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Research Article

SELECTION AND USE OF EFFICIENT BACTERIAL STRAINS FOR CHROMIUM BIOSORPTION IN TANNERY EFFLUENT

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ABSTRACT

Tanning industry is one of the large-scaled industry in India which generals high turbid, colour and foul smelling wastewater together with toxic heavy metals including chromium and thus leading to environmental pollution. The present investigation emphasise the need of biological treatment of tannery effluent as an alternative a cost-efficient and eco-friendly approach for the efficient removal of chromium toxicity. In the present study a total of eight bacterial (4 gram positive and 4 gram negative) were isolated and the chromium resistance of the same was determined. Gram positive bacteria exhibited higher chromium tolerance than Gram negative bacteria whereas *Bacillus* and *Staphylococcus*, as Gram positive bacteria exhibited the higher chromium tolerance. The biosorption of Cr (VI) by these isolates revealed that the isolate *Bacillus* adsorbed the chromium ions to a higher level than Staphylococcus strains and the pH and temperature plays a vital role in biosorption of Cr (VI). It is concluded that the biological treatment of tannery wastewater together with chromium resistant bacterial strains will be a cost-effective, eco-friendly, alternative approach for the bioremediation of chromium in heavy metal.

INTRODUCTION

Tanning is the chemical process that converts animal hides and skin into leather and related products. The transformation of hides into leather is usually done by means of tanning agents and the process generates highly turbid, coloured and foul smelling wastewater (Bulian and Kral, 2011). The major components of the tannery effluent include sulphide, chromium, volatile organic compounds, large quantities of solid waste, suspended solids like animal hair and trimmings. For every kilogram of hides processed, 30 liters of effluent is generated and the total quantity of effluent discharged by Indian industries is about 50,000 m^3 /day (Jalandhar, 2008).

Chromium salts used during the tanning process generate two forms of chrome; Hexavalent chromium and trivalent chromium. Hexavalent chromium is highly toxic to living organisms even at low concentration causing carcinogenic effect (Frankfurt, 2002). Trivalent chromium may be present in the waste or can be produced from the Hexavalent chromium by chemical treatment. Soluble trivalent chromium causes toxicity in anaerobic digestion due to the accumulation of the metal in the intracellular fraction of biomass (Jalandhar, 2008). The environmental protection regulations stipulate that industries are not allowed to emit chromium in the wastewater. Effluents from raw hide processing tanneries, which produce wet blue, crust leather or finished leather, contain compounds of trivalent chromium (Cr) in most cases. Thus, removal of chromium from the wastewater is very important. The uncontrolled release of tannery effluents to natural water bodies increases health risks for human beings and environmental pollution. Tannery wastewater is difficult to treat because of complex characteristics like high BOD, COD, suspended solids, sulphide and chromium. Among the different treatments of tannery effluent, the biological treatment of tannery effluent is considered as a cost-effective and eco-friendly attractive alternative to the present physico-chemical methods of treatment (Reid *et al*., 1985).

Microorganisms possess a metabolic machinery of immense versatility aiding the breakdown of pollutants. In the course of evolution and rapid industrialization, microorganisms have been exposed to numerous chemicals and thereby adapted to their presence by developing necessary enzymes which aid in metabolizing such compounds. Biological reductions of chromium using indigenous microorganisms offer a cost-effective and environmentally compatible technology (Camargo *et al*., 2003) Megharag *et al.* (2003), worked on the

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reduction of chromium using *Arthrobacter* sp. and *Bacillus* sp. from tannery effluents in Australia. Most of the researchers who done their investigations using chromium aqueous solutions, have not shown significant or complete removal of the metal. The occurrence of metal resistant bacteria in anthropogenically polluted sites is also documented (Diels and Mergeay, 1990).

Biosorption is widely used for metal removal from industrial effluents and subsequent recovery (Vieira and Volesky, 2000). The microbial biomass has an inherent capacity to adsorb chromium due to the presence of functional groups such as -NH_2, -COOH, -SH, and -OH on microbial cell walls, which act as binding sites for interaction of metal ions (Kuyucak and Volesky, 1998). The major advantages of biosorption technology are their effectiveness in reducing the concentration of chromium metal ions to very low levels and the use of inexpensive biosorbent materials (Volesky, 1994).

Hence, the present study has been undertaken with an aim to screen the natural bacterial strains from the tannery effluent for chromium resistance and use the same for the development of a bioremediation technology.

MATERIALS AND METHODS

Collection of tannery effluent

Effluent sample was collected in the plastic containers from the outlet of a tannery industry situated in vaniyambadi of vellore District, Tamil Nadu. The physico-chemical characteristics of the same were analyzed using standard methods (APHA, 2005). The effluent was stored at 4 C in order to avoid changes in the characteristics, during storage.

Table 3 Physico-chemical characteristics of raw tannery effluent

S.no	Parameter	Initial values
1.	pH	9.5
2.	TDS mg/L	12,200
3.	Electrical Conductivity ms/cm^2	19,000
4.	Odour	Objectionable
5.	Colour	Brownish black
6.	Turbidity NTU	390
7.	Total Hardness mg/L	5400
8.	Chlorides	1462
9.	DO mg/L	18
10.	BOD mg/L	1260
11.	Chromium mg/L	140

Isolation and identification of Cr-resistant bacteria

Tannery effluent, samples were serially diluted upto 10^{-6} in sterile phosphate buffer (pH 7.2) and inoculated into Nutrient agar medium amended with 50 µg/ml of Cr (VI) by spread plate method. A filter-sterilized solution of $K_2Cr_2O_7$ was used as the source of Cr (VI), which was added to the sterile melted Nutrient agar medium to prevent problems associated with autoclaving chromate containing solutions (Babich *et al*., 1982). The inoculated plates were incubated at 37 C for 48 hrs. After the incubation period, the plates were observed for the growth of microorganisms. Morphologically different colonies were picked and purified by many round of restreaking. The isolates were subjected to different morphological, physiological and biochemical characterization as mentioned in Bergey's manual of determinative bacteriology, 8th edition for genus and species identification.

Growth of efficient bacterial strains on nutrient broth medium with different concentrations of chromium

Hundred ml of nutrient broth medium was prepared, autoclaved and amended with different concentrations (50, 100, 150, 200 and 250 mg/L) of filter streilised $K_2Cr_2\ O_7$ solution and dispensed in 250 ml conical flasks, separately. One ml (1 x 10^7 CFU/mL) of each bacterial isolates was added into the medium, separately and incubated at 35 C for 24 hr under shaking condition (150 rpm). Bacterial growth was monitored by reading the absorbance in spectrophotometer at 660 nm.

Growth of microorganisms and Biosorption

Bacillus sp. and *staphylococcus* sp. was inoculated separately to sterilized nutrient broth, incubated at 37 C for 24 hrs in under shaking condition (150 rpm). At the end of incubation, the biomass was separated individually from medium by centrifugation (5000 rpm x g) for five minutes and it was kept in the oven at 50 C to remove the free water as much as possible. Then, the cells were suspended in deionized water separately in order to use it in the biosorption experiment. 100 ml solutions containing 100 mg/L Cr^{6+} were prepared from stock solution containing 1g/L Cr^{6+} ($K_2Cr_2O_7$). Then 2.0 g microorganism was added to the solution and adsorption of metal was investigated at different pH values adjusted by using 1N HCl and 1N NaOH at 37 C. The solution containing the biomass was agitated in a shaker of 150 rpm during the adsorption.

Table 2 Morphological and biochemical characteristics of chromium resistant bacteria

Isolate No	Colony Character	Colony morphology	Cell Shape	Gram Staining	Spore staining	Motility	Starch Hydrolysis	Gelatin liquefication	Casein hydrolysis	Catalse test	Oxidase test	Indole test	Methyl Red test	Citrate utilization test	H2S Production	Nitrate reduction	Urease	Tentative identificatioon
VB-1	Cream colour	Irregular	Rod	+	+	+	+	+	–	+	+	+	+	+	–	–	–	*Bacilus megatherium*
VB-2	White	Flat	Rod	+	+	+	+	+	–	+	+	–	+	–	–	+	–	*Bacillus cereus*
VB-3	Slimy cream colour	Raised	Rod	+	+	–	+	+	+	–	–	–	–	+	–	–	–	*Lactobacillus sp.*
VB-4	White	Flat	Coccal	+	–	+	+	–	–	–	–	–	+	–	+	+	–	*Staphylococcus sp.*
VB-5	Slimy	Flat	Rod	–	–	+	–	+	+	+	+	–	–	+	–	–	–	*Pseudomonas putida*
VB-6	Slimy	Flat	Rod	–	–	+	–	+	+	+	+	–	–	+	–	+	–	*Pseudomonas erginosa*
VB-7	White	Flat	Rod	–	–	–	–	+	–	+	–	+	–	–	+	+	–	*Flavobacterium sp.*
VB-8	Light cream	Irregular	Rod	–	–	+	–	–	–	+	+	–	–	+	–	–	–	*Alcaligenes sp.*

Samples taken at predetermined intervals were centrifuged and supernatants were analyzed. The analysis of Cr^{6+} ion was carried out by using Atomic Adsorption Spectrophotometer (Perkin-Elmer) at 0.01 ppm sensitivity level after dilution of the samples (Sultan and Hasnain, 2007). By taking the determined optimum conditions into consideration, the capacity of microorganism to remove the mentioned metal from the tannery effluent was determined with the same method.

Table 3 Growth of bacterial strains on Chromium amended solid medium at different concentrations

ISOLATES	$K_2Cr_2O_7$ (mg/L)				
	50	100	150	200	250
VB 1	+	+	+	-	-
VB 2	+	+	+	+	+
VB 3	+	+	-	-	-
VB 4	+	+	+	+	+
VB 5	+	+	-	-	-
VB 6	+	+	-	-	-
VB 7	+	-	-	-	-
VB 8	+	-	-	-	-

RESULT AND DISCUSSION

The tannery effluent sample was collected in the plastic containers from the outlet of a tannery industry situated in vaniyambadi of vellore district, Tamilnadu. The effluent released from the main outlet of tannery is brownish black in colour with an offensive odour whereas the temperature is about 38ºC. The physico-chemical analysis of the tannery effluent revealed that the pH of the effluent sample was found to be 9.5 whereas the chromium level in raw effluent was found be 140 mg/L which are above the BIS limit, (2009) (Table 3). Fadali *et al*., (2005) reported that the pH of the tannery effluent was found to be 10 which was above the permissible limit and suggested that this alkaline pH of the effluent could affect the biological property of the receiving water bodies. Chromium level in the raw effluent was found to be 140 mg/L which was higher than the amount prescribed by BIS (0.5 mg/L). The extremely high concentration of chromium in the raw tannery effluent has already been reported by Krantz and Kifferstein (2002) and Tripathi *et al.* (2011).

Isoltion and selection of Chromium tolerant bacterial strains

In the present study 8 indigenous bacterial strains were isolated from tannery effluent and designated as "VB" series and numbered randomly (VB-1 to VB-8). The genus and species characterization of the isolates were done according to Bergey's manual of determinative bacteriology 8th edition and they were identified as *Bacillus megaterium, Bacillus cereus, Lactobacillus sp., staphylococcus sp., Pseudomonas putida., Pseudomoans aeruginosa, Flavobacterium sp.* and *Alcaligenes sp,* respectively. Among the 8 bacterial strains, the strain *Bacillus cereus* and the strain *Staphylococcus sp*. were found to tolerate the chromium level upto 250 mg/L amended in nutrient agar medium. Interestingly, it was observed that the gram positive bacterial strains were able to tolerate higher concentration of chromium level in tannery effluent when compared to the gram negative bacterial strains. The occurrence and activities of chromium tolerant bacterial strains from tannery effluent has already been reported by Basu *et al*. (1997), Srinath *et al*. (2002), Megharaj *et al*. (2003), Zhiguo Hea *et al*. (2009), Soha and Sahar (2010).

Temperature and pH play a vital role in the biosorption efficiency of Cr (VI) ions by the effluent bacterial isolates. From the optimization study, it was observed that the biosorption of Cr (VI) by the bacterial isolates varied with different levels of pH and temperature, tested. The pH at 7.0 level and temperature at 35º C recorded the highest biosorption of Cr (VI) by the two bacterial isolates, namely, *Bacillus cereus* and *Staphylococcus* sp.

In general, potential microorganism's especially bacterial species could remove heavy metals, including, chromium 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 (Sultan and Hasnain, 2007; Pattanapipitpaisal *et al.*, 2002). 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 the sites which exhibit chemical affinity for the metal. It is a passive accumulation process which may include adsorption, ion-exchange, complexation, chelation, and microprecipitation.

Chromium resistant bacteria have been isolated from tannery effluents by several groups (Rehman *et al*., 2008). In the present investigation both *Bacillus* sp. and *Staphylococcus* sp. were found to be highly resistant to chromium ions. Under the optimum conditions (Table 4 & 5) the highest biosorption efficiency was 97 percent (pH 7) and 98 percent (Temp 35º C) for *Bacillus* sp. and 92 percent and 93 percent were seen at pH 7 and temperature 35º C, respectively for *Staphylococcus* sp. It was observed that both bacteria not only exhibited the ability to survive in contaminated wastewater but also demonstrated a marked increased in remediation of toxic Cr (VI)

Table 4 Effect of pH on biosorption of Cr (VI) by Bacillus spp and Staphylococcus sp

pH	Cr (VI)* adsorption efficiency (%)	
	Bacillus	*Staphylococcus*
5.0	88	83
6.0	92	87
7.0	97	92
8.0	95	88

*Initial concentration of chromium (VI) 150 mg/L

Table5 Effect of temperature on biosorption of Cr (VI) by *Bacillus* spp and *Staphylococcus* spp

Temperature ºC	Cr (VI)* adsorption efficiency (%)	
	Bacillus	*Staphylococcus*
25	92	82
30	94	85
35	98	93
40	95	87

*Initial concentration of chromium (VI) 150 mg/L

Microbially catalyzed reduction of Cr (III), was first reported with *Pseudomonas* sp. (Romanenko and Korenken, 1997). Since then, significant progress has been made towards the understanding of the processes that controlling the enzymatic reduction of Cr (VI) in gram-negative bacteria, especially those belonging to the genera *Pseudomonas, Desulfovibrio* and *Shewanella* (Ackerley *et al*., 2004). Several gram-positive bacteria have also been known to reduce Cr (VI) including several members of the genus *Bacillus* (Camargo *et al*., 2003). The cell wall of gram positive bacteria is highly efficient metal chelator 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 sp.* is also a Gram positive bacterium and has similar cell wall properties as of other Gram-positive bacteria.

CONCLUSION

The results of the present study clearly revealed the positive role of gram positive bacteria, namely, *Bacillus* sp. and *Staphylococcus* sp. on the biosorption of chromium in tannery effluent. This biological treatment technology facilitates the tanners in tackling the pollution problem of tannery wastewater. Moreover, the bioprocesses are found to be more cost effective, eco-friendly and sustainable. However, further research is needed at molecular and physiological levels of the microorganisms regarding the chromium biosorption.

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