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Biosorption of Cr (VI) with *Trichoderma viride* immobilized fungal biomass and cell free Ca-alginate beads

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Ability of Cr (VI) biosorption with immobilized *Trichoderma viride* biomass and cell free Ca-alginate beads was studied in the present study. Biosorption efficiency in the powdered fungal biomass entrapped in polymeric matric of calcium alginate compared with cell free calcium alginate beads. Effect of *p*H, initial metal ion concentration, time and biomass dose on the Cr (VI) removal by immobilized and cell free Ca-alginate beads were also determined. Biosorption of Cr (VI) was *p*H dependent and the maximum adsorption was observed at *p*H 2.0. The adsorption equilibrium was reached in 90 min. The maximum adsorption capacity of 16.075 mgg^{-1} was observed at dose 0.2 mg in 100 ml of Cr (VI) solution. The high value of kinetics rate constant K_{ad} (3.73×10^{-2}) with immobilized fungal biomass and (3.75×10^{-2}) with cell free Ca-alginate beads showed that the sorption of Cr (VI) ions on immobilized biomass and cell free Ca-alginate beads followed pseudo first order kinetics. The experimental results were fitted satisfactory to the Langmuir and Freundlich isotherm models. The hydroxyl (-OH) and amino (-NH) functional groups were responsible in biosorption of Cr (VI) with fungal biomass spp. *Trichoderma viride* analysed using Fourier Transform Infrared (FTIR) Spectrometer.

Keywords: Biosorption, Immobilized, Calcium alginate, *Trichoderma viride*, Chromium (VI).

Contamination of wastewater by toxic heavy metallic cations is a worldwide environmental problem. Pollution by Cr (VI) usually comes from several industrial processes such as electroplating, fertilizers, pigments, tanning, mining and metallurgical[1, 2]. The removal of toxic or economically important heavy metal ions from wastewater is of great importance from an environmental and industrial view point. The most commonly used procedures for removing Cr (VI) ions from dilute aqueous streams are chemical reduction, precipitation, electrochemical treatment, ion exchange, reverse osmosis and evaporative recovery etc. However, these processes have significant disadvantages, including incomplete metal removal, the need for expensive equipment and monitoring system, high energy requirements, generation of toxic sludge or other waste products that require disposal[3]. Such processes are ineffective or extremely expensive, when initial heavy metal concentration was in the range of 10-100 mgl^{-1}. New technologies are required that can reduce heavy metal concentrations to environmentally acceptable level at affordable costs. Therefore, much attention has been given to the removal of metal ions by microorganisms due to its applications in environmental protection and recovery of toxic or strategic heavy metals[4-6]. Certain types of microbial biomass are considered to retain relatively high quantities of metals by means of passive process known as biosorption. Biosorption technology based on the utilization of dead biomass offers certain major advantages such as lack of toxicity constraints, non-requirement of nutrient supply and recovery of bound metal species by an appropriate desorption method[7]. Cr (VI), because of its mutagenic and carcinogenic properties was selected for bioremediation studies by many researchers employing *Rhizopus*[8, 9] *Aspergillus niger*, *Penicillium janthinellum*[10]. Biosorption mechanisms involved in the process may include ion exchange, co-ordination, complexation, adsorption and microprecipitation[11,12]. Binding sites for metal ions are carboxylic, hydroxylic and phosphate group of lipids, proteins and polysaccharides localized at the cell surface[13,14]. Physical pre-treatment methods such as heating, autoclaving, freeze-drying, boiling and chemical pre-treatment such as using acids, alkali and organic chemical showed enhancement or reduction in metal biosorption, depending on the fungal strains and treatment procedures used[15-17]. Therefore, for

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successful detoxification of toxic heavy metals, the native biomass needs to be immobilized to improve its mechanical strength and resistance to the various chemical constituents of aqueous waste. The immobilized biomass beads could be regenerated and reused in more than 25 cycles and the regeneration efficiency was 75-78%[18]. The aim of this study was to investigate the Cr (VI) biosorption efficiency with immobilized *T. viride* biomass and cell free Ca-alginate beads. The adsorption capacity of the entrapped biomass and cell free Ca-alginate beads were explained by the Freundlich and Langmuir isotherm model and Lagergren first order kinetics.

Materials and Methods

Isolation of the strain—Fungal strain of *T. viride* (G_2S) was isolated from electro-plating industrial soil samples. Fungal strain of *T. viride* was maintained on Rose Bengal medium comprising (gl^{-1})-dextrose, 10; peptone, 5; $MgSO_4$, 0.5; KH_2PO_4, 1; rose bengal, 0.03; streptomycin antibacterial, 0.003 and agar 15.0.

Biomass production—For the production of biomass *T. viride* (G_2S) was cultivated in broth liquid medium on rotating incubator shaker. The *T. viride* (G_2S) spores were transferred to 250 ml Erlenmeyer flask filled with 100 ml of a culture medium composed of (gl^{-1})–bactodextrose, 20; bactopeptone, 10; NaCl, 0.2; $CaCl_2.2H_2O$, 0.1; KCl, 0.1; K_2HPO_4, 0.5; $NaHCO_3$, 0.05; $MgSO_4$, 0.25; and $FeSO_4.7H_2O$, 0.005. The liquid phase *p*H was adjusted to 5.0 by the use of 1N HCl. The flasks were placed on a rotatory incubator shaker at 125 rpm on 25°C. *T. viride* was, thus, cultured aerobically.

Growth of the fungus was observed after two days and it grew as pellicles. The pellicles increased in diameter on subsequent days. The biomass was harvested by filtering through muslin cloth after four days of growth. It was then washed thoroughly with deionized water to remove the growth medium sticking on its surface[19]. The obtained biomass was dried at 60°C in oven for 48 h. The dried biomass was powdered using mortar and pastel. The powder biomass was passed through 300 µm sieve and used for immobilization.

Entrapment in calcium alginate—The powdered biomass was immobilized by entrapment in polymer matric of Na-alginate. 2% (w/v) slurry of sodium alginate was prepared in distilled water. After cooling, 5% (w/v) of biomass were added and stirred on magnetic stirrer. The alginate biomass slurry was introduced into 0.1*M* $CaCl_2.2H_2O$ for polymerization and bead formation using 5 ml syringe. The resultant beads were of 4 mm diameter. The fungus entrapped beads were cured in this solution for 1 h and then washed twice with 200 ml of sterile distilled water[20]. Plain alginate beads were also prepared and stored at 4°C in 5 m*M* of $CaCl_2$ solution until use.

Biosorption experiment—Biosorption studies were conducted in batch process to evaluate the effect of *p*H, initial ion concentrations and contact time at different concentration of biomass dose on removal of Cr (VI). Stock solution Cr (VI) (1000 mgl^{-1}) was prepared by dissolving $K_2Cr_2O_7$ in deionized distilled water. All biosorption experiments were conducted in Erlenmeyer flask (250 ml) on a rotatory BOD incubator shaker (125 rpm) at 25°C. All the experiments were conducted in triplicate and mean value ± standard deviation were used in the analysis of data. The amount of metal uptake (mgg^{-1}) was obtained using the equation[21]:

$$q_e = \frac{(C_0 - C_e)\, v}{w} \quad \ldots (1)$$

where q_e is the equilibrium uptake (mgg^{-1}); C_0 the initial metal ion concentrations (mgl^{-1}); C_e is the equilibrium metal ion concentrations (mgl^{-1}); v the volume of the solution (l); w is the mass of the sorbent (g).

Effect of *p*H on the biosorption rate of the fungal biomass and cell free Ca-alginate beads were investigated in the initial *p*H range from 2.0 to 10.0 at 25°C. 100 ml of Cr (VI) ions solution (50 mgl^{-1}) were prepared from stock solution containing 1000 mgl^{-1} Cr (VI) solution. The *p*H of the solution was adjusted using 1N NaOH and 1 N HCl. 0.4 g of immobilized fungal biomass and cell free Ca-alginate beads were transferred to this medium and the reaction mixture was shaken on an orbital incubator shaker at 125 rpm for 60 min. The initial Cr (VI) ions were varied from 25 mgl^{-1} to 200 mgl^{-1} at optimum *p*H, for 60 min contact time. Similarly the contact time was varied from 30 min to 180 min to determine the optimum biosorption time at optimum *p*H with different Cr (VI) ion concentrations (50, 100, 150 mgl^{-1}). Pseudo first order rate kinetic Lagergren applied and the value of rate constant K_{ad} were derived from Lagergren plots for different Cr (VI) ions concentration. Effect of biosorbent dose was also investigated. Biomass dose was varied from 0.2-1.2 gl^{-1} in 100 ml of 50 mgl^{-1} Cr (VI) solution.

Adsorption isotherms—The adsorption isotherm is the initial experimental test step to determine feasibility of adsorption treatment and whether further test work should be conducted. It is a batch equilibrium test, which provides data relating adsorbate adsorbed per unit weight to the amount of adsorbate remaining in the solution. Adsorption data for wide range of adsorbate concentrations are most conveniently described by the various adsorption isotherms, such as Langmuir or Freundlich isotherm.

The Langmuir model can be described as[9, 22, and 10]

$$q_e = Q_o\, b\, C_e / 1 + b\, C_e \quad \ldots (2)$$

where, q_e is the uptake of metal ions per unit weight of the adsorbent; Q_o is the moles of solute sorbed per unit weight of adsorbent; b is the constant related to affinity between the biosorbents and biosorbate; C_e is the equilibrium (residual) concentration of ions.

The constants, Q_o and b are evaluated from the linear plot of the logarithmic equation.

$$1/q_e = 1/Q_o + 1/bQ_o * 1/C_e \quad \ldots (3)$$

The Langmuir model is based on the assumption that maximum adsorption occurs, when a saturated monolayer of solute molecule is present on the adsorption surface. The energy of adsorption is constant and there is no migration of adsorbate molecule in the surface plane.

The Freundlich isotherm has the form[9, 10]

$$q_e = k\, C_e^{1/n} \quad \ldots (4)$$

The logarithmic form of the equation is given below:

$$\log q_e = \log k + 1/n \log C_e \quad \ldots (5)$$

where, q_e is the uptake of metal ions per unit weight of biosorbent; C_e is the equilibrium concentration of metal ions in solution; k is the Freundlich constants denoting adsorption capacity; n is the empirical constant, is a measure of adsorption intensity.

The value of k and 1/n were found by plotting the graph between log q_e and log C_e. The value of log k is the intercept and value of 1/n is the slope of the plot. After finding the log k, its antilog is found out to calculate k. A high 'k' and 'n' value is indication of high absorption through out the concentration range. A low 'k' and high 'n' indicates low adsorption throughout the studied concentration range. A low 'n' value indicates high adsorption at strong solute concentration. The Freundlich model is basically empirical and was developed for heterogeneous surfaces. The model is a useful means of data description.

Surface analysis—The main functional group present on the cell wall of fungal biomass sp. *Trichoderma viride* was carried out in solid phase. The spectra were recorded in a FTIR-820 IPC with the samples prepared as KBr discs.

Results and Discussion

Biosorption of Cr (VI) with immobilized fungal biomass and cell free Ca-alginate beads without biomass has been found to be influenced by several factors like *p*H, initial Cr (VI) ions concentration, biomass dose, contact time with varying Cr (VI) ions concentration.

*Effect of p*H—The effect of *p*H on Cr (VI) biosorption with immobilized biomass and cell free Ca-alginate beads were studied with varying *p*H 2.0 to 10.0 at 25°C (Fig. 1A). The maximum biosorption of Cr (VI) on both adsorbent were observed at *p*H 2.0 and significantly decreased by increasing the pH values from 2.0 to 4.0. It was observed removal of Cr (VI) 71.13 ± 2.37% with immobilized and 55.21 ± 2.52% with cell free Ca-alginate beads at *p*H 2.0. At each increment of *p*H values, the percent removal of Cr (VI) was decreased up to 5-20% respectively. At low *p*H value, the H^+ ions compete with metal cation for the exchange sites in the system thereby partially releasing the metal cations[23]. The *p*H affects both cell surface metal binding sites and metal chemistry in solution. The dominant species of Cr ions in solution are $HCrO_4^-$, $Cr_2O_7^{2-}$, $Cr_3O_{10}^{2-}$ and $Cr_4O_{13}^{2-}$. These chromate anions would be expected to interact more strongly with positively charged amines of the chitosan in *Rhizopus* cell wall[24, 9].

Effect of initial Cr (VI) ion concentration—The biosorption of Cr (VI) on immobilized fungal biomass and cell free Ca-alginate beads was carried out at different initial Cr (VI) ion concentration 25-200 mgl^{-1}. The per cent adsorption of Cr (VI) was increased as the initial concentration increased up to 25-75 mgl^{-1}, after that percent removal was decreased. Maximum 80.55±2.60% adsorption with immobilized and 70.69±2.23% with cell free Ca-alginate beads was observed at 75 mgl^{-1} (Fig. 1B). At lower concentration, all Cr (VI) ions present in solution could interact with binding sites. Nearly 50-30% reduction in adsorption was observed when the

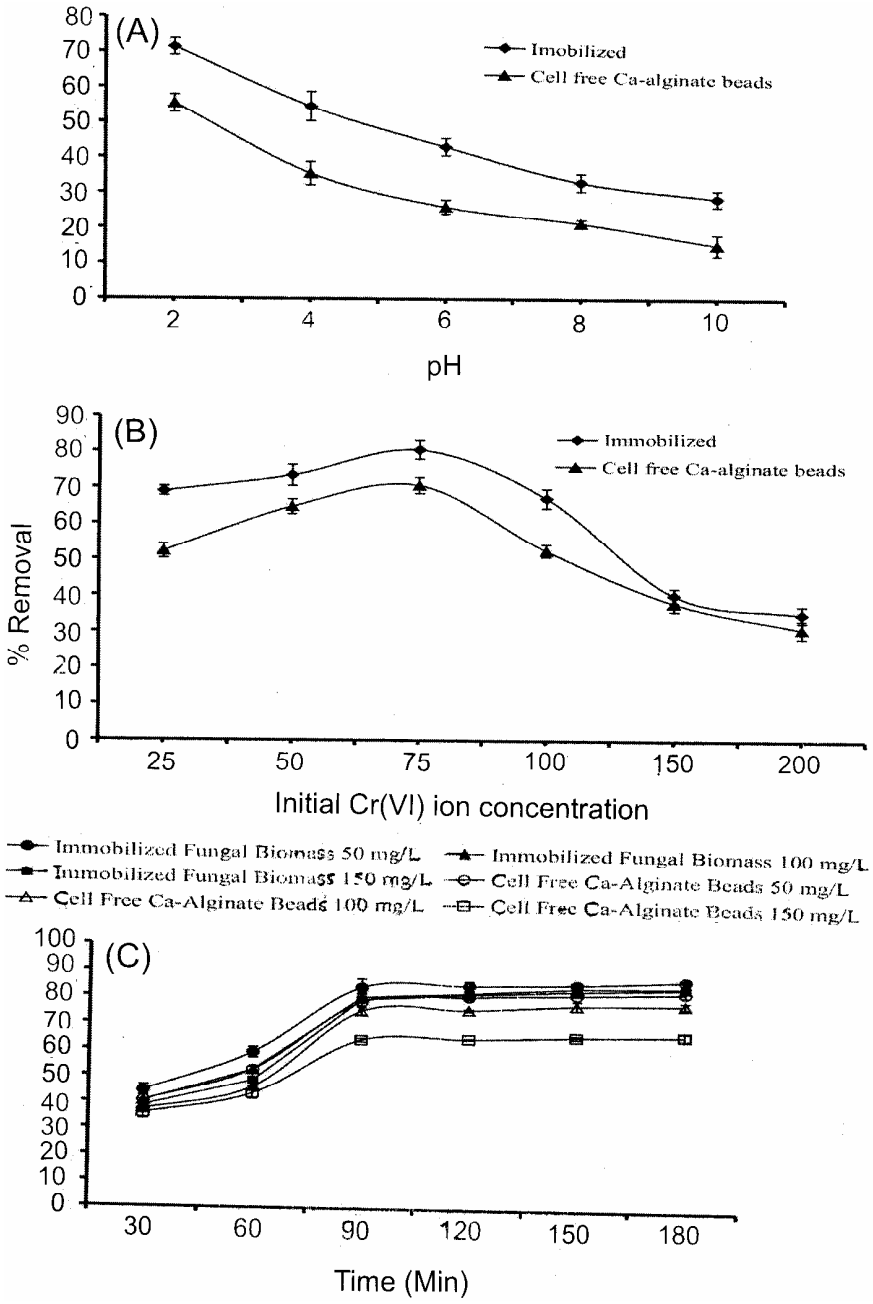

Fig. 1—(A) Effect of *p*H, (B) initial Cr (VI) ions concentration (mgl^{-1}), (C) contact time on Cr (VI) removal with immobilized fungal biomass and cell free Ca-alginate beads

concentration increased from 25-200 mgl^{-1}. This could be because at higher concentration, as more ions are competing for the available binding sites[18]. At higher concentration, more Cr (VI) ions are left unadsorbed in solution due to the saturation of adsorption sites. However, Cr (VI) mgg^{-1} uptake increased at higher concentration of Cr (VI) ions. The number of ions absorbed from solutions of higher concentration is more than that from less concentration solution[9].

Effect of contact time—Maximum Cr (VI) removal with immobilized fungal and cell free beads biomass was in initial period of contact time up to 90 min. After that no applicable amount of Cr (VI) removal was found with both biosorbents (Fig. 1C). This rapid sorption stage indicate that surface sorption occur on the fungal cell beads surface. The extent of adsorption efficiency increases sharply with time and attains equilibrium at about 90 min with both adsorbents for

all the concentration studied (50, 100 and 150 mgl^{-1}). The contact time of 2 h as the equilibrium time for sorption was observed of Ni^{2+} on NaOH treated and untreated bacterial dead *Streptomyces ramous* biomass[2]. After this length of time, the residual concentration was constant. The kinetics of metal adsorption on the cell surface is usually rapid during the initially period of contact time[9]. In order to analyze the sorption of Cr (VI) on immobilized fungal biomass and cell free Ca-alginate beads, follows Pseudo first order Lagergren rate equation for different concentration (50, 100 , 150 mgl^{-1}) of Cr (VI) ions.

Lagergren Pseudo First Order Kinetics[25]

$$\text{Log } (q_e - q_t) = \log q_e - \frac{K_{ad}}{2.303} \times t \qquad \ldots (6)$$

where, K_{ad} (min) is the rate constant of adsorption; q_e and q_t are the amount of Cr (VI) adsorbed (mgg^{-1}) at equilibrium and any time t (min) (Fig. 2A, B). The K_{ad} values are calculated from the graph plotted between log ($q_e - q_t$) and time't' (min). The straight lines confirm that the adsorption process follow first order rate kinetics in each case. The values of adsorption rate constant (K_{ad}) for each concentration and correlation coefficients were given in Table 1. The value of rate constant K_{ad} and correlation regression coefficients (r^2) indicated that the sorption of Cr (VI) ions was higher with immobilized biomass and cell free Ca-alginate beads at concentration of 100 mgl^{-1}.

Effect of adsorbent dose—The Cr (VI) adsorption on the amount of adsorbent dose is studied at 25°C temperature and *p*H 2.0 by varying the adsorbent amount from 0.2 to 1.2 g, while keeping the volume and concentration of the metal solution constant (Fig. 3). But it is apparent that the percent removal of Cr (VI) increases rapidly with increase in the dose due to great availability of the biosorbent. Adsorption is maximum with 1.2 g of immobilized fungal biomass than cell free beads. Fig. 3 shows, the biosorbent concentration was increased 0.2 to 1.2 g in 100 ml, the metal uptake capacity was decreased approximately from 16.075 to 4.06 mgg^{-1}. This may be attributed to reduction of total area of biosorbent, due to probably by aggregation during biosorption and modification of the biomass surface depending on

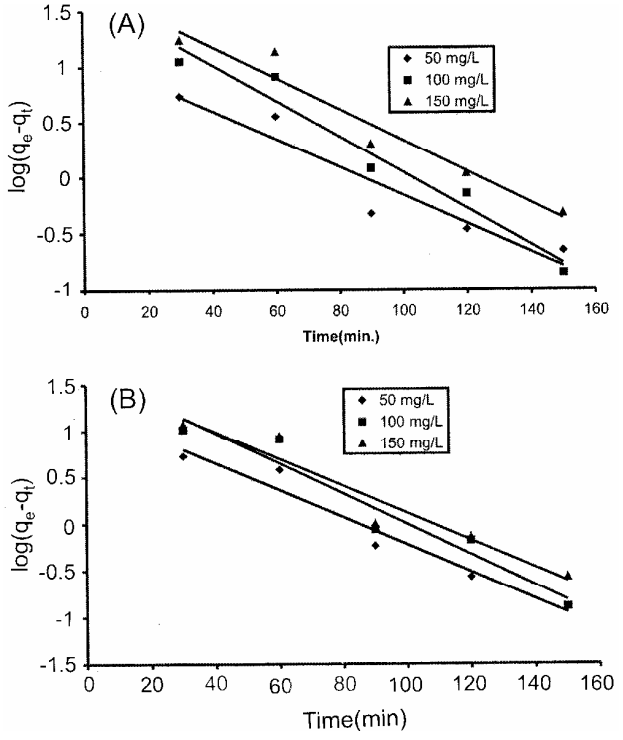

Fig. 2—Lagergren Pseudo first order kinetics for Cr (VI) removal at different Cr (VI) ions concentration (A) immobilized biomass (B) cell free Ca-alginate beads at different Cr (VI) ion concentrations

Table 1—Lagergren pseudo first order kinetics, isotherm constants and coefficient of correlation

Biosorbent	Concentration (mgl^{-1})	Lagergren Constant	
		K_{ad}	r^2
Immobilized Biomass beads	50	2.92×10^{-2}	0.91
	100	3.73×10^{-2}	0.96
	150	3.24×10^{-2}	0.95
Cell free Ca-alginate beads	50	3.36×10^{-2}	0.96
	100	3.75×10^{-2}	0.94
	150	3.36×10^{-2}	0.93

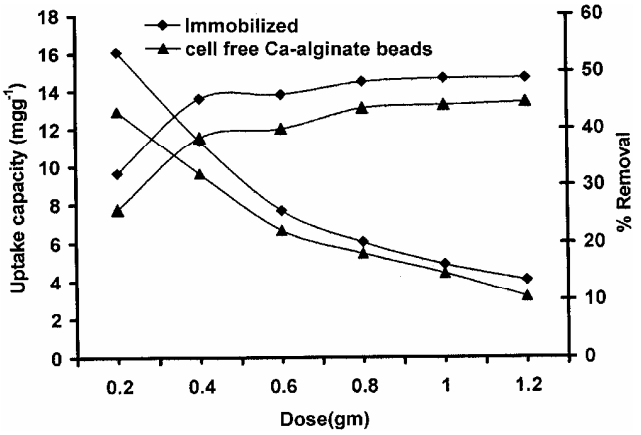

Fig. 3—Effect of adsorbent dose on uptake capacity (mg g^{-1}) and percent removal with immobilized fungal biomass and cell free Ca-alginate beads

the experimental conditions such as *p*H, ionic strength, temperature[26].

Adsorption isotherms of Cr (VI)—The Cr (VI) adsorption isotherm followed Freundlich and Langmuir model. The value of Freundlich constant (k and n) and Langmuir constants (Q_0 and b) were evaluated from Figs 4 and 5 and presented in Table 2. The high value of k (4.76 mgg^{-1}) and n (2.28) with immobilized fungal biomass than cell free Ca-alginate beads k (1.37 mgg^{-1}) and n (1.36) were indicated high adsorption. The high value of Langmuir constant Q_0 (5.39 mgg^{-1}) and b (0.058) with immobilized biomass than cell free Ca-alginate beads i.e. Q_0 (0.93 mgg^{-1}) and b (0.024 lmg^{-1}) were also indicated for better adsorption capacity. The high values of correlation coefficient ($r^2 > 0.90$) also indicate that there is a strong positive relationship in the data. Figures 4 and 5 show typical sorption isotherms for chromium-adsorbent systems. Higher the values of k and n, and lower the value of b indicate better the affinity of the biomass[27-29]. Thus, data indicated that immobilized fungal biomass was better adsorbent than cell free Ca-alginate beads for Cr (VI). This may be due to the more and more binding sites were available on immobilized biomass.

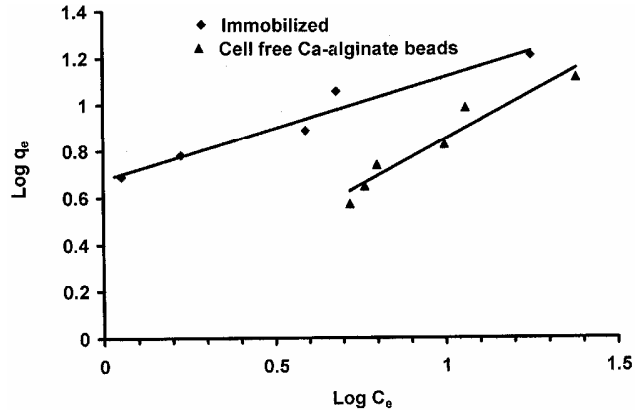

Fig. 4—Freundlich isotherm for Cr (VI) removal with immobilized biomass and cell free Ca-alginate beads

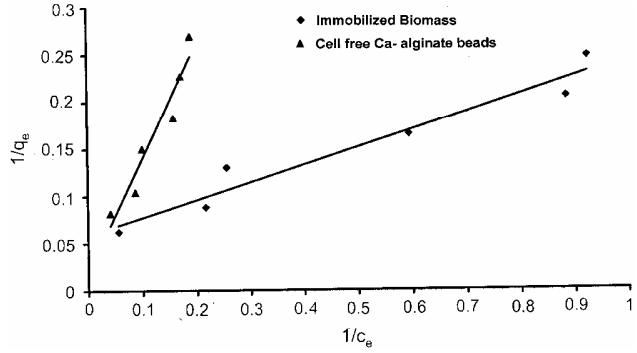

Fig. 5—Langmuir isotherm for Cr (VI) removal with immobilized biomass and cell free Ca-alginate beads

Table 2—Langmuir and Freundlich isotherm constants, coefficient of correlation

Biosorbent	Freundlich constants			Langmuir constants		
	k (mgg^{-1})	n	r^2	Q_0 (mgg^{-1})	b (lmg^{-1})	r^2
Immobilized biomass	4.76	2.28	0.95	5.39	0.0588	0.98
Cell free Ca-alginate beads	1.37	1.36	0.96	0.93	0.024	0.94

IR spectral analysis—Figure 6 shows the IR spectra and the various functional groups of which some participate in the adsorption process. According to adsorption bands significant changes were observed in the functional groups before and after biosorption of Cr (VI). IR spectra give some idea on the nature of the cell wall of *T. viride*. Frequency of the adsorption band and corresponding functional groups are presented in Table 3. The functional groups were observed amide group (-NH), hydroxyl group (-OH), carboxylate anions (-COO), carbonyl groups (-CO), C-F and C-Br mainly responsible in *T. viride* fungal biomass. The broad adsorption peaks 2925.8, 3820.7, 3853.5, 3284.5 cm^{-1} indicative of existence of hydroxyl groups (-OH) and amide groups (- NH) were responsible in biosorption of Cr (VI). In the Cr (VI) loaded biomass of *Eichhornia crassipes* observation peak was also observed 3419 cm^{-1}. The peak of amino and hydroxyl group stretching vibration showed shifting from around 3304 cm^{-1} also indicate the existence of bonded hydroxyl group[30]. The peak of amino and hydroxyl group stretching absorption peaks showed shifting from 3304 cm^{-1} to 3285 cm^{-1} of intact biomass of *S. maxima* and lead loaded biosorbents[31].

In this study, adsorption of Cr (VI) on immobilized biomass and cell free beads has been investigated. The data obtained through this work supports that immobilized fungal biomass is an effective biosorbent for removal of Cr (VI) from aqueous solution than cell free alginate beads. The adsorption of Cr (VI) is dependent on the *p*H, metal ion concentration, contact time and biosorbent dose. The equilibrium adsorption data are correlated by Freundlich and Langmuir isotherm equation. High value of Freundlich constants i.e. k (4.76mgg^{-1}) and n (2.28) and Langmuir constants i.e. Q_0 (5.39 mgg^{-1}) and b (0.058 lmg^{-1}) with

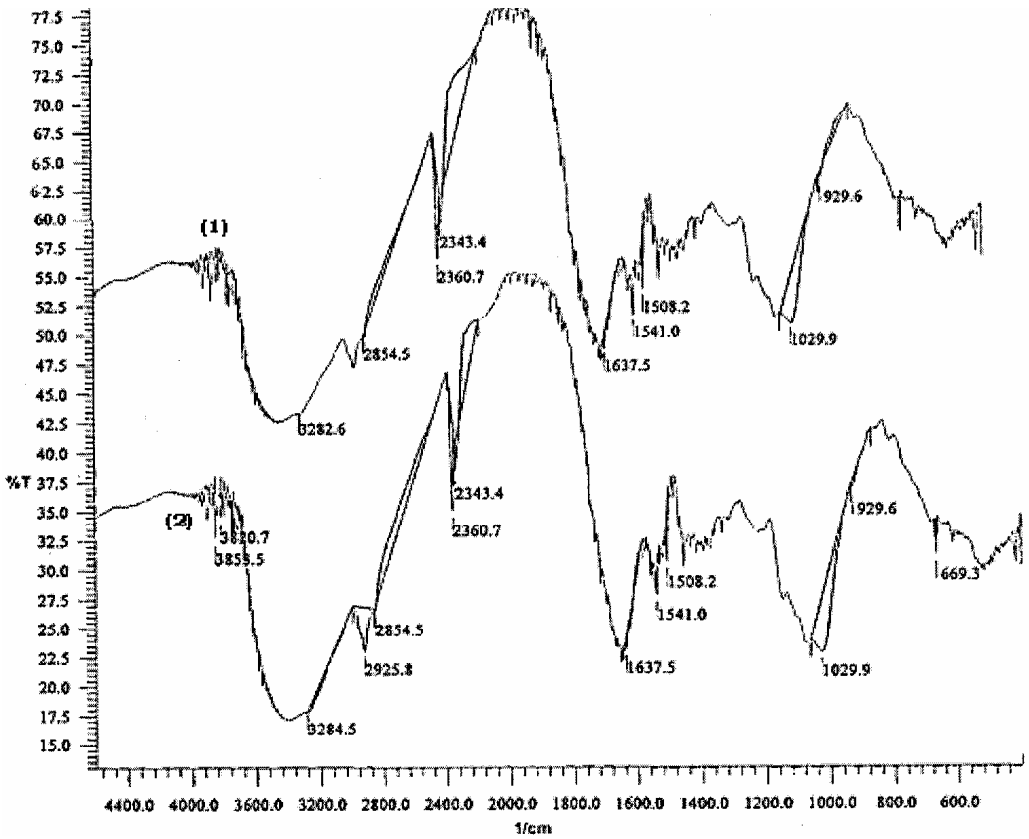

Fig. 6—FTIR spectra (1) before biosorption of Cr (VI (2) after biosorption of Cr (VI)

Table 3—IR adsorption band and corresponding functional groups

Frequency (cm^{-1})	Functional groups
3253.5, 3282.6, 3284.5	N-H, -OH (chelate compound)
2360.7, 2854.8, 2925.8	$>CH_2$, -OH
1508, 1541.0, 1637.5	-NH, C=O, C=C
1029.9	C-F, C-Br
929.6	-OH

immobilized biomass than cell free Ca-alginate beads indicates better adsorption intensity as well as better adsorption capacity. The presence of hydroxyl (-OH) and amino (-NH) functional groups in the *T. viride* fungal biomass before and after Cr (VI) biosorption has been confirmed with IR spectrum.

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References

1 Kaewsarn P & Yu Q, Cadmium (II) removal from aqueous solutions by pre-treated biomass of marine alga *Pudina sp*., *Environ Poll*, 112 (2001) 209.

2 Selatnia A, Madani A, Bakhti M Z, Kertous L, Monasouri Y & Yous R, Biosorption of Ni^{2+} from aqueous solution by a NaOH-treated bacterial dead *Streptomyces rimosus* biomass, *Minerals Engg*, 17 (2004) 903.

3 Dursun A Y, Uslu G, Tepe O, Cuci Y & Ekiz H I, A comparative investigation on the bioaccumulation of heavy metal ions by growing *Rhizopus arrihizus* and *Aspergillus niger*, *Biochem Engg*, 15 (2003) 87.

4 Vokesky B, Removal and recovery of heavy metals by biosorption. In, *Biosorption of heavy metals*, edited by B. Volesky, (CRC Press, Boca Raton, FL) 1990, 7.

5 Chang J S & Hong J, Biosorption of mercury by the inactivated cells of *Pseudomonas aeruginosa* UP21, *Biotech Bioengg*, 44 (1994) 999.

6 Liu H L, Chen B Y, Lan Y W & Cheng Y C, Biosorption of Zn (II) and Cu (II) by the indigenous *Thiobicillus thiooxidans*, *Chem Engg J*, 97 (2004) 195.

7 Gadd G M, Fungi and yeast for metal accumulation. In *Microbial Mineral Recovery*, edited by H.L. Ehrlich and C.L. Baierley, (McGraw Hill, New York) 1990, 249.

8 Niyogi S, Abraham T E & Ramakrishna S, Removal of Cr (VI) ions from industrial effluents by immobilized biomass of *Rhizopus arrihzus*, *J Sci Ind Res*, 57 (1998) 809.

9 Bai S R & Abraham T E, Biosorption of Cr (VI) from aqueous solution by *Rhizopus nigricans*, *Biores Tech*, 79 (2001) 73.

10 Bishnoi N R & Garima, Fungus— an alterantive for bioremediation of heavy metal containing wastewater: A review, *J Sci Ind Res*, 64 (2005) 93.

11 Guibal E, Roulph C & LeCloirec P, Uranium biosorption by

filamentous fungus *Mucor miehei*, *p*H effect on mechanisms and performance of uptake, *Water Res*, 26 (1992) 1139.
12 Fourest E & Roux J C, Heavy metal biosorption by fungal myclial byproducts, mechanism and influence of *p*H, *Appl Microbiol Biotechnol*, 37 (1992) 399.
13 Brown M & Lester J N, Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge, *Water Res*, 16 (1982) 1539.
14 Scott J & Palmer S, Cadmium biosorption by bacterial exopolysaccharide, *Biotech Lett*, 1 (1988) 21.
15 Huang C, Westman D, Quirk K & Huang C P, The removal of cadmium (II) from dilute solutions by fungal biomass, *Water Sci Tech*, 20 (1988), 369.
16 Kuyucak N & Volesky B, Biosorbents for recovery of metals from industrial solutions, *Biotech Lett*, 10 (1998) 137.
17 Kapoor A & Viraraghavan T, Heavy metal biosorption sites in *Aspergillus niger*, *Biores Tech*, 61 (1997) 221.
18 Bai R S & Abraham T E, Studies on Cr (VI) adsorption-desorption using immobilized fungal biomass, *Biores Tech*, 87 (2003) 17.
19 Rao J R & Viraraghvan T, Biosorption of phenol from an aqueous solution by *Aspergillus niger* biomass, *Biores Tech*, 85 (2002) 165.
20 Arica M Y, Kacar Y & Genc O, Entrapment of white-rot fungus *Trametes versicolor* in Ca-alginate beads, Preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution, *Biores Tech*, 80 (2001) 121.
21 Abu Al-Rub F A, El-Naas M K, Benyachia F & Abhour Z, Biosorption of nickel on blank alginate beads, free and immobilized algal cells, *Process Biochem*, 39 (2004) 1767.
22 Shuttle-worth K L & Unz R F, Sorption of heavy metals to the filamentous bacterium *Thiothrix* strain AI, *Appl Enviorn Microbiol*, 59 (1993) 1274.
23 Ajmal M D, Rao R A K, Ahmad R & Ahmad J, Adsorption studies on Citrus reticulate (fruit peel of orange), removal and recovery of Ni (II) from electroplating wastewater, *J Hazard Mater*, 379 (2000) 117.
24 Mor S, Khaiwal R & Bishnoi N R, Adsorption of chromium from aqueous solution by activated alumina and activated charcol, *Biores Tech*, (2007) 957.
25 Namasivayam C & Ranganathan K, Removal of Fe(II) by waste Fe(III)/Cr(III) hydroxide from aqueous solution and electroplating industry waste water, *Indian J Chem Tech*, 1 (1995) 351.
26 Veligo F & Beolchini F, Removal of metals by biosorption—A review, *Hydrometallurgy*, 44 (1997) 301.
27 Saravanane R, Sundararajan T & Sivamurthyreddy S, Efficiency of chemically modified low cost adsorbents for the removal of heavy metals from wastewater: A comparative study, *Indian J Env Hlth*, 44 (2002) 78.
28 Asku Z, Kutsal T, Gun S, Haciosmanoglu N & Gholminejad M, Investigation of biosorption of Cu (II), Ni (II) and Cr (VI) ions to activated sludge bacteria, *Environ Tech*, 12 (1991) 915.
29 Jalaliet R, Ghafourian H, Asef Y, Davarpanah S & Sepehr S, Removal and recovery of lead using nonliving biomass of marine algae, *J Hazar Mater*, 92 (2002) 253.
30 Mohanty K, Jha M, Meikap B C & Biswas M N, Biosorption of Cr (VI) from aqueous solutions by *Eichornia Crassipes*,*Chem Eng J*,117 (2006) 77.
31 Gong R, Ding Y, Liu H, Chen Q & Liu Z, Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass, *Chemos*, 58 (2005) 125.