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Bio-detoxification of chromium from industrial wastewater by fungal strains

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Chromium is one of the most toxic heavy metals to be abundant in tannery wastewater effluents. In order to study the possibility of chromium detoxification, chromium tolerant fungal strains were isolated from the tannery effluent of eastern Calcutta and their chromium tolerance limits were detected. The chromium accumulating capacity of these strains was studied by culturing them in nutrient media supplemented with chromate salts. Depending on the pH of the concerned media, chromium accumulating capacity was found to be different for these fungi. Though most strains had shown better biosorption in lower pH, *Aspergillus clavatus* accumulated more chromium in alkaline conditions. These strains were further screened for their biosorption capacity to remove chromium from the raw effluent and the nutrient supplemented effluent. As the reduction of hexavalent chromium to its trivalent form is an efficient remediation for reducing toxicity of wastewater, it was also studied to evaluate the detoxification capability of these strains. *Aspergillus* strains were found most efficient for detoxification, doing more than sixty percent conversion. These fungal strains could be used for biorecovery and detoxification of chromium.

Key words: chromium, detoxification, tannery, fungi

INTRODUCTION

Chromium is one of the major sources of heavy metal pollution. Chromium has been introduced into a wide range of large-scale industrial processes like leather tanning, chrome-plating metallurgical applications, textiles and dye industries, wood impregnation, photography, lithography etc. In nature, chromium occurs mainly in two states, trivalent (Cr^{3+}) and hexavalent (Cr^{6+}). Hexavalent chromium is much more toxic than the trivalent form (Ishibashi et. al., 1990). The discharge of several industries containing high amount of chromium leads to high toxicity in plants and animals which come in contact with chromium contaminated wastewater or sludge (Guruprasad, Nandakumar, 1983). Chromium leads to abnormal interaction with intracellular proteins and nucleic acids

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thus producing chromosomal aberrations (Horitsu et al., 1978). But the toxic effects are confined to the hexavalent compounds of chromium only.

Keeping the toxic effects of chromium and the International Standard for disposal of waste in mind, the concerned industries must minimise the total chromium level in wastewater. It may be also possible to minimise the toxic Cr^{6+} level by transferring it to its trivalent form which is almost nontoxic and even an essential trace element for growth.

Conventional technologies like ion exchange and electrolyses are becoming increasingly expensive. Biosorption and biotransformation for detoxification of wastewater by application of microorganisms is a cost-effective solution. Scientists worldwide are attempting to select out suitable strains of microorganisms with a better detoxification capability. Recently, effective chromium resistant bacteria were isolated from polluted environments by several investigators (Luli et. al., 1983; Losi, Frankenberger, 1994). Biotransformation of hexavalent chromium to its trivalent form was also reported in case of bacteria (Cervantes, Silver, 1992). Though there are reports on fungal biosorption of different metals, few reports about biotransformation, especially on chromium, are found (Lewis, Kiff, 1988; Singleton et. al., 1990; Al-Asheh, Duvnjak, 1995). In the present investigation, we have isolated chromium tolerant fungal strains and detected their biosorption and biotransformation capabilities in respect to toxic hexavalent chromium.

METHODS

Chromium polluted effluent samples were collected aseptically from the drainage canal of eastern Calcutta tannery industries. These eastern Calcutta tanneries, comprising more than 300 units, are one of the largest tanning industries in India.

Samples were collected in polyethylene wide-mouth bottles of minimum sample size 500 ml. It was important to avoid surface materials during sampling. Sterilized glass bottles were used for microbial analyses (American Public Health Association, 1992). Samples were taken to the laboratory and analyzed as soon as possible, keeping them protected from direct sunlight and heat. Refrigeration at 4 °C and addition of preservatives, if necessary, were done according to APHA (American Public Health Association, 1992). Tannery wastewater effluents were aseptically collected from two different sampling sites, first, from one of the industry after their conventional treatment (TS-1) and, second, from the effluent discharge canal near Science city (TS-2) (i. e. about 500 m away from the industry).

Chemical parameters of the wastewater samples like pH, BOD_5 and Cr (VI) in effluent were estimated after filtration through Whatman No-1 according to standard methods (American Public Health Association, 1992). For microbial characterisation of the wastewater, samples were plated in plate count agar and Czapec-Dox (CD) agar media (American Public Health Association, 1992). After incubating at 30 ± 2 °C, number of colony forming units (cfu) of bacteria and fungi were enumerated in respective media. Both fungal and bacterial strains were isolated, purified and maintained in nutrient agar and Czapec-Dox agar media.

For determining chromium tolerance limit of these microbial strains, the strains were allowed to grow in nutrient agar or Czapec-Dox (CD) agar media (pH 7.3 ± 0.2 at 30 ± 2 °C) incorporating 0.22 μm Milipore filter-sterilised K_2CrO_4 containing 100, 200, 300, 400 and 500 ppm (mg/l) Cr(VI). The fungi with the capability of growing well in both CD agar and broth with even 400 ppm Cr^{6+} within 96 hours were taken for further study. The fungal strains were identified using identification manuals (Onions et al., 1981; Ellis et al., 1992).

To study chromium removal capacity of the selected fungi, the strains were grown in 100 ml Czapec Dox broth (pH 7.3) containing 50 ppm (mg/l) Cr^{6+}(as 0.22 μm Milipore filter-sterilised K_2CrO_4) for 7 days at 28 ± 2 °C. The inocula were 1–2 loopful of freshly grown culture in C-D broth. For determination of residual hexavalent chromium in the filtrate, the filtrate (passed through 0.22 μm Milipore filter) was taken out and hexavalent and residual trivalent chromium estimation was done spectrophotometrically by 1.5-Diphenylcarbazide method (American Public Health Association, 1992). In respect to the control sets, hexavalent chromium removal percentages were drawn out. This study helped in selecting better promising strains with higher Cr(VI) removal capacities. Control sets were also run to eliminate possible errors due to non-specific chromium adsorption occurring in the flasks.

The two selected strains were grown in 200 ml Czapec Dox broth (pH 7.3) containing 25 and

50 ppm Cr^{6+} (as 0.22 µm Milipore filter-sterilised K_2CrO_4) and in tannery wastewater (containing 22.3 and 46.6 mg/l $^{Cr6+}$) for 5 days at 28 ± 2 °C. The inocula were 5 ml of fresh spore suspension of inoculum strength: 4–5 × 10^3 cfu/ml. For determination of residual hexavalent chromium in the filtrate, the filtrate (passed through 0.22 µm Milipore filter) was taken out and hexavalent and residual trivalent chromium estimation was done (American Public Health Association, 1992). In respect to the control sets, hexavalent chromium removal percentages were calculated.

RESULTS AND DISCUSSION

Chemical parameters of the wastewater samples like pH and BOD_5 were not significantly different for the two samples taken (Table 1). The pH of the effluent was always slightly alkaline as these industries indiscriminately use lime to precipitate chromium as much as possible. BOD_5 in the range of 110–145 indicates that there were enough nutrients to thrive in microorganisms. Hexavalent chromium concentration was very high in comparison to statutory norm, i. e. 0.1 ppm.

Samples from the two sampling sites differ in their chemical as well as biological parameters (Table 1). The sample from effluent canal contained more bacteria and fungi as identified from their colony forming units in proper media. Bacterial and fungal load in case of TS-I and TS-II were about 1.5 × 10^4, 7.6 × 10^3 and 3.2 × 10^4 and 1.7 × 10^4, respectively. Bacterial and fungal load of the sample collected near Science City was more than double compared with the sample from industry itself, it was probably due to increasing contamination with the decrease in chromium load.

Of total 56 fungal strains isolated, after 48 hours only 6 strains had grown at 300 mg/l chromium (VI) concentrations; but after 96 hours 6 and 4 strains were found to be tolerant and capable of growing at 400 and 500 mg/l concentrations, respectively. Of total 42 bacterial strains isolated, in 48 hours incubation 7 strains had grown at 400 mg/l chromium (VI) concentration; but after 96 hours, 12 and 2 bacterial strains were found to be tolerant and capable of growing at 400 and 500 mg/l concentrations, respectively (Table 2). The rate of growth in all the cases was slow. Only six fungal strains which had shown tolerance and good growth rate up to 400 mg/l chromium were selected for further studies.

Among the strains isolated from wastewater, *Aspergillus* species were more efficient in removal of chromium (Fig. 1). While *Cladosporium* and *Penicillium* had shown removal of 59.0 and 66.8%, *Aspergillus flavus* EST_F-241 and *Aspergillus clavatus* EST_F-181 had removed 89.2 and 87.0% hexavalent chromium in this trial run. This study helped in selecting these promising strains with higher Cr(VI) removal capacities.

The rates of removal of hexavalent chromium were slightly (2%) to moderately (18%) lower in tannery wastewater than in the media of comparable chromium strength (Fig. 2). Total removal in case of *Aspergillus flavus* strain EST_F-241 was

Table 1. Physico-chemical and microbiological characteristics of tannery wastewater

Parameters	TS-I	TS-II
pH	8.1 ± 0.4	7.8 ± 0.3
BOD_5 (mg/l)	125 ± 9.4	132.5 ± 11.2
Cr (VI) (mg/l)	47.6 ± 2.7	23.2 ± 2.3
Bacterial load (cfu/ml)	1.5 × 10^4	3.2 × 10^4
Fungal load (cfu/ml)	7.6 × 10^3	1.7 × 10^4

Table 2. Hexavalent chromium Cr (VI) tolerance limit of isolated strains of fungi and bacteria

Strains	Total No.	Growth after 48 hours					Growth after 96 hours				
		Initial chromium (VI) concentrations (ppm or mg/l)									
		100	200	300	400	500	100	200	300	400	500
Fungi	56	24	17	6	–	–	34	23	18	6	4
Bacteria	42	35	30	21	7	–	38	32	25	12	2

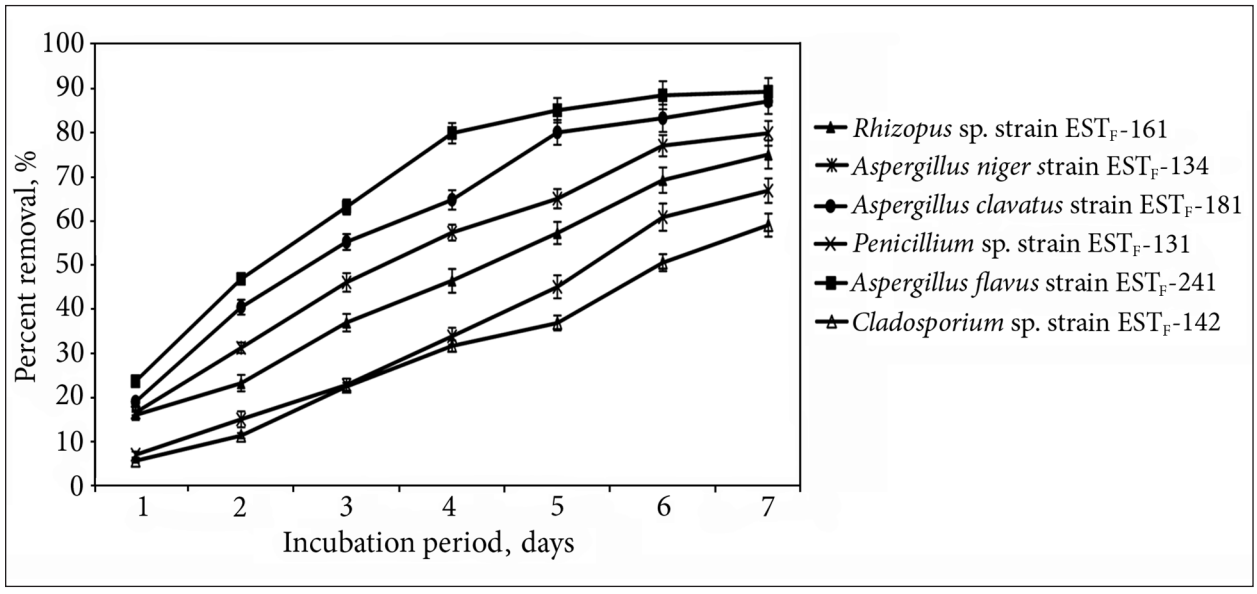

Fig. 1. Removal of chromium (VI) from 50 ppm (mg/l) Cr(VI) containing CDB (100 ml, 28 °C, pH 7.3) by fungal strains in addition to control. Control adsorption 0.4 ± 0.2%

99.6%, 88.2%, 85.2% and 81.2%, respectively in CDB-I & II and TW-I & II. Biotransformation (of total hexavalent chromium) ranged from 4–32.6%, while the remaining was biosorption. Total removal in case of *Aspergillus clavatus* strain EST$_F$-181 was 91.6%, 66.6%, 73.6% and 64.8%, respectively in CDB-I & II and TW-I & II. Biotransformation (of total hexavalent chromium) ranged from 5.6–19.6%, while the remaining was biosorption.

Aspergillus flavus strain EST$_F$-241 had a better remedial effect on wastewater for Cr(VI) removal than *Aspergillus clavatus* strain EST$_F$-181. It was

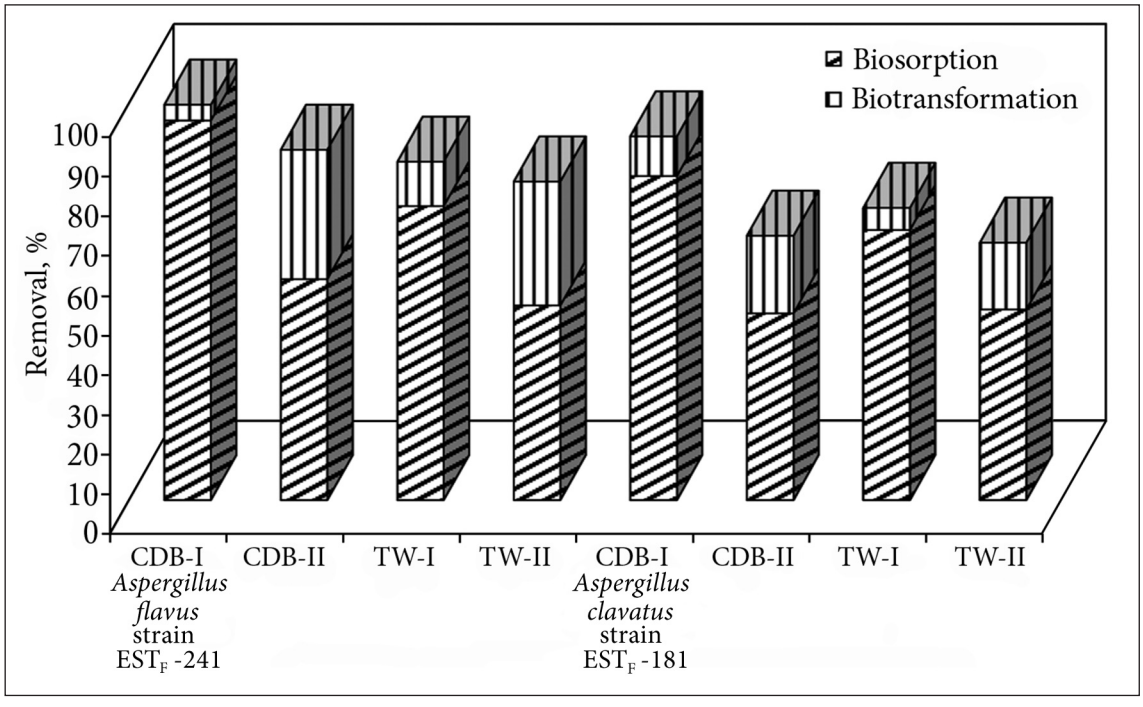

Fig. 2. Removal of chromium (VI) by biosorption and biotransformation from CD-broth (CDB-I & II) and tannery wastewater (TW-I & II) in 5 days

found that in real tannery wastewater both these strains showed moderate removal efficiencies, though about 2–30% lower than those of nutrient media with comparable chromium. Biosorption was always higher than biotransformation of Cr(VI) (Fig. 2). *Aspergillus flavus* strain EST_F-241 showed a better Cr(VI) biosorption capacity. When these strains were introduced in hexavalent chromium incorporated media or tannery effluents, hexavalent chromium was accumulated by fungal biomass and some hexavalent chromium was transformed into non-toxic trivalent form. Total bio-accumulation was higher in lower concentrations as bio-accumulation mainly depends on biomass produced and higher biomass is produced in lower concentrations of chromium. Metal bioaccumulation by such microorganisms is reported to be done by different processes, which include adsorption or micro-precipitation at the cell surface, chelation with cellular compounds, active transport and particulate ingestion or entrapment by extra-cellular organelles (Shuttleworth, Unz, 1993). Chromium bio-transformation from hexavalent to trivalent form was slightly higher in nutrient media than that of tannery effluent as there might be some constituents in effluent hampering transformation process or necessary enzyme production. Although several bacterial strains are known for such reduction of Cr^{6+}, information about fungal transformation of chromium is very sparse (Lewis, Kiff, 1988; Lovley, Phillips, 1994).

Thus, these fungi were found to be very effective in control of chromium pollution or in treatment of wastewater. *Aspergillus flavus* EST_F-241 and *Aspergillus clavatus* EST_F-181 strains in particular can effectively accumulate or adsorb chromium simultaneously with chromium reduction. They could be used to produce immobilised biomass in filtration pond or meander channels for treating chromium loaded wastewater.

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PRAMONINIŲ NUOTĖKŲ CHROMO BIODETOKSIKACIJA GRYBŲ KAMIENAIS

Santrauka

Chromas yra vienas iš labiausiai toksiškų sunkiųjų metalų, randamas odos raugyklų nuotėkų vandenyse. Ieškant galimybių chromo detoksikacijai, chromui atsparūs grybų kamienai buvo išskirti iš odos raugyklų nuotėkų Rytų Kalkutoje ir įvertintas grybų kamienų atsparumas chromui. Grybų akumuliacinių galimybių įvertinimas atliktas auginant juos chromato druskų terpėse. Priklausomai nuo pasirinktos terpės pH, chromo kaupimasis grybų kamienuose buvo skirtingas. Daugumos kamienų geresnė biosorbcija nustatyta esant žemoms pH reikšmėms. *Aspergillus clavatus* sukaupė daugiau chromo šarminėje aplinkoje. Įvertintos šio kamieno chromo pašalinimo iš nevalytų ir apdorotų nuotėkų biosorbcinės galimybės. Šešiavalenčio chromo redukcija iki trivalenčio yra veiksminga remediacijos priemonė toksiškumui mažinti nutekamuosiuose vandenyse. Labiausiai detoksikacinėmis savybėmis pasižymėjo *Aspergillus* kamienai, perdirbantys daugiau kaip 60 % nuotėkų. Šie kamienai gali būti panaudojami šalinant chromą iš nuotėkų ir biodetoksikacijai.

Raktažodžiai: chromas, detoksikacija, odų raugyklos, grybai