduce Cr (VI) including several members of the genus *Bacillus* (Camargo *et al*., 2003). The walls of Gram positive bacteria are efficient metal chelators and in *Bacillus subtilis*, the carboxyl group of the glutamic acid of peptidoglycan was the major site of metal deposition. Teichoic and teichuronic acids were important binding sites in *Bacillus licheniformis* (Gadd, 1990). *Staphylococcus* spp is also a Gram positive bacterium and it has similar cell wall properties as of other Gram-positive bacteria.

The possible mechanism of hexavalent chromium reduction by bacteria, isolated from tannery wastewater has been evaluated. *Bacillus* spp and *Staphylococcus* spp showed excellent ability to reduce hexavalent chromium to non toxic trivalent chromium, i.e. 95% and 78%. Hence, both the isolates have been identified as potential microbes for its usefulness in removing chromium from the tannery effluent. The technology when upgraded will be a boon to tanners in tackling the pollution problem of tannery wastewater. The process would not only be an economical but also eco-friendly and sustainable.

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