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Chromium Removal by Using Chosen Pseudomonads

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Abstract Tannery effluent is a major source of aquatic pollution in India. Hexavalent chromium compounds are being used in a wide variety of commercial processes and unregulated disposal of the chromium containing effluent has led to the contamination of soil, sediment, surface and ground waters. In trace amounts, chromium is considered an essential nutrient for numerous organisms, but at higher level, it is toxic and mutagenic. Therefore in the present study three strains of *Pseudomonas* such as *Pseudomonas aeruginosa*, *P. fluorescens* strain 1 and 2 were isolated from the collected tannery effluent samples. All the three strains showed more than 60 percentage of reduction for all chromium concentrations (500, 1000, 1500 and 2000ppm) tested.

Keywords: *chromium, tannery, effluent, pseudomonas*

1. Introduction

Nearly 80% of the tanneries in India are engaged in the chrome tanning processes. Heavy metals, the major constituents of industrial effluents are not usually eliminated from the aquatic systems by natural processes in contrast to most organic substances. Heavy metals, such as mercury, lead, chromium, nickel, copper, cadmium and zinc, are of considerable concern because they are non-biodegradable, highly toxic and probably carcinogen. They tend to accumulate in bottom sediments from which they may be released by various processes of remobilization and in changing form can move up the food chain and cause various chronic and acute ailments [1].

Wastewater of industries like coil coating, ferroalloys, inorganic chemicals, iron and steel, leather tanning and finishing (including electroplating), petroleum refining, porcelain enameling, textile manufacturing and timber products contain chromium [2]. The toxicology of chromium compounds has been reviewed by USNAS, IARC and Taylor [3,4,5]. Chromium (Cr) reacts with nucleic acids and other cell components to produce mutagenic and carcinogenic effects on biological systems [6].

Conventional methods of Cr removal include chemical reduction followed by ion exchange, precipitation and adsorption on activated coal, alum, kaolinite and ash. But they require large quantities of chemical reagents and high amount of energy [7]. Several treatment processes have been suggested for the removal of heavy metals from aqueous waste streams: adsorption, biosorption, ion exchange, chemical precipitation and electrochemical methods: electrowinning, electrodialysis, electrodeionization, membrane-less electrostatic shielding, electrodialysis and electrocoagulation which are highly expensive. But microbial methods are cost effective and efficient[8]. In the present work, an attempt has been made to employ chosen bacteria *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* in the bioremediation of tannery effluents.

2. Materials and Methods

The tannery effluent samples were collected from the leather industries at Tiruchirapalli and Dindigul in sterile containers. They were brought to the laboratory immediately and analysed for the concentration of chromium using Atomic Absorption Spectrophotometer (AAS). The effluent samples were serially diluted with sterile distilled water and plated on to nutrient agar (Himedia, Bombay) medium. The plates were incubated at 37 ℃ for 24 hours. Among the bacterial colonies grown, three dominant colonies were selected and cultivated in nutrient broth for eight hours. This three isolated organisms were subjected to biochemical tests for tentative identification following Bergy's manual of Systematic Bacteriology [9].

After testing the resistance of the chosen bacterial strains to different concentrations of chromium prepared by dissolving required amount of potassium chromate ($K_2Cr_2O_7$), 500, 1000, 1500 and 2000 ppm concentrations were selected for the experiment. Minimal broth (Dipotassium hydrogen phosphate 7g; Potassium dihydrogen phosphate 2g; Ammonium sulphate 1g; Glucose 1g; Sodium citrate 0.5g; Magnesium sulphate 0.1g made upto 1litre.) was prepared and autoclaved at 115℃ for 15 minutes and was cooled in a water bath at 45-50℃. In 250ml Erlenmeyer flasks, 100ml minimal broth was taken along with the above mentioned concentration of chromium. Under aseptic conditions, the three chosen organisms were inoculated individually into these flasks with 10^9 cells (0.1ml). The flasks were incubated at room temperature. Uninoculated control flasks were also maintained in the same manner. After

forty days, samples were taken from each flask and centrifuged at 10,000rpm for ten minutes. The supernatants were analysed with AAS for chromium concentration adopting standard methods [10]. Percentage reduction in chromium concentration was calculated for each chromium concentration based on the initial and final readings.

3. Results and Discussion

Table 1 shows the concentration of chromium present in the samples of tannery effluent collected from Tiruchirapally and Dindigul. Among them, the samples collected from Dindigul exhibited higher levels than that of Tiruchirappalli. Table 2 divulges the culture characteristics of the isolated strains. Based on Gram's staining, motility and other biochemical tests, the three strains were tentatively identified as *Pseudomonas aeruginosa*, *P. fluorescens* strain 1 and 2.

Table 1. Concentration of Chromium in the Tannery Effluent Samples Collected from Trichirapalli and Dindigul

Sl.No	Samples	Cr concentration(ppm)
1	T1	1120
2	T2	1200
3	D1	1520
4	D2	1532

T=Trichirapalli; D=Dindigul

Table 2. Culture Characteristics of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* isolated from Tannery Effluent

Name of the Test	Test Organisms		
	Pseudomonas aeruginosa	*Pseudomonas fluorescens*-strain 1	*Pseudomonas fluorescens*-strain 2
Morphology	G +	G +	G +
Motility	+	+	+
Indole Production	+	-	-
Methyl Red	-	+	+
Voges Proskaeur	+	+	+
Citrate Utilization	+	-	-
H_2S Production	-	+	+
Glucose Fermentation	+	-	-
Lactose Fermentation	-	-	-
Sucrose Fermentation	-	-	-
Coagulase	-	-	-
Catalase	+	+	+
Oxidase	+	+	+
Urease	+	-	-
Esculin Hydrolysis	-	-	-
Nitrate Reduction	+	-	-

G + Gram Positive; + Positive; - Negative

All the three microorganisms tested for chromium removal were capable of removing chromium (Table 3). Among them, maximum removal was exhibited by *P. aeruginosa* for 500 ppm of initial concentration. For 2000 ppm of initial chromium concentration, maximum removal of chromium was noticed for *P. aeruginosa* followed by *P. fluorescens* strain 1 and 2. All the three strains showed more than 60 percentage of reduction for all chromium concentrations tested.

Table 3. Reduction in Chromium Concentration After40 days of Treatment

Bacterial strains	Initial concentration (ppm)	Final concentration (ppm)	Percentage of reduction
Pseudomonas aeruginosa	500	44.06	91.20
	1000	205.20	80.70
	1500	489.60	77.40
	2000	733.60	72.30
Pseudomonas fluorescens strain1	500	62.49	87.20
	1000	243.80	75.62
	1500	463.80	67.36
	2000	733.60	63.32
Pseudomonas fluorescens strain2	500	64.00	87.20
	1000	241.00	75.90
	1500	456.80	69.50
	2000	730.80	62.90

Heavy metals released into the environment as a consequence of rapid industrialization pose a serious environmental threat as they derail the normal physiology by forming metabolically unspecific complexes. Their continuous presence in nature has in turn resulted in the development of metal resistant strains. These strains counteract the heavy metal toxicity by complexation or precipitation or accumulation of heavy metals inside the cell. As chemical methods are costly, bioremediation is a cheaper way to remove chromium from effluents. It is a process of trapping of metal ions outside or inside the cell. Different types of microbes like bacteria, algae, fungi and yeast are involved in bioremediation. They differ in their intrinsic capabilities and mechanisms of metal removed and interact with heavy metals by precipitation, intracellular accumulation, metal transformation and extracellular metal complexation [11].

In the present work both the isolates of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were able to cause the removal of chromium. Among them *Pseudomonas aeruginosa* is better and in all the three strains increase in Cr concentration hindered the process of Cr removal. But even at 2000ppm Cr concentration all of them exhibited more than 60% reduction in the level of chromium in solution. Even at 2000ppm they were able to remove chromium, as they were isolated from tannery effluent contaminated sites having Cr concentration as high as 2000ppm and they would have adapted to such high concentrations.

Cr^{6+} concentration affects the growth of bacteria. *Bacillus* spp. was able to grow even above 1000ppm of $Cr6^{+}$ but its growth decreased to 50% at 1500ppm [12]. At 10 and 12ppm Cr^{6+} exerts toxic and mutagenic effects on microbes and inhibits growth of soil bacteria in liquid media. Such toxic effects are due to the alteration of genetic material and altered metabolic and physiological reaction [13].

Chromate is a strong oxidizing agent and gets reduced intracellularly to Cr^{5+} which can react with nucleic acids and other cell components to cause mutagenic and carcinogenic effects [6]. Though some microbes like *Pseudomonas fluorescens* [14], *Enterobacter cloacae* and *Bacillus* spp.,[15] posses the capacity to reduce Cr^{6+} to Cr^{3+}, the potential for bioremediation of chromium wastes is limited because of some microbes losing their viability in high concentration of chromate [16].

Microbes with the ability to tolerate and reduce hexavalent chromium can be used for detoxification of environments contaminated with Cr^{6+}. Reduction of hexavalent chromium into trivalent chromium is a

potentially useful process for remediation of chromium-contaminated environments [17]. Bioreduction of hexavalent chromium can occur directly as a result of microbial metabolism through enzymes or indirectly, by a bacterial metabolite like H_2S [13]. In bacteria, chromium reduction is dependent on pH, temperature and Cr concentration. The optimum pH is found to be 7 to 9. As hexavalent chromium reduction is enzyme-mediated, changes in pH will affect the degree of ionization of the enzyme, changing the protein's conformation and affecting the enzyme activity [18].

In many instances chromate resistance is due to the presence of efflux mechanisms, which allow resistant strains to extrude chromate ions. Hence some of the microbes like *Pseudomonas aeruginosa, Pseudomonas fluorescens, Alcaligenes eutrophus* and *Enterobacter cloacae* exhibited low uptake [16]. It was suggested that bacterial chromate reduction and resistance are independent processes. Two enzymatic mechanisms were identified in microbe-mediated Cr (VI) reduction [19].

Reduced chromium is complexed with soluble organic compounds like electronegative exopolymers liberated from capsules [20]. In bacteria like *Pseudomonas fluorescens* LB300, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Alcaligenes eutrophus*, Cr resistance was offered by a plasmid via decreased uptake of the metal [21,22]. In *Pseudomonas aeruginosa* and *Alcaligenes eutrophus*, Chr A, a membrane protein confers tolerance by extrusion of chromate ions [23] while in *P. ambigua* G1, a capsule inhibits the entry of chromate ions [24].

In the chromate reducing *Pseudomonad*, cell envelope and capsule exopolymer chromium complexation inhibited the metal from entering the cytoplasm. When Cr (VI) is reduced to Cr (III), it is free to bind to the electronegative charged surface functional groups on the cell surface, which serve as nucleation sites for further precipitation so that chromate is removed from solution [25]. The isolates tested in this work show promise for chromium removal and they can be employed in the bioremediation of chromium-contaminated sites.

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