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Research Article

Biosorption and biotransformation of Cr (VI) by hexavalent chromium-stressed environmental fungal isolates from wastewater

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ABSTRACT

As conventional physico-chemical heavy-metal remediation technologies like ion-exchange, reverse osmosis, electrodialysis etc. are becoming extremely expensive, the possibility of use of microorganisms in bioremediation or biorecovery of different heavy metals from industrial effluents is extensively explored by different workers. Hexavalent chromium stressed environmental fungal isolates from tannery wastewater were assessed to biosorb and biotransform Cr(VI) from nutrient media and wastewater. Out of total 56 fungal strains isolated only six fungal strains had shown tolerance and good growth rate to up to 300 mg/l chromium(VI). Among the strains isolated from wastewater, *Aspergillus* species were more efficient in removal of chromium. It was observed that *Aspergillus flavus* strain EST_F–241 and *Aspergillus clavatus* strain EST_F–181 showed more than 85% Cr(VI) removal within a week. Removal rates were higher for the first four days. In real tannery wastewater both these strains showed moderate removal efficiencies, though about 2-30% lower than in nutrient media with comparable chromium (VI). Biosorption was always higher than biotransformation of Cr(VI). *Aspergillus flavus* strain EST_F – 241 showed better Cr(VI) removal capacity.

Key words: Fungus, *Aspergillus flavus*, biosorption, biotransformation, chromium, wastewater

INTRODUCTION

Hexavalent chromate [Cr(VI)] compounds are used in important industries like chrome leather tanning, metallurgy, textiles, ceramics, photography and photoengraving [1-3]. Wastewater from such industries contains moderate to excessive amounts of hexavalent chromate compounds. Removal of chromium (VI) from such effluent is absolutely necessary as Cr(VI) is highly toxic and known to cause lung cancer, chromate ulcer, nasal septum perforations, damage to kidney in humans [4,5].

As conventional physico-chemical heavy-metal remediation technologies like ion-exchange, reverse osmosis, electrodialysis etc. are becoming extremely expensive, the possibility of use of microorganisms in bioremediation or biorecovery of different heavy metals from industrial effluents is extensively explored by different workers [3,6,7].

Several chromium(VI) resistant microorganisms have been isolated from diversified environments [5,8,9]. Biosorption was reported in several cases of bacteria [4, 10]. Though there were reports of active fungal biosorption of chromium, fungal transformation of hexavalent chromium was insignificantly reported [11-14]. In present study, hexavalent chromium stressed environmental fungal isolates from tannery wastewater were assessed to biosorb and biotransform Cr(VI) from nutrient media and wastewater.

MATERIALS AND METHODS

Sampling and Preservation

Tannery wastewater effluents were aseptically collected from two different sampling sites, firstly from one of the industry (Bengal Reptiles Exporting

Company, situated at Topsia Road, Kolkata) after their conventional treatment (TS-1) and lastly from the effluent discharge canal near Science city (TS-2) (*i.e.* about 400m away from the industry). Samples were collected in polyethylene wide-mouth bottles of minimum sample size 500ml. It was important to avoid surface materials during sampling. Sterilized glass bottles were used for microbial analyses [15]. Samples were taken to laboratory keeping it protected from direct sunlight and heat and analyzed as early as possible. Refrigeration at 4ºC and addition of preservatives, if necessary, were done according to APHA [15].

Physico-chemical and microbial analyses of wastewater of Tannery industries effluent canal

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 [15].

For microbial characterisation of the effluent, samples were plated in plate count agar and Czapec-Dox agar (CDA) media [15]. After incubating at 30±2°C, number of colony forming units (cfu) was enumerated. the fungal strains were isolated, identified, purified and maintained in CDA media.

Selection of tolerant strains

For determining chromium tolerance limit of these microbial strains, the strains were allowed to grow in Czapec-Dox agar media incorporating 0.22 μm Milipore filter-sterilised K_2CrO_4 containing 100, 200, 300, 400 and 500 ppm Cr(VI). After appropriate incubating periods tolerant strains were detected and identified by visible prominence of growth *i.e* colony formation and size.

Organisms used

For chromium bioremediation studies only tolerant fungal strains were selected from wastewater. Fungal strains were: *Rhizopus* sp. strain EST_F –161, *Penicillium* sp. strain EST_F –131, *Aspergillus niger* strain EST_F –134, *Aspergillus flavus* strain EST_F – 241, *Aspergillus clavatus* strain EST_F –181 and *Cladosporium* sp. strain EST_F –142. The strains were maintained on Czapec Dox agar and broth media. The strains were cultured on same broth at ambient temperature of 28±2°C if not stated otherwise, under static condition with occasional stirring. The composition of the Czapec Dox medium was as follows (g/l.): sucrose,30; $NaNO_3$,3.0; K_2HPO_4,1.0; $MgSO_4$, 0.5; KCl,0.5; $FeSO_4$,0.01 with a pH of 7.3. All the chemicals used were of analytical grade.

Hexavalent Chromium removal by selected strains

To study chromium removal capacity of selected fungi, the strains were grown in 100 ml. Czapec Dox broth (pH 7.3) containing 50 ppm Cr^{6+}(as 0.22 μm Milipore filter-sterilised K_2CrO_4) for 7 days at 28±2ºC. The inocula were 2 ml. of fresh spore suspention of inoculum strength: 4-5x 10^3 c.f.u./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 spectrophotometrically by 1,5-Diphenylcarbazide method [15]. In respect with the control sets, hexavalent chromium removal percentages were drawn out. This study helped in selecting better promising strains with higher Cr(VI) removal capacities.

Hexavalent Chromium biosorption and biotransformation

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±2ºC. The inocula were 5 ml. of fresh spore suspention of inoculum strength: 4-5x 10^3 c.f.u./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[15]. In respect with the control sets, hexavalent chromium removal percentages were calculated.

RESULTS AND DISCUSSION

Bioremediation of metal containing wastewater is though a very new concept, still it is really advancing field of research. Different types of interactions like biotransformation, biosorption and binding of heavy metals with extracellular or intracellular organelles are effectively used and scientifically manipulated to perform with higher effectiveness.

Chemical parameters of the wastewater samples like pH and BOD_5 were not significantly different for the two samples taken from different locations (Table-1). The pH was slightly alkaline in nature. Hexavalent chromium was higher and almost double in the sample taken from the exit-point of tannery industry than in wastewater canal. But both values (47.6 and 23.2 mg/l) were too high than the permissible limit (0.1 mg/l) of hexavalent chromium in wastewater. Sample from wastewater canal contained more bacteria and fungi as identified from their colony-forming units in proper media. Samples from the two sampling sites differ in their chemical as well as

biological parameters. 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.. Bacterial and fungal load of the sample collected near Science City were more than double than the sample from industry itself probably due to more and more contamination with decrease in chromium load.

For determination of chromate tolerance, all the isolated bacteria and fungi were grown in Cr-supplemented media. The results were shown in Table 2. It was evident from the table that bacteria are comparatively more tolerant to Cr(VI) than fungi. Six out of 56 fungi were able to grow even at 400 ppm Cr(VI) within 4 days of incubation, while 12 out of 42 bacteria showed growth at the same concentration.

Only the six fungal strains, which had shown tolerance and good growth rate to up to 300 mg./l chromium, were selected for further studies. Among the strains isolated from wastewater, *Aspergillus* species were more efficient in removal of chromium (Figure-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 respectively in this experiment within a week. Removal rates were higher for the first four days (Fig. 1).

Biosorption and biotransformation rates for these two strains were assessed in tannery wastewater too. It was 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 (Figure 2). Total removal in case of *Aspergillus flavus* strain EST_F –241 were 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 rest was biosorption. Total removal in case of *Aspergillus clavatus* strain EST_F –181 were 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 rest was biosorption. Biosorption was always higher than biotransformation of Cr(VI). 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 bio-accumulation by such micro-organisms 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 [6]. 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 little [16]. *Aspergillus flavus* strain EST_F –241 showed better Cr(VI) removal capacity and found to be very effective in control of chromium pollution or in treatment of tannery wastewater.

CONCLUSION

The identification of promising organism and better understanding of optimum physico-chemical parameters regulating biosorption and biotransformation capacity would assist in the optimisation of performance of the new biosorbent materials in detoxifying metal-bearing industrial effluents [5]. In developing countries such an effluent treatment system where little infrastructure, manpower and recurrent cost is involved, is of immense importance in pollution abatement technology [17]. *Aspergillus* species were found more efficient in removal of chromium even in wastewater. These strains could be used for hexavalent chromium abatement after detailed study.

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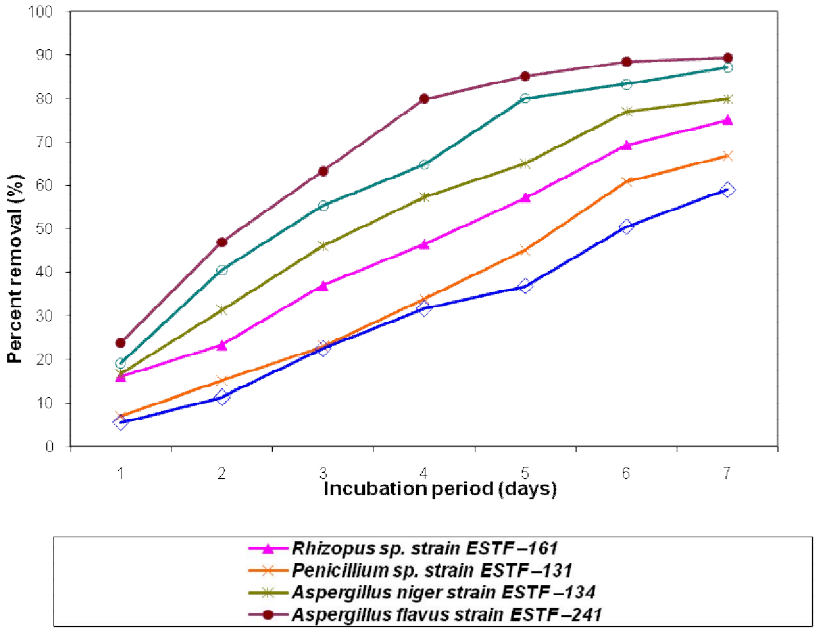
Table-1
Physico-chemical and microbiological characteristics of tannery wastewater

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±0.4) $x10^4$	(3.2±0.5) $x10^4$
Fungal load (cfu/ml)	(7.6±0.7) $x10^3$	(1.7±0.3) $x10^4$

Table-2
Hexavalent Chromium (CrVI) 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



Control adsorption 0.4±0.2%.
Fig. 1
Removal of Chromium(VI) from 50ppm(mg/l) CrVI containing CDB (100ml,28C,pH7.3) by fungal strains in addition to control.

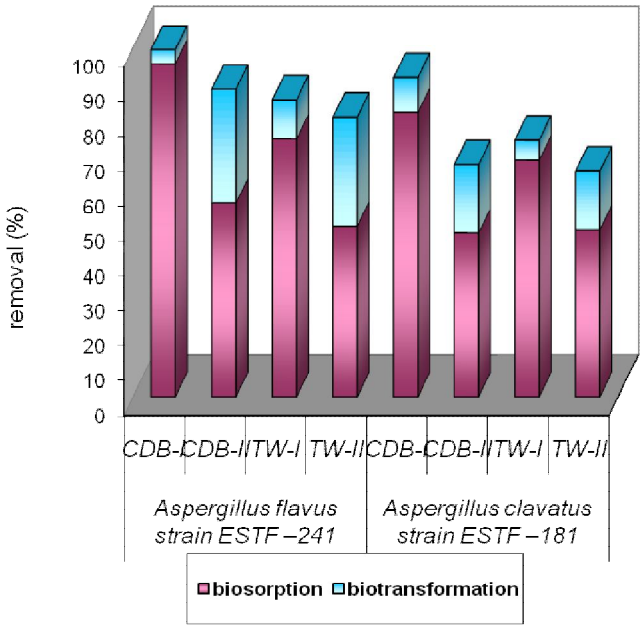


Fig. 2
Removal of chromium (VI) by biosorption and biotransformation from CD-broth (CDB-I & II) and tannery wastewater(TW-I & II) in 5 days

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