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Bioremediation of Chromium (Vi) from Textile Industry's Effluent and Contaminated Soil Using *Pseudomonas putida*

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Abstract: Nine bacterial colonies were screened for the Cr(VI) removal efficiency and out of these three bacterial strains *Pseudomonas putida*, *Pseudomonas aeruginosa* and *Bacillus sp*. were isolated from soil and used to remove Cr(VI) from aqueous solution. The effect of time and concentrations on the removal rate of hexavalent chromium were studied using batch experiment. Maximum Cr (VI) removal was noted 75.0% by *Bacillus sp.* at 10mg/l, 69.70% by *Pseudomonas aeruginosa* at 40mg/l and 90.88% by *Pseudomonas putida* at 10mg/l of synthetic solution, during 96 hours. Among these three bacteria, the maximum Cr(VI) removal was reported by *Pseudomonas putida* on lower concentration. On the basis of highest removal rate, *Pseudomonas putida* was selected and used for further chromium removal from samples. It was found to be removed the highest Cr(VI) by 82.92%, from effluent and 74.41% from soil during 96 hours. The present study depicts that bacteria removes chromium efficiently and this could be used for industrial waste management and other environmental contaminants.

Key words: Hexavalent chromium % Bioremediation % Pseudomonas % Bacillus % Textile industry effluent % Soil

INTRODUCTION

Effluents released from the textile industries contain various organic dyestuffs, chrome dyes and other chemicals during various operations and produce a large quantity of solid and liquid waste containing hexavalent chromium, salts of zinc, sulphates, copper, sodium and potassium etc. The treatment of these wastes is essential before discharging them to the environment because of the toxicity and carcinogenicity. In trace amounts, Cr(VI) is considered as essential nutrient but it is more toxic, carcinogenic and mutagenic at elevated levels and it is also toxic to human beings, animals and plants. Concentration of hexavalent chromium should not exceed 0.05 mg/l in drinking water [1]. Its compounds can cause irritation in the lining of the nose, breathing problems, allergy reactions, skin rashes, reproductive problems, anemia, irritation and ulcer in small intestine and sometimes cancer and tumors in stomach, intestinal tract and lung, etc. [2, 3]. Chromium concentration above 2 ppm found to have inhibitory effects on plant growth, seed germination, necrosis and leads diminished photosynthesis and change in chloroplast structure in vegetable and fruit trees, growing in the neighborhood of chromium discharging factories [4-7].

In Indian context the discharge concentration of chromium should not exceed from 0.1 mg/l as per waste water discharge standard of Central Pollution Control Board [8]. However, in India some sites are identified as most polluted sites namely Sukinda valley in Orissa, Vapi in Gujarat, Mahad in Maharashtra, Noraiakheda, Kanpur, Uttar Pradesh and Ranipet in Tamilnadu as these places have chrome mining, tanneries, textile and other industries etc and discharge effluent containing higher concentration as per data released by Blacksmith Institute, Newyork, USA [9]. Chromium pollution in the ground waters due to the environmental impacts of industrial effluent irrigation from a tanning industrial cluster in Bangalore, India was reported [10]. The Haryana State Pollution Control Board, India detected ground water contamination containing excessive amount of Hexavalent chromium and total chromium in Village Sehraul and surrounding areas due to the intensive industrial activity in that area [11]. Hexavalent chromium is not confined to India rather it is a more global problem and spread all over the world. A number of scientists had

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worked in different part of world and found a dreadful situation of chromium contamination. In West Berkeley, hexavalent chromium occurs in the groundwater as a result of historical industrial activities [12]. It was observed the chromium pollution in drinking water of certain wells in Jinzhou, China due to Jinzhou iron alloy plant [13]. It was found chromium contamination in groundwater of the Ljubijansko Poije aquifer in Slovenia due to industries in near by area [14].

Many technologies are being developed and used to clean up heavy metal contamination such as excavation/pumping of the contaminated material, addition of chemical reductant, precipitation followed by sedimentation, or ion exchange and/or adsorption. These methods suffer from some drawback such as high capital and operational costs or the treatment and disposal of the residual metal sludge. Conventional treatment technologies become less effective and more expensive when metal concentrations are in range of 10-100mg/l [15]. However, non-conventional technologies are proved to be effective in removal of metal under this range such as 99.9% of chromium was removed in the 10 mg/l chromium solution by Bengal Gram (*Cicer arientinum*) husk [16]. Hence, conventional and less effective physico-chemical methods are being replaced by the more effective biological methods such as biosorption for the removal of hexavalent chromium from aqueous solution [16], biostimulation for the Cr(VI) [17], bioreduction for the Cr(VI) contamination in soil and ground water by Cr(VI) reducing bacteria, *Shewanella oneidensis* MR-1 [18] etc., which include the use of eco-friendly and easily available materials such as sea weed [19], algal biomass [20], husk of *Cicer arientinum* [16], *Eucalyptus* bark [21], oyster shells, cedar bark, vermiculite, cocoa shells and peanut shells [22], Cocolumber (*Cocos nucifera*) sawdust [23] that can remove hexavalent chromium actively and economically. A number of studies are reported on the removal of chromium by *Psudomonas sp. Pseudomonas* sp. was used to remove Cr(VI), Cu(II), Cd(II) and Ni(II) [24, 25]. In another study, *Pseudomonas aeruginosa* was used for the removal of Cr, Cu, Mn and Zn [26], while, Murugesan and Maheswari used *Pseudomonas* sp for the removal of Cr(VI) [27]. Removal efficiency of Cr(VI) by *Bacillus subtilis*, *Pseudomonas aeruginosa* was also observed by some workers [28,29]. Cr(VI) removal efficiency was also observed in *Bacillus sp.* and *Pseudomonas fluorescens* [30]. These bacteria were found very effective in bioremediation of heavy metals because metals are directly or indirectly involved in all aspects of microbial growth, metabolism. Bioremediation of heavy metals by bacterial cells has been recognized as a potential alternative to existing technologies for recovery of heavy metals from industrial wastes. This is an attempt to explore innovative, cost effective and environment friendly technology for the bioremediation of Cr (VI) contamination using microorganisms.

MATERIALS AND METHODS

For the bioremediation of Cr(VI), soil samples were collected from the dumping site and nearby areas of textile industry (Rishabh Valvleen Ltd.), at Bahadarabad, Hardwar, India and the effluent samples were taken from the effluent treatment plant (ETP) of the same industry. The samples were transported to laboratory at 4°C as in accordance with the standard methods [31]. Analysis of physico-chemical characteristics in soil was done by preparing 1: 5 soil suspension by taking 20 g of soil in 100 ml of aerated distilled water and shaking mechanically for one hour.

Analysis of the Effluent and Soil Samples: The physicochemical parameters (pH, Colour, Electrical Conductivity (EC), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total solids (TS), Chlorides, Sulphates, Nitrates, Phosphates, hardness, calcium, magnesium, sodium, potassium and heavy metal ions) were determined as soon as the samples were brought to the laboratory. pH was determined by electronic digital pH meter (Model-Century CP 901). EC was determined by digital conductivity meter (Model-P/N 44600-00 Hach maker). DO was determined by Winkler's iodometric methods while COD was determined by reflux method. Chlorides and sulphates were determined by titrimetric method and turbidity method, respectively. Nitrate and phosphates were quantifies by using Spectrophotometer (Model-Spectronic 21 D Milton Roy Company). Hardness, calcium and magnesium were analyzed by titrimetric method. Sodium and potassium were used by using Flame photometer (Model Toshniwal TMF 45).

In soil samples nitrogen was determined by Kjeldahl method and organic carbon and organic matter were determined by Walkey and Black methods. Different metal ions in the effluent and soils samples were determined by Atomic Absorption Spectrophotometer (Model-Perkin Elmer 3110) except Cr (VI) analysed by UV spectrophotometer as per standard methods [31]. For metals analysis effluent sample was digested with HNO_3 and soil sample was digested with HF (10 ml) and Aqua regia (1 ml) as per method described by Berrow and Mitchell [32].

Table 1: Selection of suitable bacterial isolates

S. No.	Cr Concentration	Bacterial Colonies
1.	50.0 mg/l	B1, B2, B3, B4, B5, B6, B9
2.	100.0 mg/l	B2, B4, B5, B7, B8, B9
3.	150.0 mg/l	B2, B3, B5, B9
4.	200.0 mg/l	Nil
5.	250.0 mg/l	Nil

Most efficient bacterial colonies: B2, B5 and B9

Isolation and Identification of Bacteria

Preparation of Nutrient Broth: 100 ml of nutrient broth (Hi Media Laboratory, Mumbai) was taken in a 250 ml conical flask having different concentrations of hexavalent chromium (50, 100, 150, 200 and 250 mg/l) and further, 10 g of soil contaminated with textile industry's effluent was added for the isolation of microbes and kept in incubator for 24 hours at 28 °C for the growth of microbes. After incubation, samples from the conical flasks were plated for the isolation of bacteria using nutrient agar media. The isolation was carried out by pour plate method, based on dilution principle.

Isolation of Bacteria: Nutrient agar medium (Hi Media Laboratory, Mumbai) was used for the isolation of bacteria from the soil sample. Serial dilutions from 10^{-1} to 10^{-7} were prepared by pipetteing appropriate amount of water suspension in 1 ml of sample. Then 1 ml of aliquot from 10^{-4} to 10^{-7} dilutions were pipetted into sterilized petri dishes and 20 ml of nutrient agar having a temperature of 45°C ± 1°C was poured. The plates were rotated slowly clockwise and anticlockwise at least 5 times to mix the suspension with agar. The plating was done in duplicate for each dilution. After solidification of agar, the plates were incubated at 28-30°C in an inverted position for 3 days. The plates were removed after completion of incubation period.

Selection of Bacterial Isolates: Bacterial sensitivity to metal toxicity was determined using a total of nine colonies, which were isolated from the selected soil samples, only three bacterial colonies B2, B5 and B9 show maximum tolerance even at 150.0 mg/l of Cr (VI) solution (Table 1). These bacterial colonies namely B2, B5 and B9 were used for bio-remediation of Cr (VI) from effluent and contaminated soil.

Characteristics of Different Bacterial Isolates Used for the Bio-remediation: The bacterial colonies were identified on the basis of morphological characteristics like shape, size and biochemical tests such as oxidase test, oxidation fermentation glucose test and starch agar test [33]: These colonies were identified as B2-*Bacillus species*; B5-*Pseudomonas aeruginosa* and B9-*Pseudomonas putida*. Bacterial cultures were prepared by these bacterial colonies for bio-remediation experiment.

Growth Studies: The bacterial growth was quantified in terms of optical density (OD) in the culture medium (100 ml) taken in side arm flask with the addition of 2 ml of Cr(VI) solutions having concentrations of 10, 20, 30, 40 and 50 mg/l, by measuring the absorbance at 690 nm against a blank, taken at regular intervals of 24 hours as per standard method [34]. The medium without addition of metal served as control.

Effect of Time and Concentration on Cr(VI) Removal: Five concentrates of Cr(VI)10, 20, 30, 40 and 50 mg/l (synthetic solutions) were prepared by dissolving 0.1414 g of potassium dichromate ($K_2Cr_2O_7$) in 1000 ml of distilled water. Bacterial culture was inoculated and kept in shaker incubator at 30°C for 24, 48, 72 and 96 hours at 120 rpm. 10 ml sample was withdrawn using sterilized pipette at regular time intervals of 24 hours and all the samples were stored at 4°C for 6 hours before analysis of Cr(VI). 2 ml of bacterial culture (@2.5 x 10^5 cfu/ml) was also inoculated in 100 ml of sterile effluent and soil suspension (1:5 in sterilized distilled water) and quantified for Cr(VI). This was kept in incubator at 30°C for 96 hours. Then 10 ml of sample was also withdrawn for analysis of Cr(VI) removal from effluent and soil samples.

The Cr(VI) concentrations in samples were determined colorimetrically by using spectrophotometer (Model-Spectronic 21D, Milton Roy Company) at 540 nm by diphenylcarbazide (DPC) method [31, 35].

RESULTS AND DISCUSSION

The effluent sample was taken from the effluent treatment plant (ETP) of textile industry and soil samples were collected from the dumping site of the same industry and analyzed for physico-chemical properties.

Table 2: Average values of selected physico-chemical parameters of textile industry effluent and soil (All values are Mean ±SD and range for four observations each and given in mg/l for effluent and µg/gm for soil, otherwise stated)

Sample no.	Parameters	Effluent	Soil
1.	Color	Bluish Black	Reddish brown
2.	24.93±3.62	(19.40-29.20)	38.00±1.78
			(36.00-41.00)
3.	Moisture content (%)	--	5.06±0.80
			(3.95-6.24)
4.	E.C (µS/cm)	6994.33±40.82	980.33±124.74
		(6917.00-7032.00)	(825.5-1170.4)
5.	TS	5224.97±110.61	(5103.24-5331.00)
			--
6.	pH	7.30±0.05	7.86±0.032
		(7.20-7.35)	(7.82-7.90)
7.	Alkalinity	676.95±6.28	1167.80±7.20
		(668.50-679.26)	(1160.30-1174.65)
8.	DO	ND	--
9.	BOD	1373.42±15.20	
		(1354.30-1392.0)	--
10.	COD	3985.98± 14.78	
		(3960.60-4003.66)	--
11.	Nitrate	16.79 ± 3.85	
		(12.48-23.42)	69.96±17.88
		(48.44-90.75)	
12.	Phosphate	ND	9.68±2.43
		(7.53-12.20)	
13.	Sulphates	35.15 ± 3.43	
		(30.48-40.70)	2654.00±2.66
		(2648.00-2659.00)	
14.	Chlorides	217.90 ± 7.16	
		(209.60-226.00)	995.00±20.04
		(980.00-1025.00)	
15.	Hardness	1771.53 ± 41.03	
		(1709.91-1810.6)	--
16.	Calcium	427.06±7.64	4025.40±.337.45
		(417.25-437.3)	(3728.67-4623.3)
17.	Magnesium	172.47±7. 37	2838.79±96.93
		(164.20-185.00)	(2721.20-2924.0)
18.	Sodium	222.82 ± 6.41	185.98±32.06
		(214.74-231.75)	(152.85-240.30)
19.	Potassium	19.88 ± 2.74	126.53±38.64
		(15.90-23.50)	(86.84-175.65)
20.	Organic Matter (%)	--	2.45±0.011
			(2.43-2.46)
21.	Organic carbon (%)	--	1.42±0.002
			(1.41-1.42)
22.	Kjeldahl-Nitrogen	--	13.50±0.818
			(13.20-13.80)

Table 3: Average values of metals in effluent of textile industry and soil (All values are Mean ±SD and range for four observations each)

Sample no.	Metals	Effluent (mg/l)	Soil (µg/g)
1.	Cr	2.38 ± 0.005	568.00±4.163
		(2.37-2.38)	(562-572)
2.	Fe	1.70 ± 0.017	308.40±3.02
		(1.65-1.74)	(298-332.12)
3.	Mn	0.57 ± 0.005	668.80±0.559
		(0.56-0.57)	(668.43-669.09)
4.	Cu	0.01 ± 0.004	109.54±0.315
		(0.007-0.015)	(109.12-109.85)
5.	Pb	ND	191.25±19.35
			(175-208)
6.	Cd	0.018 ± 4.472	83.62±0.119
		(0.012-0.022)	(83.48-83.75)
7.	Ni	ND	ND

The pH of the effluent and soil was 7.30 and 7.86, respectively and the effluent was characterized by a high biological oxygen demand and chemical oxygen demand (Table 2). Higher values of BOD and COD were observed in effluent as 1373.42 and 3985.98mg/l, respectively. A high value of BOD and COD will cause depletion of dissolved oxygen in water [36]. Higher values of hardness were also observed. The bacterial cultures exhibited removal even at higher levels of Cr (VI) and the bacterial growth decreased with increase in the metal concentration.

Similarly, effluent and soil samples were analyzed for heavy metals (Table 3). The average concentrations of heavy metals were reported within the permissible limits except chromium.

Removal of Cr (VI) from Synthetic Samples: Nine different bacterial species were screened on the basis of morphological characteristics which grew in 10-50 mg/l of Cr(VI) concentration. Out of these, three bacterial species i.e. *Bacillus sp., Pseudomonas aeruginosa and Pseudomonas putida* showed maximum tolerance even at 50.0 mg/l of Cr(VI) and isolated for experiment. After screening, *Pseudomonas putida* was found capable to remove chromium and used for further study. It showed consistent growth, both in nutrient broth and nutrient broth containing $K_2Cr_2O_7$.

Effect of Time on Removal of Cr (VI) in Synthetic Solution: The data was observed for the uptake of Cr metal ions vs contact time for different concentrations (10, 20, 30, 40 and 50 mg/l) at natural pH, up to 96 hours. The metal removal efficiency increased with increase in time. However, a remarkably increased in percent Cr(VI) removal was estimated 75.0 ± 2.27% by *Bacillus sp.* at 10mg/l, 69.70 ± 0.80% by *Pseudomonas aeruginosa* at 40mg/l and 90.88 ± 0.87 % by *Pseudomonas putida* at 10mg/l of test concentrations, during 96 hours (Fig. 1). *Bacillus sp.* and *Pseudomonas aeruginosa* removed considerable amount of chromium ions and showed significant efficiency for bioremediation. However, *Pseudomonas putida* showed highest removal of Cr(VI), corresponding to increase in time and reached a maximum value at a particular time; which is termed as equilibrium time that was 96 hours. Thereafter, the removal efficiency becomes constant. At equilibrium, removal of metal ions attains a constant value because adsorption and desorption balance each other [37]. Another explanation was given for the initial rise in adsorption of Cr(VI) ions that it is due to bigger driving force and lesser surface

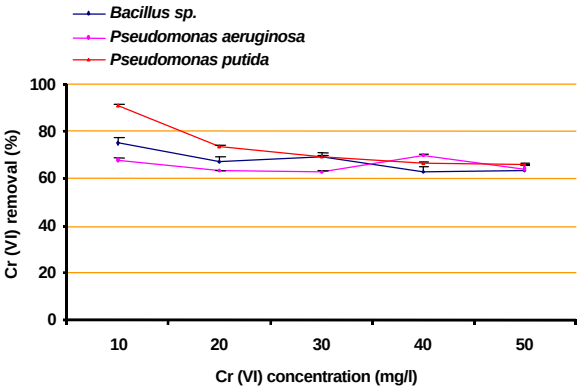

Fig. 1: Cr (VI) removal by different bacterial strains from synthetic solution during 96 hours

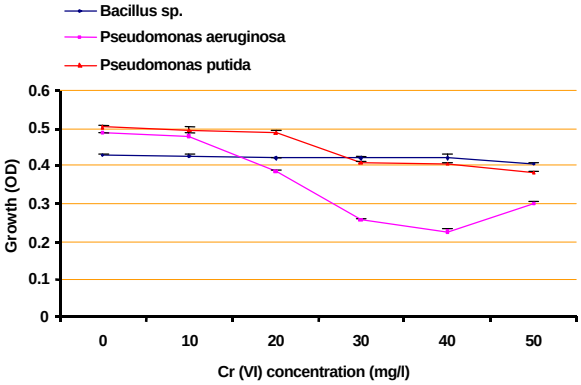

Fig. 2: Growth pattern of different bacterial strains in synthetic solution during 96 hours

area; while decrease in adsorption may be due to intra-particle diffusion process dominating over adsorption [38].

Effect of Concentration on Removal of Cr (VI) in Synthetic Solution: Most of the hexavalent chromium (90.88%) absorbed by *Pseudomonas putida* from synthetic Cr (VI) solution at 10 mg/l during 96 hours of incubation period (Fig. 1) and it was found that with the increase in concentration the metal removal efficiency gradually decreased. This is because of microbial population in the system can affect chromium removal due to saturation of metal binding sites of the biosorbent.

Thus, sorption increased with increase in concentration as long as binding sites were available. Cr binding was rapid initially, but also reaches capacity or equilibrium afterwards [39]. In a previous study it was found the maximum metal removal by 80.16% at 10 mg/l of Cr (VI) through *Pseudomonas sp.*, after 72 hours and

Table 4: Cr (VI) removal of by *Pseudomonas putida* from effluent and associated soil. (All values are Mean ± SD and range of 4 samples each)

			Percent removal (%)			
Sample no.	Initial Cr (VI) concentration		24 hrs	48 hrs	72 hrs	96 hrs
1	Effluent (mg/l)	2.383±0.01	23.24±2.4	59.67±2.41	71.34±0.92	82.92±2.07
		(2.379-2.389)	(21.30-25.94)	(57.38-62.48)	(70.56-72.35)	(80.82-84.95)
2	Soil (µg/g)	568.00±4.16	22.02±2.29	42.77±2.59	65.05±3.35	74.41±2.07
		(562.0-572.0)	(20.59-24.67)	(40.46-45.50)	(62.83-68.90)	(72.24-76.35)

suggested that it was due to higher concentrations, as more ions are competing for the available binding sites, the rate of adsorption decreased, resulting in lower adsorption percentage [40]. Sethuraman and Balasubramanian also noted maximum removal percentage was *Pseudomonas aeruginosa* (78%) and *Bacillus subtilis* (33.5%) at 25 mg/l [29]. Correspondingly, it was also reported that an increase in chromium concentration results in reduction in adsorption [41]. It might be due to lack of availability of active sites on the adsorbent. Further, exposure of chromium to bacterial strain causes no change. The loss in Cr(VI) reduction capacity among bacterial cells may be attributed to the mutagenic and toxic effects of hexavalent chromium [42].

The present study recites that *Pseudomonas putida* removes a maximum of 90.88% of Cr(VI) at 96 hours which is higher than other bacteria. *Pseudomonas putida* found more efficient than *Bacillus sp.* and *Pseudomonas aeruginosa* for the removal of Cr(VI) from synthetic solutions and selected for further removal of Cr(VI) from effluent and soil.

Effect of Time and Cr(VI) Concentration on Bacterial Growth Pattern: In order to study the effects of Cr(VI) concentrations on bacterial growth, different concentrations of Cr(VI) were mixed with bacterial culture. *Bacillus sp*. do not show change in growth over the tested concentration range. On the other hand, *Pseudomonas putida* and *P. aeruginosa* shows higher growth at 10 mg/l. However at higher concentrations growth of *P. putida* decreases continuously while growth of *P. aeruginosa* decreases from 10 mg/l to 40 mg/l but then increases at 50 mg/l (Fig. 2). Bacteria shows growth up to 96 hours after that growth subsequently decreases. It was also reported by some workers that higher concentration of chromium show inhibitory effect on growth of bacteria [43, 44]. While, out of three bacterial isolates the highest growth was observed by *Pseudomonas putida* at 10 mg/l (OD-0.496) which also supports that the bacteria is suitable for removal of hexavalent chromium.

Removal of Cr (VI) from Effluent and Soil: During this study, it was found that *Pseudomonas putida* removed hexavalent chromium by 82.92 % in effluent and 74.41% in soil during 96 hours. Results presented in Table 4 showed excellent removal efficiency of *Pseudomonas putida* than other studied bacteria. Hence, the bioremediation of hexavalent chromium from effluent and soil by *Pseudomonas putida* looks environmentally and economically feasible and can be applied in field.

CONCLUSION

It can be concluded from the present study that *Pseudomonas putida* has a great potential to remove hexavalent chromium from aqueous solution of chromium as well as effluent and soil. From the above results it is clear that it is a good biosorbent and can be used for removal of heavy metals from industrial wastes and that further study for application of this technology in field is recommended.

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