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TRICHODERMA HARZIANUM: ADSORPTION, DESORPTION, ISOTHERM AND FTIR STUDIES

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ABSTRACTS

Metal removal capability of indigenous soil fungus of Pakistan i.e. *Trichoderma harzianum* (Rifai) was determined through laboratory bioassays. Experiments were conducted in flasks by taking 0.1 g of powdered fungal biomass in 100 mL (25 mg/L) of metal solution kept at 150 rpm for 3 hours. Results showed that the biomass of *T. harzianum* exhibited significantly greater adsorption efficiency of 65% (capacity: 16.33 mg/g) for uptake of Cr(VI) ions from the aqueous solution. FTIR spectroscopy of the fungal biomass revealed the involvement of chitin and chitosan in metal adsorption process. Adsorption efficiency was significantly decreased (70-50%) and capacity increased (15-50 mg/g) with increase in Cr(VI) concentration from 20, 40…….100 mg/L. Batch data was well-defined by Langmuir and Freundlich isotherms. Solution pH evaluated in the range of 2, 4…..10 found to favor adsorption at pH 2 (efficiency: 70%, capacity 50 mg/g). Adsorption/desorption trials using four acids indicted, maximum desorption of metal was occurred with HNO_3 (q_{des}: 10 mg/g) followed by HCl (9 mg/g), CH_3COOH (8 mg/g) and H_2SO_4 (7 mg/g). Adsorption based trials summarized that dried powdered biomass of *T. harzianum* is suitable and easily available indigenous biomaterial with viable application in the removal of Cr(VI) from the metal-bearing solution within the concentration of 20-100 mg/L.

Keywords: Heavy metal, Cr (VI), waste water, adsorption, Fungus, *Trichoderma harzianum.*

INTRODUCTION

Chromium (Cr) is enlist amongst the priority toxic heavy metals, that occurs in different valence states, but Cr(III) and Cr(VI) are the most dominant. In comparison to Cr(III), the chromate anions of Cr(VI) are highly soluble and are 500 times more toxic because of carcinogenic and mutagenic properties (Kara and Demirbel, 2012). The metal compound gain access to ground and surface water through wastewater of leather tanning, metal finishing, electroplating and chromate preparation. The untreated effluent from electroplating industry contains approximately 100 mg/L of Cr(VI) (Javaid *et al*., 2010), which is much higher than the permissible limit of 0.05 mg/L in drinking water (Baral *et al*., 2006).

Many pysico-chemical treatment methods for Cr(VI)-contaminated water are commonly practiced all over the world. However, these methods are discouraged by the biologists and environmentalists due to prerequisite of excessive chemicals, production of sludge and secondary pollution issues (Deng *et al*., 2009). Ecological friendly biological process named as biosorption is getting attention of scientists from the last two-decades as an acceptable option to clean up the environment without producing harmful waste by-products (Volesky, 2007). Biosorption is a technique that required any entity e.g. microorganisms, algae, plants, agricultural waste etc. to sequester heavy metals from the polluted wastewater. This technique has been proved advance, economical, eco-friendly (low installation and operational costs) and efficient for the remediation of metal bearing wastewater (Volesky and Naja, 2005). Various bacteria, fungi, yeast and algae are known to be capable of adsorbing Cr ions. Ever since, fungal biomasses are the best biosorbents, as they can easily be grown in excessive amount and can be manipulated morphologically and genetically (Park *et al*., 2005). The filamentous morphology and high percentage of cell wall material make fungi as an efficient adsorbent that can work over wide range of pH, temperature and different peaks of metal concentration etc. (Javaid *et al*., 2010). So far, literature revealed tremendous ability of the fungal biomass of *Aspergillus niger*, *A. sydoni* and *Penicillium janthinellum* (Rajender *et al*., 2008), *Trichoderma* species (Vankar and Bajpai, 2008) and *Aspergillus awamori* (Gochev *et al*., 2010) to remove Cr(VI) from aqueous solution.

Besides, the economy of biosorption technique could be more attractive for its users if it is applied along with desorption of adsorbed heavy metal from the adsorbents. Desorption studies provide useful insights into the nature and reversibility of the metal ions through repeated adsorption-desorption processes (Klimiuk and Kuczajowska-Zadrozna, 2002). Such studies will also be effective in predicting the consequences of incorporation of used-up adsorbents into the ecosystems (Ngah and

Hanafiah, 2008) and therefore critical in the design of remediation strategies.

In this study, Cr(VI) ions uptake ability of the dried powdered biomass of *T. harzianum* was assessed from the aqueous solution. FTIR spectroscopy of the fungal biomass was performed to assess the functionality of different metal binding sites. Role of key parameters i.e. pH and initial metal ions concentration were studied along with Langmuir and Freundlich assessments. Reversibility of the sorption reaction through desorption process with different acids were also evaluated.

MATERIALS AND METHODS

Metal: High purity chemicals (MERCK) were utilized in recent investigation. pH of each experiment was adjusted using either NaOH or HCl. To prepare 1000 mg/L of Cr(VI) solution (stock solution), calculated amount of $K_2Cr_2O_7$ (Merck Germany) was added in one liter of double distilled water. Subsequent dilutions of 20, 40, 60 and 100 mg/L were prepared by suitably diluting stock solution with double distilled water.

Adsorbent: *T. harzianum*-FCBP 0139 culture was acquired from the First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, University of the Punjab, Lahore. Pakistan. Fungal biomass was prepared on 2% malt extract and was separated from the media after 7th day of incubation. Collected fungal biomass was rinsed with double distilled water, dried at 60 °C in oven and powdered prior to utilization.

Batch Experiments: Metal removal capacity of the powered biomass of *T. harzianum* was evaluated by taking 0.1 g of adsorbent in 100 mL of 25 mg/L of metal solution at pH 4 in an orbital shaker at 150 rpm for 3 hours in 250 mL Erlenmeyer flasks (Shoaib *et al*., 2012). By keeping pH 4 and temperature constant (25°C), the influence of initial metal ion concentration was investigated over the range of 20, 60…..100 mg/L. pH assessment trials were performed at values of 2, 4, ….10 at the similar metal concentration as mentioned above. After each trial, biomass was filtered and filtrate was analyzed for remaining metal concentration on Atomic absorption spectrophotometer for determination of residual metal ions in the aqueous phase.

For desorption of adsorbed metal, four desorbing acids viz. HNO_3, HCl, CH_3COOH and H_2SO_4 in normality of 0.1N and 0.5N were employed. Residual metal ions were estimated by stirring 100 mL of metal solution at 400 rpm for 60 minutes at 25+1 °C in 250 mL Erlenmeyer flasks followed by Atomic absorption spectrophotometery. The desorption percentage ratio is calculated by calculating acquired metal per gram of biomass (q_{des}) from the concentration of metal desorbents (C_{des}) in the solution (Bayramoglu *et al*., 2005).

$$q_{des} = C_{des}\frac{V}{W} \qquad \%\, desorption\, ratio = \frac{q_{des}}{q_a}\, x100$$

V= volume of solution; W= weight of biosorbent

FTIR analysis: Sample for FTIR, prepared by the method defined by Patty and Feist (2001). By using hexachlorobutadiene and chlorofluorocarbon oils as complementary mulling agents spectra of the biosorbent were studied prior to and after Cr(VI) adsorption.

Data evaluation: The amount of Cr(VI) ions accumulated by the fungal adsorbent and efficiency of biosorption was estimated through following equations (Holan and Volesky, 1994).

$$q = \left(\frac{C_i - C_f}{m}\right)V \qquad E = \left(\frac{C_i - C_f}{C_i}\right)*100$$

$\mathbf{C_i}$ = initial metallic ion concentration (mg/L); $\mathbf{C_f}$ = final metallic ion concentration (mg/L); **m** = dried mass of the biosorbent (g); **V** = volume of solution (mL). Isotherms constitute the equilibrium relationship between metal uptake by adsorbent and the final metal ions concentration in the aqueous solution.

Langmuir (1916) and Freundlich (1906), adsorption isotherm models were applied to acquired investigational data during varied concentration of metal ions.

$$\text{Langmuir model } q_{eq} = \frac{q_{max}\, bC_{eq}}{1 + bC_{eq}} \qquad \text{Freundlich model } q_e = K_F(C_e)^{1/n}$$

Where, q_{eq}= metallic ions adsorbed per unit of weight of adsorbents at equilibrium (mg/g); q_{max}=maximum possible amount of metallic ions adsorbed per unit of weight of adsorbents (mg/g); ***b***= constant related to the affinity of binding sites for metal ions (L/mg); C_{eq}= equilibrium concentration (mg/L); K_F= Freundlich characteristic constant of the system, incorporating parameters affecting the adsorption process, such as adsorption capacity; ***n*** = Freundlich characteristic constant of the system represents the adsorption intensity.

Data regarding various parameters were analyzed through Duncan's Multiple Range Test ($P \leq 0.05$) to separate mean differences (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Fourier transforms infrared spectroscopy: *T. harzianum* is most frequently saprobic soil inhabitant belongs to ascomycota. Fungus cell wall comprised of chitin-a β-(1,4)-linked *N*-acetylglucosamine and β-(1,3) glucan, both are together embedded in the amorphous fraction of α-glucans, galactomannans and other carbohydrate polymers (Latgé, 2007). The polymers of fungal cell wall have many functional groups that could facilitate in metal binding. The evidence of the functionality of these polymers in metal bindings was was confirmed during present FTIR spectroscopy (Fig I A & B). IR spectra of raw biomass *T. harzianum* revealed the broad band at 3300 and 3700 cm^{-1} of H bound hydroxyl (carbohydrate), -NH stretching of amine (protein) and the acetamido group (chitin). Changes in the intensity of 3300 to 3700 cm^{-1} in metal-loaded spectra indicated that free OH and NH groups probably get bound with metal. Alteration in wave number at 2919, 2852, 2358, 2158, 2182 and 2021 cm^{-1} in metal-bounded biomass could be assigned to participation of C-H, C=O and C=N groups in biosorption process. Dropping in wave number in Cr(VI) laden biomass (1729 cm^{-1}) in comparison to raw biomass (1743 cm^{-1}) highlighted the role of C=O stretching of carboxylates in metal uptake. Band position at 1630 cm^{-1} in untreated biomass belongs to *N*-acetyl glucosamine (polymer of the protein peptide bond) manifested significant variation after metal treatment. Likewise, NH of amide-II at finger print region in raw biomass (1547 cm^{-1}) likely supported in electrostatic bindings with negatively charged chromate ions as evidenced by band shifting to low wave number (1516 cm^{-1}) in metal treated biomass. Band at 1371 cm^{-1} revealed the presence of amide-III or sulfamide band. Vibrational shifting of several other functional groups like: C-C, C-O, C-O-P and C-O-C stretching vibrations of polysaccharides at 1234, 1150 and 1020 cm^{-1} to various modes of higher and low stretching frequencies indicated their contribution in Cr(VI) binding onto metal loaded biomass. The bands at < 1000 cm^{-1} correspond to finger print zone of phosphate and sulphur groups.

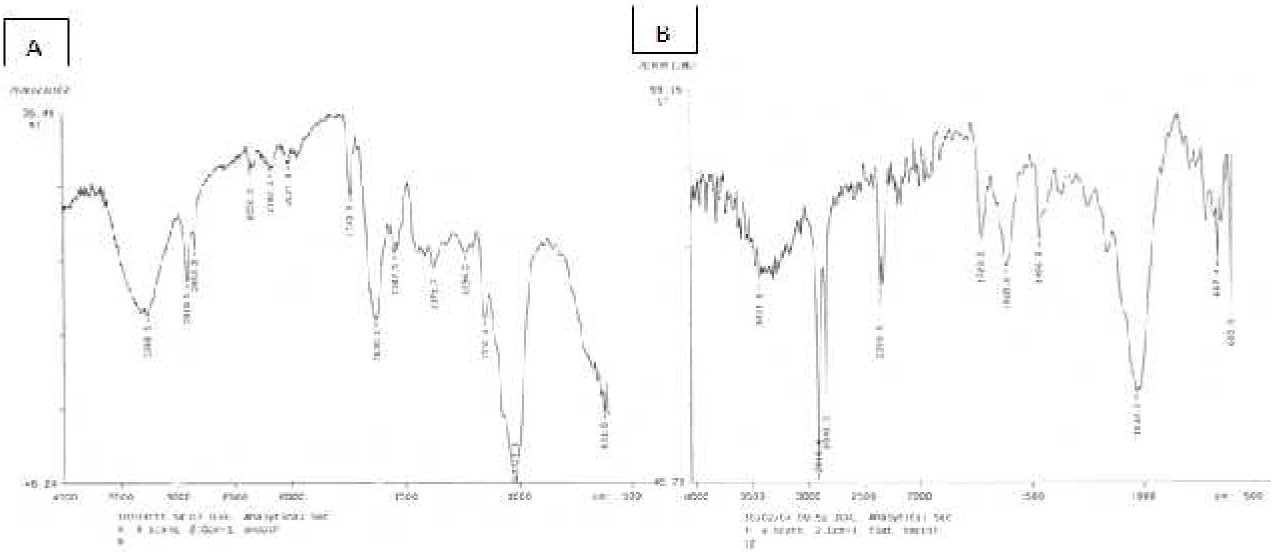

Fig. I: Comparison of Infra red spectra of raw (A) and Cr(VI)-loaded (B) biomass of *T. harzianum*.

Influence of metal ion concentrations: When Cr(VI) ions concentration was raised from 20, 40…..100 mg/L, adsorption efficiency was significantly dropped from 70% to 50% and adsorption capacity was significantly increased from 14 mg/g to 49 mg/g (Fig. II). Occurrence of more unoccupied surface binding sites on the adsorbent at low concentration of metal ions could possibly be responsible for higher adsorption efficiency. Whereas, on increasing metal concentration equilibrium between metal ions and adsorbent's active site probably established very soon thus efficiency decreased due to competitive effect of metal ions for adsorption site. While, adsorption capacity was significantly increased with rising Cr(VI) concentration in the aqueous phase, it could be due to utilization of all active functional groups accessible for the adsorption even at higher metal concentration (Shoaib *et al*., 2012).

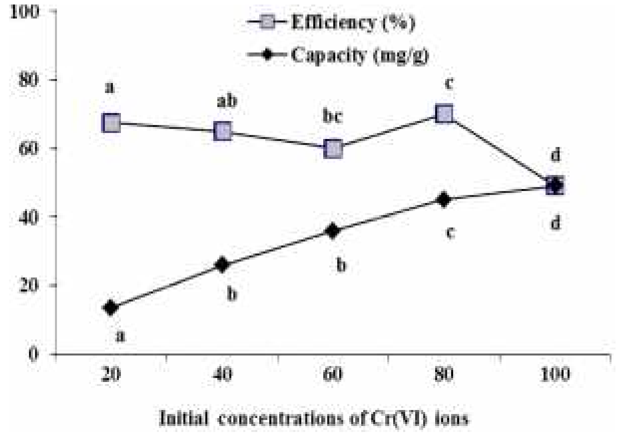

Fig. II: Influence of Cr(VI) concentrations on adsorption efficiency and capacity of the *T. harzianum*. Different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

Langmuir and Freundlich isotherms: The adsorption of hexavalent chromium onto biomass was studied in the light of Langmuir (1916) and Freundlich isotherm (1906). The R^2 (> 0.99) and b (> 0.05) values determined from the Langmuir isotherm showed that the experimental data confirmed well to model (Table I). Adsorption capacity of 50 mg/g was calculated from the Langumir plot is very close to experimental value. Whereas, the stability complex "*b*" formed between adsorbents cell wall and metals ions under specific experimental conditions clearly satisfied the small value i.e., 0.05 mg/L. Ho (2006) related the Langmuir equation with the coverage of adsorbate on a solid surface to concentration of a medium above the solid surface at a constant temperature. Mulgund *et al.* (2011) elucidated the interactions between adsorbing ion per binding site and homogeneous surface of the biosorbent comprises of only one type of binding site.

The Freundlich expression is an experiential equation based on heterogeneous surfaces. Presently, the value of R^2 : 0.97 exhibited the suitability of Freundlich model on the experimental data. The value of n (greater than 1) is accomplishing the condition for favorable adsorption and $K_{F:}$ 0.64 showed easy separations of metal ions from the aqueous medium (Table I). The experimental equilibrium data fitted well to both models with correlation coefficient values greater than 0.9 as reported in a related work (Kehinde *et al*., 2008). Thus, the Langmuir and Freundlich isotherms endow with a flexible logical scaffold for adsorption mechanisms. Isotherm uses a single value to describe maximum adsorption capacity and does not require fitted adsorption capacity values at diverse pH values (Jeppu and Clement, 2012).

Table 1. Langmuir and Freundlich isotherm model parameters for biosorption of Cr(VI) ions onto fungal biomass.

Langmuir isotherm model parameters			
q_{exp} (mg g^{-1})	***q_{max} (mg/g)***	***b (mg/L)***	***R^2***
51	50	0.05	0.99
Freundlich isotherm model parameters			
K_f	***n***	***R^2***	
0.64	1.56	0.97	

pH effect on biosorption of Cr(VI) : Among the five pH values (2, 4, 6, 8, 10), the biomass of *T. harzianum* exhibited the maximum removal efficiency of 70% at pH 2.0 (Fig. III). On increasing pH (pH > 2.0), a downward trend in adsorption efficiency (70-20%) was observed. The results were strongly supported by previous findings of Sivaprakash *et al.* (2009). According to them, at low pH, negatively charged chromate or dichromate binds with the support of electrostatic magnetism to positively charged functional groups (e.g. amine) on the biosorbents surface. When pH increased the whole charge on the adsorbent surface turned into negative and deprotonated carboxylate reduced adsorption efficiency of the fungal biomass. Furthermore, solution chemistry of Cr(VI) ions may affect the biosorption process. Previous investigation of Mohan and Pittma (2006) showed that Cr displayed different types of pH dependent equilibria in the solutions. Biosorbate and Cr found to form stable complexes such as $Cr_2O_7{}^{2-}$, CrO_4^{2-}, $HCrO^4$ and HCr_2O^{7-}, the fraction of any particular species is truly dependent on the pH and concentration of the chromium.

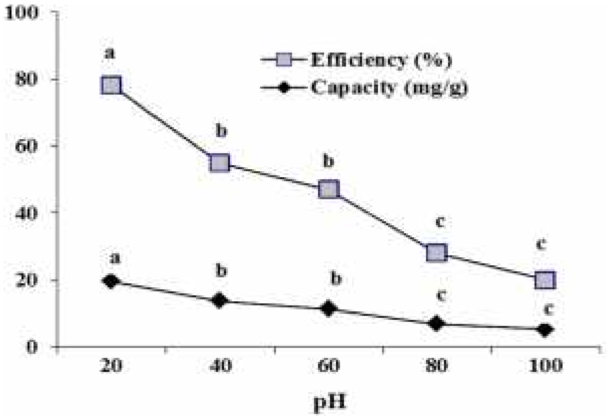

Fig. III: Influence of pH on removal of Cr(VI) ions by *T. harzianum*. Different letters show significant difference (P≤ 0.05) as determined by Duncan's Multiple Range Test.

Desorption: Desorption experiments were performed on the general assumption that restoration of adsorbent prop up the economic of adsorption treatment plant. Adsorption-desorption trial with biomass of *T. harzianum* exhibited adsorption capacity (q) of 16.33 mg/g from 25 mg/L of metal solution. When this metal-loaded biomass was treated with different desorbing agents, a maximum desorption of Cr(VI) was achieved with 0.1M HNO_3 (q_{des}: 11.5 mg/g) followed by 0.5M HNO_3 (11.4 mg/g), 0.1M HNO_3 (9.5 mg/g), 0.1M HCl (9.3 mg/g), 0.5M HCl(9 mg/g), 0.1M H_2SO_4 (8.1 mg/g), 0.1M CH_3COOH (8 mg/g), 0.5M CH_3COOH (7.5 mg/g) and 0.5 M H_2SO_4 (6.1 mg/g) (Fig. IV). Mineral acids like nitric acid and hydrochloric acid seemed to be the most practicably applicable eluting agents. Probably a new low-pH equilibrium attained in desorption system during weak acidity that could possibly prevented the arrant elution and discharge of the adsorbate metal ions into the solution. It has been stated that the comparatively small individual metal affinity differences did not play any role when the concentration of protons is very high, the protons just actually embroiled the metals away from the sorbent (Diniz and Volesky, 2006).

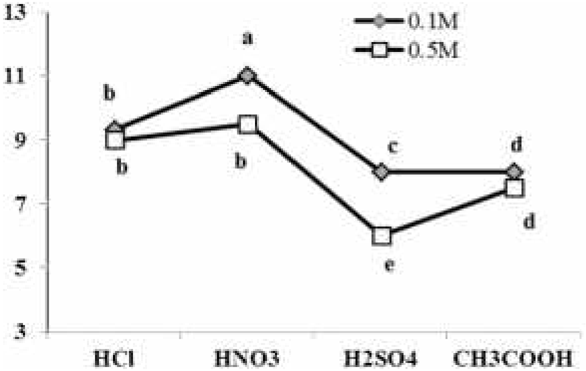

Fig. IV: Ability of different acids to desorp Cr(VI). Different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

CONCLUSIONS: Biomass of *T. harzianum* exhibited considerable potential to uptake Cr(VI) ions from the aqueous solution. FTIR spectroscopy revealed key role of amine of chitin and chitosan of fungal cell wall in metal binding. On increasing metal concentration form 20-100 mg/L, the adsortion efficiency decreased and capacity increased. The well-fitted isotherms viz., Langumir and Freundlich explained that chromium adsorption is favorable for *T. harzianum* biomass. Adsorption/desorption trials showed that Cr(VI) can be effectively desorbed from the fungal biomass using nitric acid. Thus study proposes *T. harzianum* as a prospective and economic adsorbent that can be utilize for the treatment of chromium loaded wastewater.

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