

ISOLATION AND CHARACTERIZATION OF CHROMIUM REMOVING BACTERIA FROM TANNERY EFFLUENT DISPOSAL SITE

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ABSTRACT

Heavy metals found in wastewaters are harmful to the environment and their effects on biological system are very severe. An efficient and economic treatment for their removal and reuse needs to be developed. Microbial metal bioremediation is an efficient strategy due to its low cost, high efficiency and ecofriendly nature. Recently advances have been made in understanding metal-microbe interaction and their application for metal detoxification. Microorganisms in soils are sensitive to the high concentrations of heavy metals like zinc, manganese, cobalt, copper, chromium, cadmium, mercury and silver. The present study was focussed with an objective to remediate the tannery effluent contaminated soil by microorganisms. The leather tanning effluent contaminated soil was collected and analyzed. It was found to have higher pH and large amount of total suspended and total dissolved solids, minerals and metals like sodium, potassium, chromium, zinc and copper. Biochemical tests were performed for the microorganisms isolated from the tannery effluent. As per the present study the isolated *Bacillus* sp was found to reduce 85.9% of chromium from the medium after 96 h. The results also showed that the isolated *Bacillus* sp has the capacity to remove other heavy metals (Ni, Cr, Cu, Zn and Cd) in the tannery effluent. The metal removing capacity increased with increase in concentration of the metals.

Keywords: Tannery effluent, effluent recycling, *Bacillus* sp, heavy metal removal

[I]INTRODUCTION

Heavy metal pollution of water is a major environmental problem facing the modern world. The global heavy metal concentration

in various environments is increasing in the environment due to increase in number of industries. Most of the industrial wastewaters contain heavy metals like cadmium, lead,

zinc, cobalt and chromium. Among heavy metals chromium is the major pollutant of the leather tanning industry and is toxic to plants and animals around the environment [9].

The damage to the environment by the hazardous tannery effluent is becoming an acute problem in several countries. The chrome tanning process results in toxic metals, especially chromium III passing to waste water and are not easily eliminated by ordinary treatment process [13]. Tannery waste waters are mainly characterized by high salinity, high organic loading and specific pollutants such as chromium [10]. The industrial effluent released directly or indirectly into natural water resources, mostly without proper treatment, poses a major threat to the environment.

Among the different forms of chromium, the hexavalent chromium Cr^{6+} is the most toxic and carcinogenic due to its high solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids. The heavy metals in general cannot be biologically transformed to more or less toxic products and hence persist in the environment indefinitely [35]. They are significantly toxic even in small amounts and can cause diseases in humans and animals as they cause irreversible changes in the body, especially in the Central Nervous System [26]. Soil contamination by heavy metals is often irreversible and may repress or even kill parts of the microbial community and it is generally assumed that the exposure to metals leads to the establishment of a tolerant/resistant microbial population [32].

Microorganisms play a significant and vital role in bioremediation of heavy metal contaminated soil and wastewater. Indigenous soil microbes appear well suited for Chromium (VI) transformation in highly

contaminated soil and may accumulate chromium within its cells by adaptation to the high concentration of the metal. Very stable final chromium forms can be achieved as a result of microbial activity, with minimal risk of re-release of Cr (VI) [22]. In this paper an effort has been made to remove heavy metals from tannery effluent using microorganisms.

[II] MATERIALS AND METHODS

2.1 Tannery Effluent

The effluent was collected from a selected leather processing industry situated at Dindigul in Tamil Nadu, India at weekly intervals for five weeks, pooled together and stored at 4 °C for analysis. The collected tannery effluent was analyzed for physicochemical properties like colour, odour, turbidity, pH, total suspended solids, total dissolved salts, chemical oxygen demand (COD), biological oxygen demand (BOD) [1], carbonate, bicarbonate, sodium, potassium [20], chromium, copper, cadmium, nickel and zinc [1].

2.2 Isolation of the microorganism from tannery effluent contaminated soil

For isolation of chromium resistant bacteria, 1.0 g effluent treated soil sample was dispersed in 100 mL of the sterile distilled water. A serial dilution was made up to 10^{-5} from the effluent treated soil sample. 100 μL of each dilution was spread on to nutrient plates containing 10 μg potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) per mL of the medium. The growth of the bacterial colonies was observed after 24 h of incubation at 37 °C. It was subcultured on nutrient agar plate containing 10 μg of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) per mL. Biochemical tests like Gram staining [27], dextrose fermentation, sucrose fermentation, IMViC, catalase activity, starch hydrolysis [11] and gelatin

liquefaction [15] were carried out to identify the isolated microorganisms.

2.3 Optimum growth conditions

For the determination of optimum temperature for the growth of the isolated bacteria, test tubes containing 5 mL nutrient broth were taken in four sets, autoclaved and inoculated with 20 μ L of freshly prepared culture of the isolate. The four sets of tubes were incubated each at 25, 30, 37, 45 $^{\circ}$ C respectively. After an incubation period of 12 hours, the absorbance was measured at 600 nm. For the determination of optimum pH, test tubes having 5 mL nutrient broth were taken in 9 test tubes and then, the pH was adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0. The test tubes were inoculated with 20 μ L of freshly prepared culture of the isolate. After 12 hours of incubation, the absorbance was measured at 600 nm.

2.4 Growth rate of bacterial isolates with and without chromium

Growth curves of bacterial isolates were determined with 10 μ g $K_2Cr_2O_7$ per mL and without $K_2Cr_2O_7$ (control). For bacterial isolates, 50 mL nutrient broth was taken in one set consisting of three flasks, autoclaved and then inoculated with 20 μ L of the freshly prepared inoculum. These cultures were incubated at 37 $^{\circ}$ C in a shaker at 30 rpm. The absorbance of the cultures was measured at 600 nm at intervals of 2 h up to 24 h after inoculation.

2.5 Removal of chromium by bacterial isolates

In order to determine the ability of bacterial isolate to reduce Cr^{6+} to Cr^{3+} , spectrophotometric method was used. 5 mL samples were taken from the culture after 24 h, spun down at 10,000 rpm for 5 min and the supernatant was used for the estimation of chromium left in the medium. From this 1.0 mL sample was taken and diluted with 9

mL of distilled water in the test tubes. The mixture was kept at room temperature for 10 min and OD was taken at 600 nm.

2.6 Removal of heavy metals by isolated bacterium

20 mL of the sterile medium containing nutrient agar 15 g, K_2HPO_4 0.5 g, K_2PO_4 1.5 g, glucose 0.05 g and distilled water to 1000 mL was poured into sterile petriplates and was allowed to solidify. Then each isolated microorganism was swabbed on the medium. Four wells were made on each petriplate using well puncture. Different concentrations (0.5, 1.0, 1.5 and 2 mg/mL) of metals (Cu, Cd, Cr, Ni, and Zn) were made and loaded on the wells. Then the plates were incubated for 24 h at 37 $^{\circ}$ C

[3] RESULTS

3.1 Tannery Effluent

The collected leather tanning industrial effluent was assessed for its physicochemical properties and toxic metal levels (Table 1). The effluent sample analyzed had a pH value of 10.5. The total suspended solids (2300 mg/L) and total dissolved salts (12,900 mg/L) in the effluent sample were found to be very high when compared to BIS standards. COD and BOD in the selected effluent sample were found to be 3180 mg/L and 1300 mg/L respectively.

In the effluent sample analyzed, the presence of carbonates and bicarbonates were found to be very high, 7250 mg/L and 10,238 mg/L respectively. Chromium is the widely used heavy metal in tannery industries and was found to be 179 mg/L in the effluent. Other metals like cadmium, chromium, copper, nickel and zinc were present at levels of 4.81, 179, 64.32, 132, and 171 mg/L respectively (Table 2).

3.2 Isolation of microorganisms from the effluent contaminated soil

The chromium resistant bacteria were isolated from the effluent treated soil by serial dilution method, each isolated colonies were picked up and were sub-cultured. The biochemical characterizations were carried out to identify the microorganisms. Cr (VI) resistant bacteria were isolated on the basis of growth in Cr (VI) enriched medium [7]. All the soil samples yielded a large number of colony-forming units/mL (cfu/mL). The serial dilution method indicated the different colonies in dilution of 10^{-5} were found to be Gram positive bacteria and gave positive result for catalase, starch hydrolysis and gelatine liquefaction tests and negative result for IMViC test indicating it to be *Bacillus* sp.

3.3 Optimum growth characteristics of chromium resistant bacteria

In the present study, the optimum temperature for chromium resistant bacteria *Bacillus* sp, was found to be 37°C (Figure 1). The chromium resistant bacteria, *Bacillus* sp, showed maximum growth at pH 7 (Figure 2). The growth curve of isolated microorganism in the presence of chromium ($K_2Cr_2O_7$) 10 µg/mL was compared with the control (i.e no metal ions were added). There was no significant difference between control and chromium treated culture (Figure 3). But the growth of the isolate was decreased in after 18 hours in presence of chromium.

3.4 Tannery effluent contaminated soil

Both the control and effluent contaminated soil samples were analysed for essential parameters (Table 2) and it is evident that the effluent contaminated soil has a greater amount of mineral and metal content than the control soil. Chromium reducing capability of bacterial isolate was analysed by adding Potassium dichromate ($K_2Cr_2O_7$) at 10 µg/mL in the culture medium. The isolated *Bacillus*

sp was found to reduce 85.9% of chromium from the medium within 96 h. It was also capable to reduce chromium (10 µg/mL) by 35.8%, 68.9% and 74.9% from the medium after 24, 48 and 72 hours respectively (Figure 3). The heavy metal degradation capacity of *Bacillus* sp was observed by well diffusion method (metal content ranging from 0.5, 1.0, 2.0 mg/mL) respectively. The degradation capacity of *Bacillus* sp was found to be maximum at 2.0 mg/mL for chromium, copper, nickel, zinc and cadmium. The zone of degradation was found to be highest in nickel. The heavy metal degradation capacity of *Bacillus* sp showed maximum resistance against nickel, and the reduction for other heavy metals was in the order of Ni > Cr > Cu > Zn > Cd.

[IV] DISCUSSION

4.1 Physiochemical properties of Effluent

The effluent released from tannery industry was turbid, brown in colour and had an offensive odour. The colour of the effluent might be due to the presence of biodegradable and non-biodegradable high molecular weight organic compounds and high amount of inorganic chemicals like sodium and chromium used during the processing and the odour may be due to putrefaction of the organic residues from the processed skin and hides. The yellowish brown colour might be hindering the penetration of sunlight causing depletion in the rate of oxidation process [37;21]. The turbidity of the effluent might be due to the discharge of high concentrations of carbonates, bicarbonates and chlorides of calcium, magnesium and sodium [8].

The normal pH range of water should be between 6.0 and 8.0 [4]. The effluent had a high pH when compared to normal water indicating the alkaline nature of the effluent

due to the presence of high concentrations of salts of sodium, potassium, chromium etc. [33].

The presence of higher level of total suspended solids and total dissolved salts in the effluent might be due to the presence of insoluble organic matter from the animal skin and unused inorganic salts used for tanning [19]. The record of high COD might be due to the presence of oxidizable organic matter from the animal skin [29;21].

From the study it was clear that high carbonate and bicarbonate content contributes to the total alkalinity of the sample [3]. The level of sodium and potassium in the tannery effluent was found to be 2300 mg/L and 600 mg/L respectively. Chromium and sulfide are among the most hazardous components of the tanneries effluent. The use of excessive amount of these chemicals in tanning process gives rise to their high concentrations in the effluent. Chromium VI is known to cause cancer. The recommended limit for maximum amount of chromium in the effluent samples is 1.0 mg/L [6].

4.2 Effluent Degrading Microorganisms

Thirty four bacterial strains were isolated, identified and characterized, which belonged to different bacterial genera, dominant being *Bacillus*, *Micrococcus* and *Lactobacillus* spp [30;12]. The most suitable temperature for Cr resistant bacterial isolate was found to be 37 °C. Bacterial Cr (VI) reduction was found to be maximum at 25 to 30 °C for five bacterial isolates and higher temperature (above 37 °C) severely retarded Cr (VI) bioreduction [5]. In a similar study, the optimum pH for the growth of Cr resistant bacteria was reported as pH 7 to 7.8. But Cr forms are soluble over a wide range of pH and generally mobile in soil-water systems [34]. The optimum initial pH was 7.0 to 9.0 which

corresponded to a final pH of 6.8 to 6.2. Decrease in culture pH as a result of bacterial growth was generally noted. The growth curve pattern of the isolate was not significantly different from that of the control initially, but, the growth of the isolate decreased in presence of Cr⁶⁺ [23].

Several Gram positive bacteria were also known to reduce Cr (VI) including several members of the genera *Bacillus* sp [7]. Chromium resistant bacteria capable of reducing chromate have been reported from chromium polluted environment and they were showing resistance in lower concentration [18;2]. *B. subtilis* reduced chromium (VI) under aerobic conditions. This might be due to the presence of chromium reductase enzyme. Similar chromium (VI) reduction has been reported by *B. coagulans*. Accumulation of chromium Cr (VI) by *B. circulans* has also been demonstrated [26].

Pseudomonas strains isolated from metal contaminated soil harboured resistance to chromium (III) [17]. Reduction of chromium in industrial effluent was observed in *Pseudomonas aeruginosa* strain isolated from tannery effluent [14]. Bacterial Cr (VI) reduction can occur under both aerobic or anaerobic conditions in presence of different electron acceptors such as oxygen, nitrate, sulphate and ferric iron, but the suitable condition for Cr (VI) bioremediation are aerobic at higher Cr (VI) concentrations and anaerobic at lower Cr (VI) concentrations [28]. It was concluded that bacterial colonies of *Bacillus* strain can grow successfully in chromium containing medium as it can tolerate dichromate [24]. It was concluded that *Pseudomonas* has the ability to degrade heavy metals present in industrial effluent [31]. It was reported that *Aspergillus niger*

has the potency to remove chromium from industrial effluent [25].

The average levels of sodium, potassium, chromium, zinc, cadmium, nickel and copper were found to be more in the effluent contaminated soil collected from effluent receiving sites. It was reported that the levels of exchangeable cations (sodium and potassium) in the soil irrigated with tannery waste water were found to be high [16]. Microorganisms adapt to the heavy metal polluted soil by changing their intrinsic biochemical and structural properties and physiology of the organism. Therefore, the survival of microbes in polluted soil is prone to show higher resistance to heavy metals as compared to population in non-contaminated sites [36].

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Tables and Figures:**Table 1:** Physicochemical characteristics of the tannery effluent

PARAMETERS	EFFLUENT[#]	BIS LIMITS[*]
Colour	Light brown	Absent
Odour	Offensive	Absent
Turbidity	Turbid	-
pH	10.5	6.0-9.0
Total suspended solids (mg/g)	2,300	100
Total dissolved solids (mg/g)	12,900	2,100
COD (mg/g)	218	250
BOD (mg/g)	20	30
Carbonate (mg/g)	7,250	NM
Bicarbonate (mg/g)	10,238	NM
Sodium (mg/g)	50	NM
Potassium (mg/g)	202	NM

* - Tolerance limits for textile effluent discharged into inland water source as per Bureau of Indian Standards (BIS), (2009).

#-Mean value of duplicate samples NM- Not mentioned.

Table 2: Analysis of control and effluent contaminated soil

PARAMETERS	CONTROL SOIL SAMPLE	EFFLUENT CONTAMINATED SOIL SAMPLE
pH	8.04	8.50
Sodium (mg/kg)	46	121
Potassium (mg/kg)	513	650
Chromium (mg/kg)	21	90
Zinc (mg/kg)	47	82
Cadmium (mg/kg)	3.2	6.8
Nickel (mg/kg)	90	113
Copper (mg/kg)	20	64

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Figure 1: Optimum temperature for chromium resistant bacteria

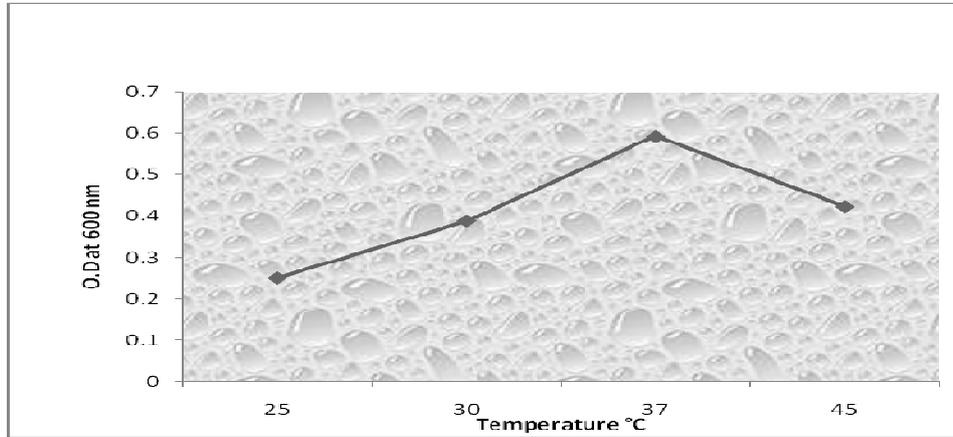


Figure 2: Optimum pH for chromium resistant bacteria

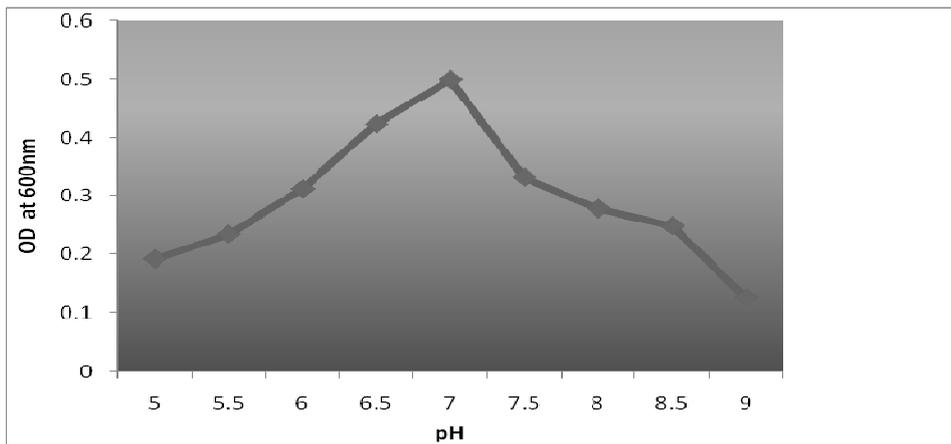


Figure 3: Growth curve of bacterial isolates were determined with (10 µg/mL) and without chromium

