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Bio-reduction of chromium using *Arthrobacter citreus* and *Brevibacterium casei* (Microbial consortium)

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ABSTRACT

Hexavalent chromium is the heavy metal constituting a major part of tannery effluent, poses a serious threat to the environment. Chromium has adverse health effects on plants, animals and human beings. It becomes a necessary task to reduce the Chromium in the environment. In the present study a preliminary attempt has been made to reduce the chromium using two selected micro organisms such as Arthrobacter citreus and Brevibacterium casei to screen the efficacy of bioremediation. In the present study instead of using the tannery effluent it has been planned to use the actual pollutant in the tannery, synthetic hexavalent chromium. From the study it is evident that in the Cell Suspension technique, using Brevibacterium casei showed 84% of Chromium(VI) reduction after 72 hours, whereas in the immobilization technique, the consortium showed 78% of Chromium(VI) reduction within 48 hours. The results indicate that immobilized consortium is effective since it reduces 78% of Chromium(VI) within 48 hours. Such type of pilot studies will form a base to identify an effective bioremediation procedure to reduce chromium in the tannery effluent.

Keywords: hexavalent Chromium, Arthrobacter citreus, Brevibacterium casei, effluent

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INTRODUCTION

Tannery effluents are ranked as the highest pollutants among all the industrial wastes. It is estimated that in India alone about 2000-3000 tons of chromium escapes into the environment annually from tannery industries, with chromium concentrations ranging between 2000 and 5000 mg/l in the aqueous effluent compared to the recommended permissible limits of 2 mg/l. The tanning industries are especially large contributors of chromium pollution in India. However, one of the major emerging environmental problems in the tanning industry is the disposal of chromium contaminated sludge produced as a by-product of wastewater treatment [5]. According to a 1965 survey of 155 people in the village of Nuerhe who had directly drunk groundwater containing high concentrations (20 mg/L) of Cr+6, symptoms including perleche, diarrhea, abdominal pain, indigestion and vomiting appeared. Elevated white cell counts, elevated juvenile cells among neutrophilic granulocytes, and shifts to the left appeared in residents of highly polluted areas. Lung cancer mortality rates between 13.17-21.39/100,000, while the average for the entire district in the same period was 11.21/100,000. Stomach cancer mortality rates were 27.68-55.17/100,000 [9].

In India, industrial units use common effluent treatment plants (CETPs) to varying degrees to treat and process waste streams. Common effluent treatment plants (CETPs) have been promoted in the region as a long-term, end of pipe solution to the environment problems arising from contaminated waste water disposal. Investigations by the Greenpeace International in Gujarat clearly showed that CETPs failed to deal with all the chemicals pollutants produced by the industries, specially the heavy metals and organic pollutants. Consequently waste water which are considered acceptable for discharge to surface water by the authorities can still contain high concentration of toxic and persistent chemicals. Tannery effluents emanating from CETP in Unnao, UP was found toxic in nature having high Biochemical oxygen Demand BOD, Chemical Oxygen Demand COD, Total Dissolved Solids TDS and Cr content (5.88 mg.l), which supported growth of chromate-tolerant bacteria [8].

Persistent Organic Pollutants (POPs) and inorganic residues in fluid form goes beyond the capacity of primary and secondary treatment in CETPs. Reverse Osmosis, Granulated Activated Carbon, Ultra-filtration, ion exchange and other tertiary treatment methods which could be effective in this case are not used by CETPs mainly for economic reasons. This concept also faced many operational and institutional problems as many participating industries started withdrawing from the scheme. Trivalent [Cr(III)] and hexavalent [Cr(VI)] forms are the dominant oxidation states of Cr that exist in the environment. Chromium(VI) is more water-soluble and more mobile than Cr(III) in soils and is therefore transported to ground waters.

The toxicity of Cr is dependent on its oxidation state. Thus, Cr(VI), a carcinogen, is highly toxic to all forms of life. Chromium(III), an essential micronutrient for many higher organisms, is relatively insoluble in water and 100 times less toxic than Cr(VI) [3] .Hence the present work has been designed to screen the efficacy of two selected microbes Arthrobacter citreus and Brevibacterium casei using synthetic hexavalent chromium .

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MATERIALS AND METHODS

Materials

Bacterial cultures of Arthrobacter citreus(MTCC3148) and Brevibacterium casei(MTCC1530) were obtained from Microbial Type Culture Collection(MTCC)institute at Chandigarh. The strains were grown in a Luria Bertani broth which contains Peptone- 5 g, Sodium chloride- 5 g, Beef extract -1.5 g, Yeast extract- 1.5 g, final pH (at 25°C) - 7.4±0.2. The 13grams of the broth powder is dissolved in 1000ml of water. The synthetic effluent was prepared using potassium dichromate salts of chromium(VI). The potassium dichromate solutions were prepared using double distilled water. 1.414gms of potassium dichromate was dissolved in 250ml of glucose minimal media to achieve 2000mg/liter of Chromium(VI) [6].

Methods

Bioremediation is done under two methods viz., Cell Suspension Technique and Immobilized Culture Technique.

Cell suspension Technique

The overnight grown culture was inoculated into each conical flask(Synthetic effluent as well as original Effluent). The organisms were inoculated as single and consortium. The flasks were kept on Incubator shaker at 150 rpm for Arthrobacter citreus and Brevibacterium casei at room temperature were maintained at 37°C for a period of 96 hours. Chromium was estimated at an interval of 24 hours to calculate the chromium depletion from the effluent by the different test organisms. The results were calculated and tabulated [9].

Immobilized Culture Technique

The cells of the exponentially growing culture were harvested and harvested cells were then homogenized. About 17.5 ml of the homogenate were measured into 250 ml conical flask containing 87.5 ml distilled water and mixed properly to ensure homogeneity. This was allowed to settle after 10 minutes and separated. The supernatant (concentrated cells) was stored at 20◦ C. Exactly 3.063 g of sodium alginate was weighed into the concentrated cells. The mixture was subsequently dropped through a sterilized syringe and a needle into a flask containing sterilized 70 ml of 0.12 M $CaCl_2$. Gel formation were achieved at room temperature as soon as the sodium alginate drops come in direct contact with the calcium solution. Complete precipitation formed spherical beads of diameter 3.75 - 4.5 mm. The beads are allowed to fully harden in 1-2 hours. The beads were washed with fresh calcium cross linking solution [7].

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Chromium Analysis

Chromium analysis were carried out using both instrumental and biochemical procedures.

Instrumental method

The collected tannery effluent was analyzed for total chromium and hexavalent chromium using Atomic Absorption Spectroscopy at Chennai Mettexlab (P)Ltd, Guindy, Chennai. (US EPA Method 7195, 1986)

Biochemical method

Chromate reducing activity was measured as the decrease of Cr(VI) with time using the colorimetric reagent diphenylcarbazide. 95 mL of the sample to be tested is transferred to a 100-mL volumetric flask. Then 2.0 mL of diphenylcarbazide solution is added to it and mixed. H2SO4 solution is added to give a pH of 2 ± 0.5, and diluted to 100 mL with reagent water, and allowed to stand for 5 to 10 min for full color development. An appropriate portion of the solution is transferred to a 1-cm absorption cell and measured for its absorbance at 540 nm. Reagent water is used as a reference. The absorbance reading of the sample is corrected by subtracting the absorbance of a blank carried through the method. An aliquot of the sample containing all reagents except diphenylcarbazide was prepared and used to correct the sample for turbidity (i.e., turbidity blank). From the corrected absorbance, the mg/L of chromium present was determined by reference to the calibration curve [1].

Results

The results of cell suspension technique showed a steep increase in the chromium reduction which gets stabilized at 96 hrs. From the (Table No 1, Fig No 1) it is evident that maximum chromium [84%] reduction was observed at 72 hrs of treatment. When we critically analyse the result Brevibacterium casei showed a maximal reduction in chromium when compared to Arthrobacter citreus and consortium.

Table no 1

Time(Hours)	Arthrobacter citreus	Brevibacterium casei	consortium
24	74	70	72
48	80	72	72
72	82	84	78
96	72	72	72

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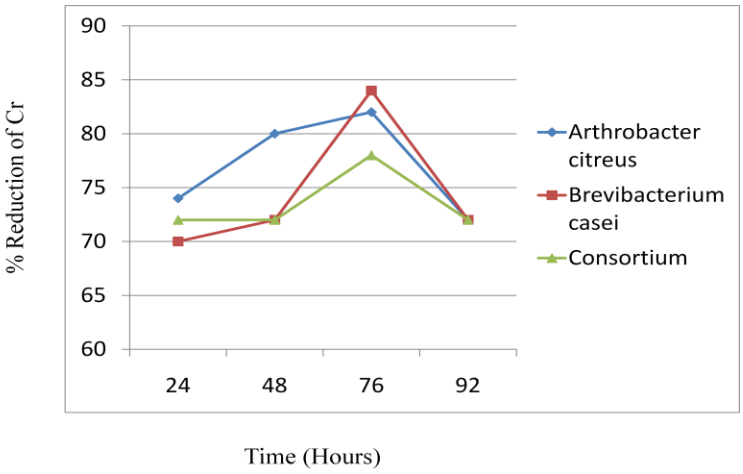

Fig 1

Regarding the second technique Cell Immobilization technique, consortium showed the maximum reduction (78%) within 48 hours of treatment (Table 2, Fig No 2). After 48 hours we see a remarkable reduction in its activity both in consortium and individual microbes.

Table 2

Time(Hours)	Arthrobacter citreus	Brevibacterium casei	consortium
24	30	28	38
48	40	38	78
72	30	24	34
96	2	0	32

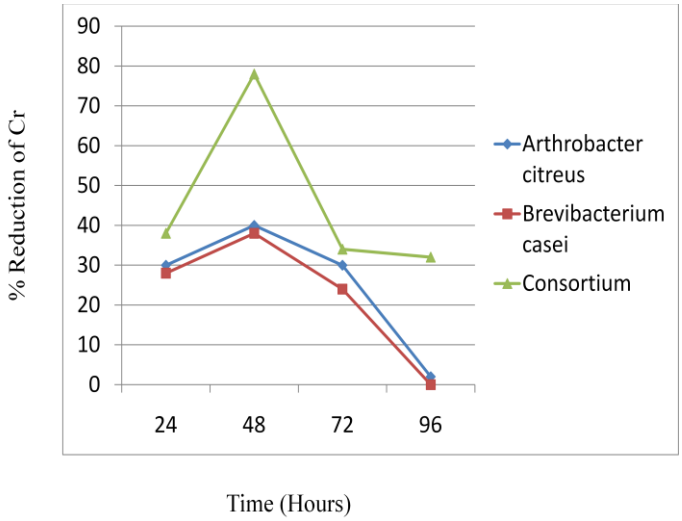

Fig 2

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DISCUSSION

The results are quite encouraging both in cell suspension and cell immobilization techniques. In accordance with the works of Sethuraman et al,2010 the results in cell suspension technique showed maximum chromium reduction(84%) in Brevibacterium casei when compare to Arthrobacter citreus and consortium. In addition to this from the data it is clear that the activity reaches maximum up to 72 hrs after which the activity steeply reduces indicates that 72 hrs treatments is very much essential to obtain maximal chromium reduction. Regarding other technique using consortium [immobilized cells] showed significant reduction in chromium which in accordance with the work of Benezir et al, 2010.this type of pilot screening on synthetic chromium reduction will form a base to identify a microbe which can reduce maximum % chromium with in a short period in the tannery effluent. It can be further refined to get a cost effective chromium reduction method for advising the government and tannery industry.

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