J. Mycopathol. Res. **46**(1) : 77-80 (2008)

Characterization of simultaneous bio-accumulation and bio-reduction of hexavalent Chromium by an *Aspergillus flavus* isolate (EST_F-241)

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Environmental pollution arising out of tannery wastewater effluent is a significant problem and a matter of great concern to these industries even as stricter pollution norms are re-introduced in recent years. Tannery industries generate huge amount of effluent with very high organic load. As the leather tanning industries commonly use the chrome-tanning method, the effluent contains very high amount of toxic chromate salts.

In eastern Kolkata area there are more than 500 small to large tanneries, most of which use chrome-liquor for leather tanning. As a result the effluent generated contain haxavalent chromate compounds in huge amount, higher than the Pollution Control Board norms. Effluent containing high amount of toxic hexavalent chromate [Cr (VI)] salts leads to high toxicity when fed to plants as well as in animals. For bioremediation of chromium containing wastewater, different types of interactions like biotransformation, bio-absorption and binding of heavy metals with extracellular or intracellular organelles are effectively used. After initial screening, simultaneous bio-accumulation and bio-reduction of Cr(VI) by a chromium resistant environmental isolate of *Aspergillus flavus* EST_F-241 was studied under different cultural conditions. In batch-culture of chromium supplemented media, effects of different parameters like pH, initial Cr(VI) concentration and additional nutrients were studied. Neutral to slightly alkaline pH was found to be best for bio-accumulation while pH 8.0 was best for reduction to Cr(III). Temperature did not seem to have any significant impact on chromium removal in range of 26-34^{0}C. The candidate strain was more efficient when applied in lower concentrations (25-50 ppm) of chromium in absence of other metal ions. Additional nutrients like peptone or sucrose promoted bio-absorption as well as bio-reduction to remove more than 99% hexavalent chromium. Even with cheaper source like sugarcane-molasses, 95% removal was possible.

Key words : Chromium, bio-accumulation, bio-reduction, tannery, *Aspergillus flavus*

INTRODUCTION

Hexavalent chromate [Cr(VI)] compounds are used in industries like chrome leather tanning, metallurgy, chrome plating, textiles, ceramics, photography and photoengraving (Komori *et al.,* 1990; Langard, 1980). Wastewater effluent from such industries contain moderate to excessive amounts of hexavalent chromate compounds. Treatment or remediation towards 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 and damage to kidney in humans (Bhide *et al.,* 1996). Due to expensive nature of the conventional physico-chemical technologies, application of microorganisms in bioremediation or biorecovery of different heavy metals from industrial effluents is thus extensively explored by different workers (Brierley *et al.,* 1989 ; Wong *et al.,* 1993).

Though there are reports of active fungal biosorption of chromium reports on fungal transformation of hexavalent chromium has not been adequetly reported (Asheh and Duvnjak, 1995 ; Ul Haq and Shakoori, 1998). In the present study we have studied simultaneous bio-absorption and bioreduction of Cr(VI) by a Cr-resistant environmental isolate of *Aspergillus flavus* (Link ex Fries) ES_FT-241 under different active cultural conditions.

MATERIALS AND METHODS

After initial screening for potential for bio-absorption and reduction of Cr(VI), a promising *Aspergillus flavus* ES_FT-241, isolated from Kolkata tannery industries wastewater was selected for detailed study.

The strain was maintained on Czapec Dox agar and broth media. The strain was cultured on the same broth at ambient temperature of 28 ±2°C if not otherwise stated, under static condition with occasional stirring. The composition of the Czapec Dox medium was al 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.

A batch culture method was used to determine simultaneous bio-absorption and bio-transformation of Cr(VI) by aforesaid strain. In 250 ml Erlenmeyer flasks Czapec Dox media of appximately 100 ml were poured and sterilised by autoclaving and supplemented with 0.22 μm Milipore filter-sterilized K_2CrO_4 aqueous solution as per the requirement of experiments. The final vulume of Cr(VI) supplemented media was maintained at 100 ml. Two sets of controls, one without fungus (to eliminate any non-specific adsorption or reduction) and one without chromium were always maintained. All the experiments were repeated at least thrice and their mean values were represented.

The media were aseptically inoculated with 1-2 loopful of freshly grown culture in C-D broth. After completion of 5 days of incubation under stated conditions the fungal biomass was harvested by filtration using 0.22 μm Milipore filter and then their dry weights were recorded. For chromium bio-absorption determination, biomass was digested in 3 : 1 HNO_3 : HCl acid mixture (Luli *et al.,* 1983) and then studied in Varian AA-575 ABQ Atomic Absorption spectrophotometer (APHA, 1992). For determination of biotransformation of hexavalent chromium to its trivalent form, the filtrate was taken out and residual hexavalent and trivalent chromium estimation were done spectrophotometrically in Milton Roy Spectronic 20 by 1,5-Diphenylcarbazide method (APHA, 1992). In respect with the control sets, hexavalent chromium accumulation and reduction percentages were calculated.

The effects of different pH, ranging 3.0 to 9.0, were studied by changing media pH by addition of 10 mM HCl or NaOH prior to inoculation. The initial Cr(VI) concentration was kept at 50 ppm.

To study the effect of initial chromium concentrations, the concentration of Cr(VI) in media was varied to 25, 50, 75 and 100 ppm having normal pH of 7.3. After incubation of 5 days, bio-absorption and reduction were measured.

Additional nutrients like sucrose, peptone and sugarcane-molasses (commercial grade) were added in form of aquous solution after filter-sterilization in 1g/L concentrations and their impact on accumulation and reduction was observed.

RESULTS AND DISCUSSION

From the preliminary study, it was evident that after the initial inhibition, there was little difference in growth rate being in CD media with or without Cr(VI).

Development of biosorption technologies requires the identification of promising organism and optimum physico-chemical parameters regulating biosorption capacity of that organism. Better understanding of the biosorption mechanism and impact of outside variables regulating heavy metals binding would assist in the optimisation of performance of the new biosorbent materials in detoxifying metal-bearing industrial effluents.

Table 1 depicts the specific values of accumulation of Cr(VI) by fungal biomass grown in a medium with respective initial pH. Growth was represented by dry weight of biomass. With the increase in initial pH, accumulation of Cr(VI) increased. The optimum pH was found to be pH 8.0 with a maximum chromium uptake of 16.81 mg g^{-1} dry weight. The high correlation of pH with accumulation indicated that at an elevated pH level more Cr(VI) binding occurred to mycelial surface. The functional groups present on mycelial surface which are chiefly responsible for bio-accumulation of metal could be influenced greatly by pH. Higher pH might result in easier transportation of chromium into the cytosol. The optimum pH for Cr uptake being 8.0, it is a matter of convenience for us as in most cases, Cr(VI) bearing tannery wastewater posses an alkaline pH of 7.5-9.0. It is also to be noted that the growth of fungus was also high in pH range of 7-8. So the total accumulation of chromium by fungal biomass was at a peak around this pH range.

Table 1 : Characteristics of simultaneous bio-accumulation and reduction of haxavalent chromium by the fungal strain. Mean values of at least three replica were represented.

Parameter	Particulars	Cr(VI) bio-accumulation (Mu%)	Bio-transformation of non-accumulated Cr(VI) to Cr (III) (M_R%)	Residual Cr (VI) (M_N%)
pH	3.0	13.06	58.48	36.10
	4.0	18.48	52.75	38.52
	5.0	27.50	53.54	33.68
	6.0	45.20	62.26	20.50
	7.0	54.70	73.16	12.16
	8.0	53.44	82.30	8.24
	9.0	38.60	60.13	24.48
Cr (VI) concn. (ppm)	25	95.80	>95.00	BDL
	50	55.60	73.42	11.80
	75	26.99	55.19	42.30
	100	18.76	43.62	91.60
Nutrient (1g/L)	Sucrose	72.80	>99.00	<0.05 ppm
	Peptone	76.24	>99.00	<0.05 ppm
	Molasses	65.48	77.11	7.90

Metal accumulation studies at varying initial Cr(VI) concentrations showed that metal uptake increased with increase in initial metal concentration at least up to 100 ppm (Table 1). The gradual increase in accumulation capacity by fungal biomass in accordance with initial chromium concentration suggested that interaction and binding affinity of chromium with metal binding or sequestering sites of organism increases with increased Cr concentration. The growth of fungus was highest in 25 ppm while it decreased with increase in Cr(VI) concentration as a result of toxicity.

Impact of additional nutrients especially that of sucrose or peptone on growth as well as on metal accumulation was very good in comparison with normal media (Table 1). In case of added nutrients whether it was sugar or protein, it always enhanced the growth as well as metal-binding capacity of biomass. This might also enhanced uptake capacity of binding sites by providing necessary energy. Microbial transformation of hexavalent chromium to non-toxic, less soluble trivalent form indicates a useful detoxification process. Advantage or speciality of applying living fungal biomass in bioremediation of hexavalent chromium was reduction of non-accumulated Cr(VI) to non-toxic Cr(III) with simultaneous accumulation of hexavalent chromium.

Just like Cr-uptake capacity, bio-transformation of reduction of Cr(VI) was also found to be dependent on different growth parameters (Table 1). In case of a change in pH of media, it was found that the reduction percentage of non-accumulated Cr(VI) was maximum at pH 8.0, where more than 80% Cr(VI) was reduced to Cr(III) leaving only 8.24% Cr(VI) in the medium. In acidic pH, growth as well as bio-reduction capacity decreased. In higher Cr(VI) concentrations very significant decrease was found in bio-transformation. In case of 25 ppm concentration almost 100 % reduction was observed although there was only 4.2 % residual Cr(VI) left for conversion after bio-accumulation. Additional nutrients like sucrose or peptone enhanced Cr(VI) conversion to Cr(III) very effectively to neutralise all Cr(VI) to non-toxic form.

From the above experiments it was evident that the *Aspergillus* concerned was a promising strain showing high accumulation of Cr(VI) simultaneously with Cr(VI) bio-transformation. Different aspects of metal detoxification by uptake or transformation were studied in some details. The strain was found more efficient in lower concentration of chromium and slightly alkaline pH range. In batch studies, the effect of additional nutrients like peptone or sucrose was excellent removing all hexavalent chromium. In underdeveloped countries like ours such an effluent treatment system where little infrastructure, manpower and recurrent cost are involved, is of immense importance in pollution abatement technology.

ACKNOWLEDGEMENT

The authors are grateful to University Grants Commission, India for financing this research work. Authors are thankful to R.S.I.C., Bose Institute for helping in A.A.S. study.

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(Accepted for publication December 18, 2007)