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ORIGINAL RESEARCH ARTICLE

Bioremediation of Chromium [Cr (VI)] In Tannery Effluent Using *Bacillus* spp and *Staphylococcus* spp

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ABSTRACT

Chromium is the most toxic and common among the heavy metal pollutants of industrial effluents. In the present study, biosorption of Chromium by microorganisms from tannery effluent was 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. From the optimization study the effective pH 7 and temperature 35ºC was maintained in tannery effluent for *Bacillus* spp and *Staphylococcus* spp. The chromium content of the effluent was around 140 mg/L before remediation, after that the metal removal percentage was 86 and 74. The best activity was observed by *Bacillus* spp followed by *Staphylococcus* spp.

Key words: Bioremediation, Cr (VI) reduction, Chromate-resistant bacteria

INTRODUCTION

Environmental pollution is one of the major problems of the world and it is increasing day by day due to urbanization and industrialization. Over the last few decades large scale usage of chemicals in various human activities has grown very fast, particularly in a country like India which has to go for rapid industrialization in order to sustain over growing large problem of population [1]. The current pattern of industrial activity alters the natural flow of materials and introduces novel chemicals into the environment. The released organic compounds and heavy metals are one of the key factors that exert negative influences on man and environment causing toxicity to plants and other forms of biotics and abiotics that are continually exposed to potentially toxic heavy metals [2].

Of the various sources of pollutants industrial effluents containing heavy metals pose a threat to the ecosystem. These metals are present in the waste water of different industries such as metal cleaning, plating baths, refineries, mining, electroplating, paper and pulp, paint, textile and tanneries [3]. Water used in these industries creates a waste that has potential hazards for our environment because of the introduction of various contaminants such as heavy metals into soil and water resources [4]. Presence of pollutants in effluent is a common environmental hazard since the toxic metal ions dissolved can ultimately reach the top of the food chain and becomes a risk factor for human beings [5].

Tanneries are typically characterized as pollution intensive industrial complexes which generate widely varying, high-strength wastewaters. Variability of tannery wastewaters are not only from the fill and draw type operation associated with tanning processes, but also from the different procedures used for hide preparation, tanning and finishing. These procedures are dictated by the kind of raw hides employed and the required characteristics of the finished product. Tanning industry also has one of the highest toxic intensity per unit of output [6]. During tanning process, at least about 300 kg chemicals are added per ton of hides [7]. Major problems are due to wastewater containing heavy metals, toxic chemicals, chloride, lime with high dissolved and suspended salts and other pollutants. Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH and high concentrations of suspended solids, BOD, COD, tannins including chromium. The growth of industrialization has encroached even to small townships and villages along with all ills of pollution.

Biosorption of heavy metals from aqueous solutions is a relatively new technology for the treatment of industrial wastewater. The major

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advantages of biosorption technology are their effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials [8,9]. Hence the present study, exploited the properties of *Bacillus* spp and *Staphylococcus* spp in the remediation of chromium.

MATERIALS AND METHODS:

Sample collection:

Effluent sample was collected in the plastic containers from the outlet of a tannery industry in Vaniyambadi at Vellore District in Tamil Nadu. Its various physico-chemical characteristics were analyzed using standard methods [10]. The effluents were stored at 4°C during storage period to avoid changes in its characteristics.

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 [11]. The inoculated plates were incubated at 37ºC for 48 hrs. After the incubation period the plates were observed for the growth of microorganisms. Two isolates were selected for further studies. Microscopic and biochemical tests were applied to this isolates according to Bergey's manual of systematic bacteriology. The genus to which the isolates belong were determined.

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 of 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 [12]. 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 AND DISCUSSION

Some physicochemical characteristics of tannery effluent were ascertained, from where chromium tolerant bacteria were isolated. The colour of the effluent sample is dark brown, temperature is 39ºC and pH is 8.4. When subjected to AAS, total chromium was estimated to be about 140 mg/L.

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). From the optimization study, it was noted that at pH 5 *Bacillus* spp and *Staphylococcus* spp showed (168.9/157.3 mg/L) biosorption activity. Whereas at pH 6 the results noted was (182.0/160.4 mg/L), followed by pH 7 (194.5/172.2 mg/L) and at pH 8 (169.7/172.3 mg/L). Similarly at temperature 25ºC the biosorption level noted was (154.6/161.2 mg/L) at 30ºC (170.5/168.4 mg/L), 35ºC (192.4/172.6 mg/L) and 40ºC (176.1/169.7 mg/L). The results showed that pH 7 and temperature 35ºC were found to be optimum for both *Bacillus* spp and *Staphylococcus* spp for the biosorption study (Table 1&2). From the optimization study the effective pH 7 and temperature 35ºC was maintained in tannery effluent for *Bacillus* spp and *Staphylococcus* spp. The efficiency of the results noted was 86 and 74%.

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 [13,14]. 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 [15]. During the present investigation *Bacillus* spp and *Staphylococcus* spp both were found to be highly resistant to chromium. Under the optimum

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conditions (**Table 1& 2**) the highest uptake was 194.5 (pH 7) and 192.4 (temp 35ºC) for *Bacillus* spp. And 172.2 and 172.6 were seen at pH 7 and temperature 35ºC for *Staphylococcus* spp. 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 [16].

One potential method was microbially catalyzed reduction of Cr (III), which was first reported with *Pseudomonas* spp [17]. 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* [18]. Several Gram-positive bacteria are also known to reduce Cr (VI) including several members of the genus *Bacillus* [19]. 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* [20]. *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. 86% and 74%. 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.

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

	Cr(VI) adsorbed 100 mg/L	
pH	***Bacillus* spp**	***Staphylococcus* spp**
5.0	168.9	157.3
6.0	182.0	160.4
7.0	194.5	172.2
8.0	189.7	169.7

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

Temperature ºC	Cr(VI) adsorbed (100 mg/L)	
	Bacillus	***Staphylococcus* spp**
25	154.6	161.2
30	170.5	168.4
35	192.4	172.6
40	176.1	159.7

CONCLUSION

The present study establishes the role and efficiencies of *Bacillus* spp and *Staphylococcus* spp in the adsorption of chromium in tannery effluents. The technology when upgraded will be a boon to tanners in tackling the pollution problem of tannery waste water. The process would not only be economical but also eco-friendly and sustainable. However, further research is needed to establish the process with specific attention.

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