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HEXAVALENT CHROMIUM REMOVAL FROM AQUEOUS SOLUTION USING *TRICHODERMA VIRIDE*

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ABSTRACT

Chromium, a toxic heavy metal released by different industries is considered to be one of the environmental pollutants. Fungi being effective in removing heavy metals due to excellent metal binding efficiency, in the present study 23 fungi were isolated from tannery effluent. Out of which *Trichoderma viride* was taken under study. Chromium biosorption ability of both live and alkali pretreated *T. viride* was compared and the conditions for chromium removal were also studied. Maximum removal was observed at pH 2, in the initial metal ion concentration of 75 mg/l at 35 ^{o}C, biosorbent dose at 1 g for alkali pretreated and 0.4 g for live biomass and contact time at 150 minutes. Under optimum conditions, maximum removal of chromium was 37 mg/g and 18 mg/g for alkali pretreated and live biomass respectively. The change in protein content was analyzed before and after biosorption of chromium and it was observed that the protein content was higher after biosorption. FTIR analysis showed that chromium-binding sites on fungal cell wall would be –OH group, as there was a shift in the stretching frequency.

KEYWORDS: **Biosorption, Chromium, FTIR, Tannery effluent, *Trichoderma viride*, Alkali pretreated**

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INTRODUCTION

The discharge of Chromium (VI) into aquatic ecosystems has become a worldwide environmental problem. Industries especially the tanneries play a significant role in introducing this pollutant into the aquatic systems. The hexavalent chromium (Cr (VI)) compounds are comparatively much more toxic than those of trivalent chromium (Cr (III)) due to its higher solubility in water, rapid permeability through biological membranes and subsequent interaction with proteins and nucleic acids[1]. Cr (VI) also causes risk to animals and humans since they are reported to be involved in mutagenicity, carcinogenicity and teratogenicity[2,3,4]. Therefore, it is essential to immediate Cr (VI) before being discharged. Conventional methods used for the removal of hexavalent chromium are not often appropriate or very expensive; often generate other wastes that require further disposal and large consumption of chemicals[5]. This led to the search of economic and ecofriendly approach for heavy metal removal In this regard, microorganisms including fungi have been considered as an excellent alternative for heavy metal bioremediation[6]. They offer the advantage of having cell wall material which shows an excellent metal-binding properties[7]. Microbes present in metal polluted sites have adapted and become tolerant to toxic heavy metals[8]. Fungi are reported to exhibit considerable tolerance towards heavy metals and become dominant organisms in some polluted habitats[9]. Fungal tolerance towards a mixture of metals is of high importance both for fungal survival and their application for industrial purposes[10]. Both live and dead fungi have been used for heavy metal removal from aqueous streams[11]. Preference of dead fungal biomass is due to various advantages over live fungi such as, no toxicity limitations, no requirement of growth & nutrient media, storage property for long time period and easy desorption of adsorbed metal ions[12]. Some *Penicillium* sp., *Aspergillus* sp. and *Trichoderma* sp. were also reported to adsorb chromium[13]. Various physical and chemical treatments can be used to enhance the biosorption capacity of the biomass, which led to removal, hiding or exposing chemical groups that bind or exchange with the adsorbed metal ions[14]. Alkali pretreatment including sodium hydroxide, potassium hydroxide, alkaline detergents or other alkaline reagents rupture the cell wall of the microbes and expose additional groups for metal binding[15]. Therefore, in the present investigation, chromium tolerance and biosorption efficacy of *T. viride* was studied.

MATERIALS AND METHODS

i) Collection of tannery effluent

The untreated tannery effluent was collected in a sterile plastic container at 4°C from the Common treatment effluent plant located in Chrompet, Chennai, India during March 2011.

ii) Isolation and identification of fungi

Fungi were isolated from tannery effluent by spread plate method using Potato dextrose agar (PDA) as media and identified using Lactophenol cotton blue[16].

iii) Hexavalent chromium biosorption using Trichoderma viride

a) Preparation of Biosorbent

- **Live biomass:** *T. viride* was inoculated in PD broth and incubated for 7 days. Then the fungal mat was harvested, filtered and was washed with distilled water and stored at -20°C.
- **Alkali pretreated biomass:** Live harvested biomass was treated with 0.5 N sodium hydroxide for 30 minutes and washed with distilled water to set neutral pH, autoclaved for 20 minutes. The pretreated biomass was dried at 60°C for 48 hours in hot air oven and powdered using mortar and pestle[17].

b) Biosorption experiment

100 ml of Cr (VI) ion solution (50 mg/l) was prepared from the stock solution containing 1000 mg/l Cr (VI) solution by dissolving Potassium di chromate ($K_2Cr_2O_7$) in deionized water and pH was adjusted to 7 using 1N NaOH or 1N HCl. 0.4 g of live and alkali pretreated *T. viride* were inoculated and the reaction mixture

was shaken on an orbital shaker at 125 rpm for 60 minutes and incubated at 25°C[18].

c) Estimation of Cr(VI) using Diphenylcarbazide

3 ml of above sample was taken, centrifuged at 6000 rpm for 10 minutes and Cr(VI) concentration was analyzed by taking 1 ml of the supernatant with 9 ml of 0.2 M sulphuric acid and 0.2 ml of 0.25% (w/v) Diphenylcarbazide (DPC) in acetone was added. The absorbance of the pink color was measured at 540 nm using distilled water as reference and the total concentration of Cr (VI) was determined[18]. Bioadsorption experiments were carried out in triplicates and average values were used in the analysis. The amount of chromium uptake (mg/g) was determined using the following equation (Narsi *et al*., 2007).

$$q_e = (C_o - C_e)\ v/w$$

Where, q_e = equilibrium uptake (mg/g)

C_o = initial metal ion concentration (mg/l)

C_e = equilibrium metal ion concentration (mg/l)

v = volume of the solution (l)

w = mass of the sorbent (g)

d) Optimization of various parameters

Effect of pH on the biosorption was investigated with pH range between 2.0 to 10.0, Cr (VI) ion concentration from 25mg/l to 200mg/l, with the temperature between 30-50°C. Biosorbent dose was investigated with varying concentration of Potassium di chromate ($K_2Cr_2O_7$) solution from 0.2-1.2 g with different contact time between 30-180 minutes.

e) Estimation of total protein

Total protein content of both alkali pretreated and live *T. viride* was estimated before and after biosorption[19].

f) Fourier Transform infrared (FTIR) analysis

Both live and alkali pretreated *T. viride* was dried to almost nil moisture and send for FT-IR analysis before and after biosorption[20].

RESULTS

a) Isolation of fungi

Fungi were isolated using PDA which includes *Trichoderma viride* -26*, Aspergillus niger* - 2, *Penicillium* sp. - 1, *Aspergillus tamarii* - 1. The results are presented in Plate 1a&b. Since *T. viride* was dominant, it was taken for further study.

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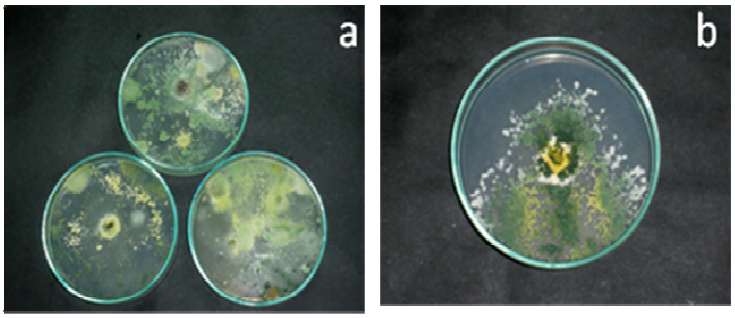

Plate 1: ***(a) Fungi isolated from tannery effluent (b) Trichoderma viride***

b) Effect of pH

The present study indicates that maximum biosorption of Cr(VI) of both live and alkali pretreated *T. viride* was observed at pH 2.0 and biosorption efficiency significantly decreased with increasing pH from 2.0 to 10.0 (Fig 1). It was observed that removal of Chromium(VI) (q_e) was 12mg/g with alkali pretreated *T. viride* and 1.5 mg/g with live fungal biomass at pH 2.0.

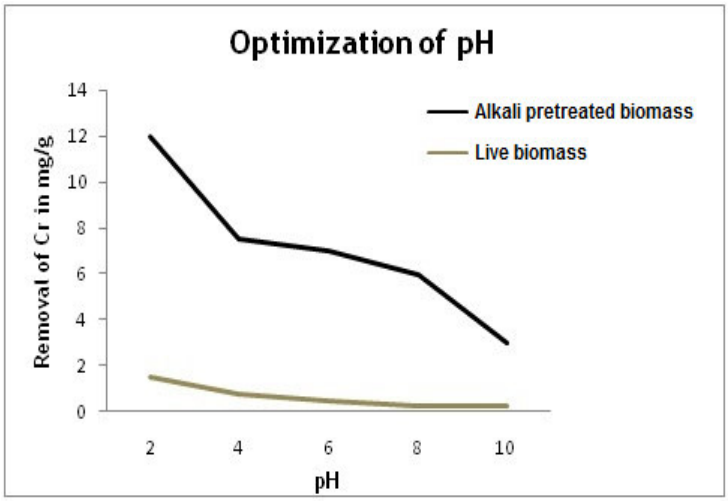

Figure 1: ***Optimization of pH using alkali pretreated and live T. viride***

c) Effect of initial metal ion concentration

The biosorption of Cr(VI) was increased as the initial concentration increased upto 75 mg/l. Then there was a decrease in chromium removal (Fig 2). 10.5 mg/g Chromium(VI) removal was observed with alkali pretreated *T. viride* and 2.25 mg/g with live fungal biomass at 75 mg/l concentration of Cr(VI).

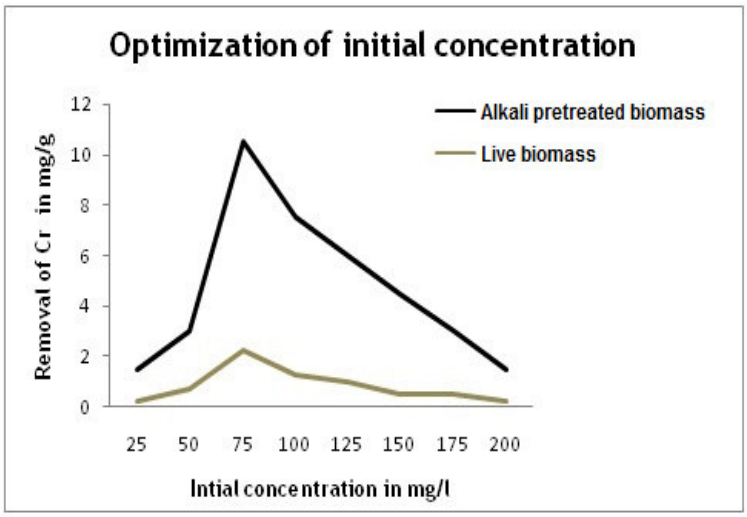

Figure 2: ***Optimization of initial metal ion concentration using alkali pretreated and live T. viride***

d) Effect of temperature

The maximum biosorption of Cr(VI) on both alkali pretreated and live *T. viride* were observed at 35 ^{o}C with 9 mg/g by alkali pretreated fungal biomass and 6.25 mg/g by live fungal biomass (Fig 3) but increase in temperature beyond 35 ^{o}C decreased biosorption of Cr(VI).

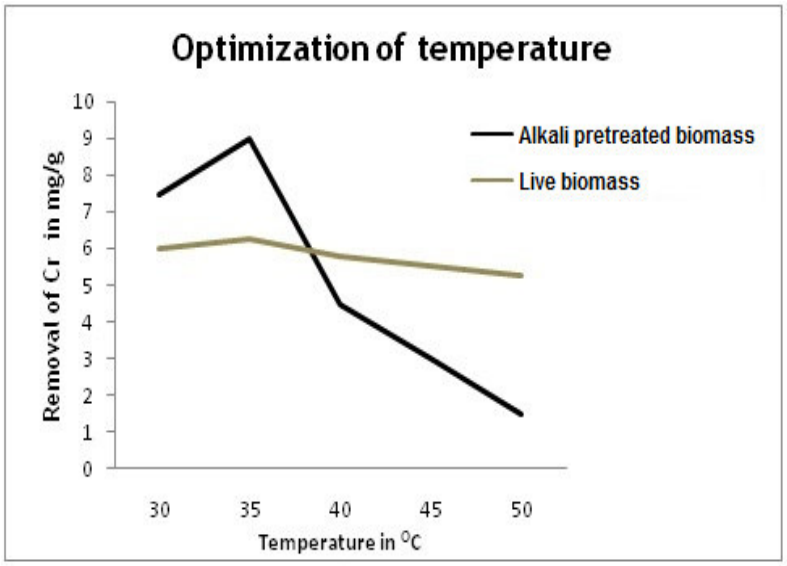

Figure 3: ***Optimization of temperature using alkali pretreated and live T. viride***

e) Effect of biosorbent dose

Adsorption is maximal with 1 g of alkali pretreated fungal biomass and 0.4 g of live fungal biomass where the amount of Cr(VI) removal was 22.5 mg/g with alkali pretreated biomass and 7.5 mg/g with live biomass (Fig 4).

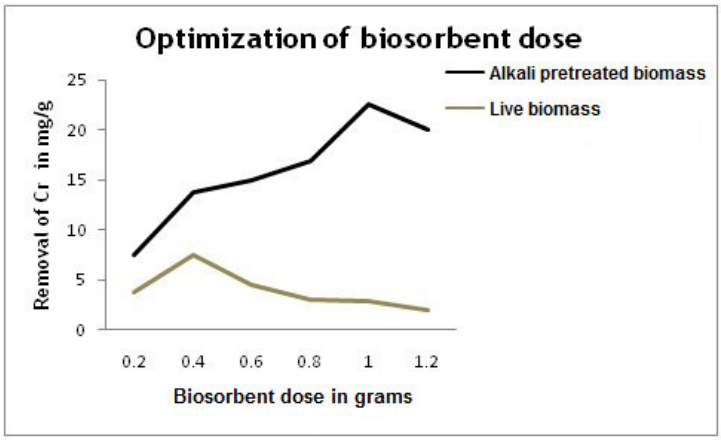

Figure 4: ***Optimization of biosorbent dose using alkali pretreated and live T. viride***

f) Effect of contact time

Maximum Cr(VI) removal was obtained at 150 minutes with 36 mg/g by alkali pretreated fungal biomass and 16.87 mg/g by live fungal biomass (Fig 5). The extent of biosorption efficiency increases sharply with time until 150 minutes and started decreasing later.

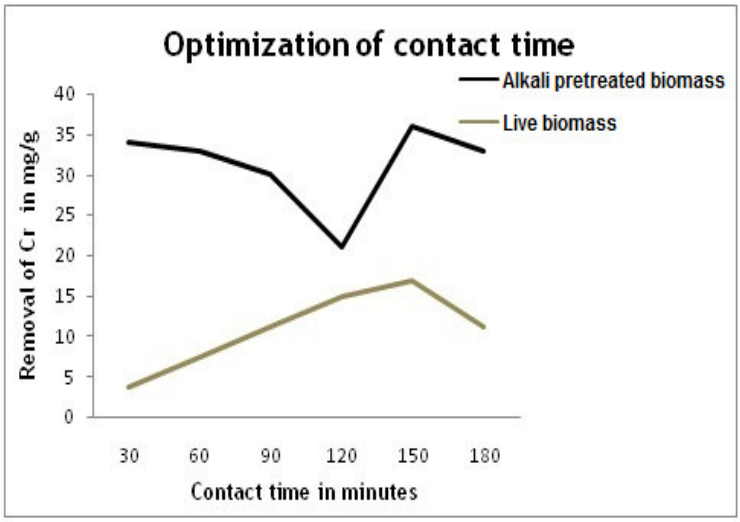

Figure 5: ***Optimization of contact time using alkali pretreated and live T. viride***

g) Hexavalent chromium removal under optimum conditions

Under all optimum conditions (pH – 2, Initial metal ion concentration – 75mg/l, Temperature - 35 ^{o}C, Biosorbent dose - 1 g for alkali pretreated *T. viride* and 0.4 g for live fungal biomass and Contact time – 150 minutes), the maximum chromium removal 37mg/g was observed in alkali pretreated fungal biomass whereas in case of live fungi, the amount of Cr(VI) biosorbed was 18 mg/g **(**Fig 6).

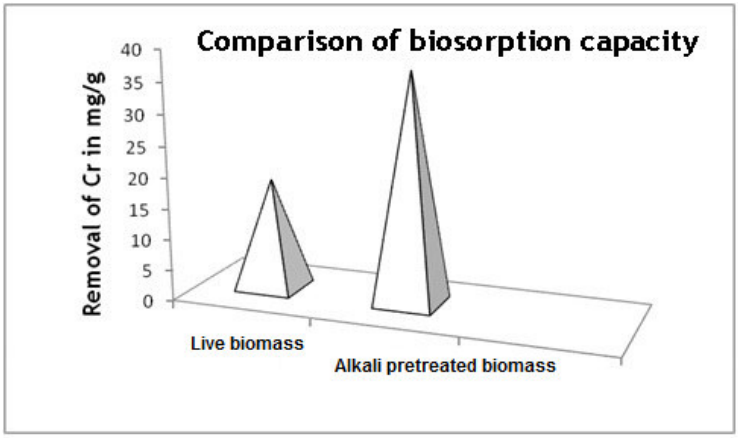

Figure 6: ***Comparison of biosorption capacity between live and alkali pretreated T. viride***

h) Estimation of total protein

The total protein content of both alkali pretreated and live fungi was estimated by Bradford's method before and after biosorption and the results were presented in Table 1.

Table 1: ***Estimation of total protein of Alkali pretreated and live T. viride***

S.No.	Biosorbent	Total protein content (mg/ml)	
		Before Cr(VI) biosorption	After Cr(VI) biosorption
1.	Alkali pretreated biomass	5	18
2.	Live biomass	17	47

i) Fourier Transform Infra Red (FTIR) analysis

Both live and alkali pretreated *T. viride* was subjected to Fourier Transform Infra Red (FTIR) analysis before and after biosorption to determine the functional groups involved in Cr(VI) biosorption. From IR spectra it was observed that the stretching frequency at 3391, 2924, 1746, 1634 cm^{-1} corresponds to hydroxyl group (-OH), alkyl group (-CH_2), carbonyl group (-CO) and alkene group (-C=C) respectively for alkali pretreated *T. viride*. For live biomass, the stretching frequency for alkyl, carbonyl and alkene groups remain the same as that of alkali pretreated biomass where as the stretching frequency at 3396 cm^{-1} corresponds to hydroxyl group (Table 2).

Table 2: ***FTIR adsorption band and corresponding functional groups***

S.No.	Functional groups	Stretching frequency			
		Alkali pretreated		Live	
		Before biosorption (Control)	After biosorption (Test)	Before biosorption (Control)	After biosorption (Test)
1.	–OH	3391	3433	3396	3410
2.	>CH2	2924, 2853	2924, 2853	2924, 2853	2924, 2853
3.	-C=O	1746	1746	1746	1746
4.	C=C	1634	1640	1650	1650

A broad stretching was observed at 3391cm^{-1} for control alkali pretreated (before biosorption) and the stretching frequency got shifted to 3433cm^{-1} for test alkali pretreated (after biosorption) (Fig. 7a & b). This clearly shows the co-ordination of chromium metal with the –OH group present in the alkali pretreated *T. viride*.

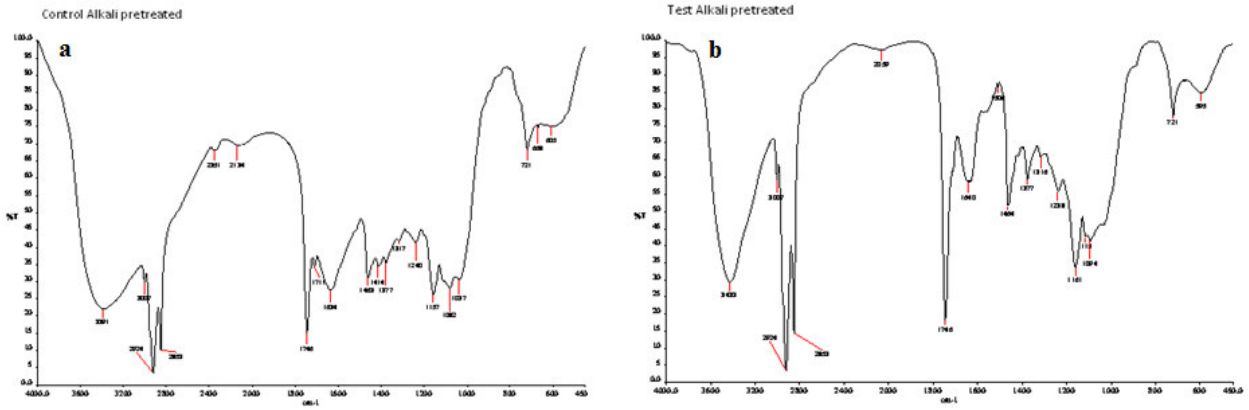

Figure 7a&b: ***FTIR analysis of alkali pretreated T. viride before (Control) and after (Test) biosorption***

In live *T. viride* after biosorption, the chromium metal seems to coordinate with the same functional group and hence there was a shift in the –OH group from 3396 to 3410cm^{-1} (Figure 8a & b) indicates that the live *T. viride* would biosorb Cr(VI). Shift in the stretching frequency of any other functional group was not observed which confirmed the involvement of only–OH group during Cr(VI) biosorption.

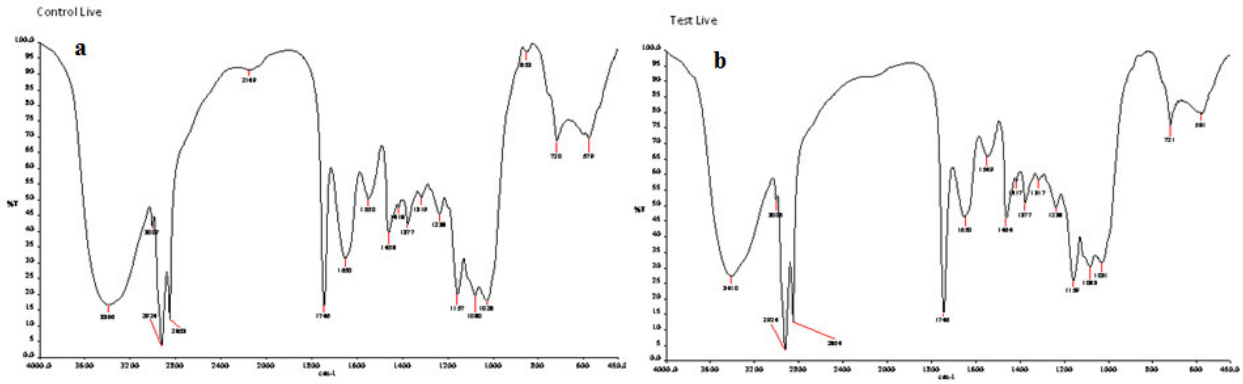

Figure 8a&b: ***FTIR analysis of live T. viride before and after biosorption***

DISCUSSION

In the present study, Fungi were isolated from tannery effluent using PDA in triplicates. Out of 4 fungal isolates, *T. viride* was dominant in all the three plates inoculated. Therefore it was selected for the present study. Chromium biosorption efficiency of *T. viride* was determined using two types of biomass. One is the live biomass and the other is the alkali pretreated biomass. Chromium biosorption using live and alkali pretreated *T. viride* is influenced by various factors such as pH, initial metal ion concentration, temperature, biosorbent dose and contact time. Metal ion adsorption is pH dependent, as the pH affects the availability of metal ions in solution as well as the metal binding sites on the cell surface[21]. In the present investigation, the effect of pH on the chromium removal rate was investigated in the range of 2 to 10. With increasing pH beyond 2, the chromium sorption rate was decreased, which might be due to osmotic changes and hydrolyzing effect[22]. At low pH value, the H^+ ions compete with metal ions for the exchange sites and lead to the partial release of the latter[23]. The rate of chromium sorption decreased with higher initial concentration due to the high competence of ions with their metal binding sites[24]. Similar results were observed with *Trichoderma viride*[18] in immobilized form where the maximum chromium removal was observed at pH 2 with the initial concentration of 75 mg/l. In the present investigation, chromium removal decreased as the temperature is increased. The same result[19] was observed in *Aspergillus* and *Micrococcus* sp. Similar results have been reported in the bioaccumulation of Cr (VI) by *Streptococcus equisimilis* and *Aspergillus niger*[25] which indicate that the temperature affects the bioremediation process in the presence of fungal cells by influencing enzymatic systems[26]. The removal of Chromium(VI) increases rapidly with an increase in the biosorbent dose due to greater availability of the biosorbent. Beyond 1g in case of alkali pretreated biomass and 0.4g in case of live biomass, a decrease in chromium biosorption was observed. This may be attributed to reduction in the total area of biosorbent due to the aggregation and modification of the biomass surface depending on the pH, ionic strength, and temperature[27]. The extent of biosorption efficiency increases sharply with time until 150 minutes and started decreasing later. Similar results were observed by Padma and Dhara[20] with *Trichoderma* sp. Shriram *et al*.[28] also observed that *Trichoderma sp.* (KF284161) has remarkable capacity for Cr removal in his study. Sodium hydroxide pretreatment is an effective method to improve the biosorption capacity for metal ions and it was reported earlier by Ahmad *et al*.[17] Javaid *et al*.[29] also noticed an increase in biosorption of Cu(II) and Ni(II) ions using *Aspergillus niger as* a result of alkali pretreatments particularly Na_2CO_3 and NaOH. Similar enhancement in metal uptake capacity of the fungal biomass regarding alkali pretreatment was recorded by Yan & Viraraghavan[15], El-sayad & El-Morsey[30]

and Das *et al*.[31]. It could be due to chemical modifications of the cell wall components. The modification of biomass probably destroys autolytic enzymes that cause putrification of biomass and remove lipids and proteins that mask the reactive sites[32]. The protein content was higher in live biomass than alkali pretreated biomass. After chromium biosorption, there was an increase in protein content from 5mg/ml to 18mg/ml for alkali pretreated biomass and for live biomass, protein content increased from 17mg/ml to 47mg/ml at 75mg/l concentration of Chromium. Murugesan and Maheswari[33] also observed the protein content to increase to 15.9 mg/ml at 48 hrs. for Pseudomonas sp. at 100 ppm chromium concentration. The increase in the protein content is due to the synthesis of metal binding protein. Andreoni *et al*.[34] also showed an increase in the protein content from 20g/ml to 357 g/ml at 1500 mg/l concentration of lead. The FTIR spectra provide an idea of nature of the cell wall of *T. viride*. The broad stretching of hydroxyl group after chromium biosorption clearly shows the co-ordination of chromium metal with the –OH group present in *T. viride*. Similar result has been reported by Narsi *et al*.[18] The shift difference was higher in alkali pretreated biomass than the live *T. viride* suggesting biosorption efficiency to be higher in the former than in the latter.

CONCLUSION

From this study, it is concluded that alkali pretreated *T. viride* may be considered as a promising alternative which can be used for Cr(VI) bioremediation especially of tannery effluent.

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