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Chromium (VI) biosorption by immobilized *Aspergillus niger* in continuous flow system with special reference to FTIR analysis

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Abstract: *Aspergillus niger was treated with acid and immobilized in calcium alginate matrix. The dynamic removal of Cr (VI) ion was studied using continuously fed column packed with immobilized biosorbent beads. Column experiments were carried out to study the effect of various bed heights (20, 30, 40 cm) under different flow rates (5, 7.5, 10 ml min^{-1}) on efficiency of biosorption. The maximum time (1020 minutes; 17 hr) before breakthrough point was observed in case of 40 cm bed height with flow rate of 5ml min^{-1}. FTIR analysis of acid treated immobilized A.niger was used for a qualitative and preliminary analysis of chemical functional groups present on its cell wall which provided the information on nature of cell wall and Cr (VI) interaction during the process of biosorption. The IR spectra of biosorbent recorded before and after chromium biosorption had shown some changes in the band patterns, which were finally analyzed and was found that chemical interaction such as ion-exchange between carboxyl (-COOH), hydroxyl (-OH) and amine ($-NH_2$) group of biosorbent and Chromium ion were mainly involved in biosorption of Cr (VI) onto A. niger cell wall surface. The biosorbed metal was eluted from biosorbent by using 0.1 M H_2SO_4 as eluant. Immobilized biosorbent could be reused for, five consecutive biosorption and desorption cycles without apparent loss of efficiency after its reconditioning. Considering all above factors together this paper discusses the efficient chromium biosorption process carried out by immobilized A. niger biosorbent.*

Key words: *Aspergillus niger, Chromium, Biosorbent, Heavy metal*
PDF of full length paper is available online

Introduction

Increased heavy metal concentration in natural systems derived from natural and anthropogenic sources pose a severe threat to environment. In many areas, natural sources are overshadowed by anthropogenic inputs from industrial effluents. Co, Pb, Cr, Cd, Ni, Zn and Hg are the common heavy metal pollutants found in industrial effluents. The toxicity of these metals, not only affects plants and animals but also human beings. Concentration of heavy metal pollutants in industrial discharge depend on manufacturing process and other factor such as house keeping, reuse and technology *etc.* Major industries which release metal containing polluted water are electroplating, metal processing, leather tanning, automotive, rubber and plastic, paint, pesticides, cement, chemical, distilleries, electrical and machinery industries *etc.* (Volesky, 2001).

Chromium is highly toxic element, regulated with respect to its oxidation state between Cr (III) and Cr (VI). Out of these Cr (VI) is most toxic form of chromium. Cr(VI) is released during many industrial processes including electroplating, leather tanning and pigment manufacturing (Faisal and Hasnain, 2004; Richard and Bourg, 1991). These industries are the major anthropogenic source of Chromium metal in water. More than 1,70,000 tones of chromium waste are discharged annually in environment as a result of industrial and manufacturing activities (Abassi *et al.*, 1998) that releases Cr(VI) in water. The permissible limit of Cr (VI) ion in waste water discharged from industrial and municipal effluent in inland water is 1.0 mg l^{-1} (CPCB, 2004). Cr (VI) is considered to be the most toxic form as it is highly soluble and thus easily taken up by the cells (Cervants *et al.*, 2001; Sarangi and Krishanan, 2008). Cr(VI) causes liver and kidney damage, internal hemorrhage, dermatitis, respiratory damage, lung cancer and skin ulcers (Harie *et al.*, 1993). It also damages DNA through interface with DNA Polymerase enzyme and free radical formation. To comply with the permissible limit, various techniques are used for the removal of chromium. The recovery of chromium using conventional techniques like Reverse osmosis, Electrodialysis, Ultrafiltration, Ion-exchange, Chemical precipitation etc. are neither economical nor eco-friendly. So effective methods have to be evolved for removal of Cr (VI) from industrial effluent before disposal, in order to avoid possible acute and chronic chromium poisoning of living beings.

In this endeavor, Biosorption has emerged as an alternative to conventional effluent treatment methods as it posses advantages of low operating cost, high effectivity in dilute solutions, generation of minimum secondary waste, completion within short time period and havingo no toxicity limit for heavy metals (Dadhich *et al.*, 2004; Ahalya *et al.*, 2005). Biosorption is the term that describes the removal of heavy metals by passive binding to nonliving biomass from aqueous solution. Biosorption is attractive since naturally occurring biomass or spent biomass from various fermentation industries can be effectively utilized (Gupta, 2000). These advantages

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have served as potential incentives for promoting biosorption as viable clean up technology for heavy metal pollution. Immobilization of biosorbent leads to its stability and it can be used repeatedly with ease for the process of adsorption/desorption (Srinath *et al*., 2003). Various biomaterials such as plant products (tree bark, peanut skin, saw dust, plant weeds *etc.*) and microbes (algae, fungi, bacteria) have been examined for their biosorptive properties. Among microbes, fungal biomass offers the advantages of having high percentage of cell wall material which shows excellent metal binding properties including short multiplication time and economical production (Gadd, 1990).

As biosorption is a process in which physico-chemical interaction between the charged surface groups of micro-organisms and ions in the solution takes place by the process of complexation, ion-exchange, microprecipitaion *etc.* (Atkinson *et al*., 1998), it is important to elucidate the chemical characteristics relevant to metallic ion sorption by the biosorbents. In the light of this, present study has been carried out on acid treated *Aspergillus niger* which is immobilized and used as biosorbent for Cr biosorption. The present paper therefore aims to: (i) depict the efficiency of biosorption process in concern to different bed heights and flow rates during continous flow process, (ii) make process more efficient and economical by carrying out the desorption of metal ion and reutilization of biosorbent, (iii) identify the possible sorption sites that were involved in Cr (VI) removal process by analyzing the associated functional groups in this interaction through FTIR spectrometer analysis which might help in exploration of Cr binding mechanism to fungal biosorbent.

Materials and Methods

Chemicals, reagents and synthetic solutions: All the chemicals used were of fine analytical grade and the chemicals were supplied by Qualigen Fine Chemicals (Bombay, India). Stock metal solutions of Cr(VI) were prepared by dissolving appropriate quantities of pure analytical grade metal salts in double distilled water. The stock solutions were diluted further with double distilled water to obtain working solutions of different concentrations. The stock solution of Cr (1000 mg l^{-1}) was prepared by dissolving 0.28 g of $K_2Cr_2O_7$ salt to one liter of double distilled water. The solution was used for further experimental preparation.

Biosrbent: Pure culture of *A. niger* was obtained from I.A.R.I., New Delhi and was routinely maintained by streaking on Rose Bengal Agar medium and incubated at 25°C.

For mass culturing, *A. niger* was cultivated in liquid medium using the shake flask method. Spores and mycelium from the spread plate cultures were transferred to 250 ml Erlenmeyer flasks containing 100 ml growth medium (g l^{-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; $Fe(SO_4)_2.7H_2O$, 0.005). The pH was adjusted to 4.5. Once inoculated, the flasks were shaken on a rotary shaker at 125 rpm for five days at 22±2°C. Harvesting of biomass was carried out by filtering the cultured medium through a 150 μm sieve. Once harvested, the biomass was washed with deionized water. Non-viable biomass was obtained from cultured cells by heating at 80°C in an oven till their weight became constant. The dried samples were ground and sieved through the pore sizes of 100 μm. The biomass thus obtained was untreated biomass.

Treatment and immobilization of biosorbent: In order to generate active site and enhanced biosorption, the biomass was treated with 0.1 M H_2SO_4 for 6 hrs at 30°C. The acid treated biomass was collected by centrifugation at 2500 rpm for 10 minutes. Then the biomass was washed twice with double distilled water. After washing, the biomass was dried at 60°C in an aluminium foil till weight of biomass become constant. It was ground and sieved through screen with pore size of 100 μm and stored. To establish the simple and cost effective granulation of biomass, it was immobilized in Ca-alginate matrix. The immobilization of fungal biosorbent via entrapment was carried out as follows: 3% (w/v) Na-alginate was dissolved in distilled water and mixed with 5% (w/v) of fungal biosorbent. The mixture was stirred for 1 hr at 30°C and then the slurry solution was dripped through a nozzle into 4% (w/v) $CaCl_2$ solution (Dong, 2004). Spherical beads containing biomass were formed immediately by a phase inversion process as the alginate was cross linked by Ca^{2+} ion. The gel beads (3.2 mm±0.1 mm) were moderately agitated in double distilled water for 2 hr at 4°C. The curing procedure hardened the beads and resulted in the formation of favorable micro porous structure (Merrin *et al*., 1998). Finally the beads were stored at 4°C in double distilled water until further use.

Chromium estimation: Chromium analysis was carried out by spectrophotometric method by using 1,5-Di-phenyl carbazide, according to APHA, 1995. This procedure measures only Cr (VI). The hexavalent chromium is determined colorimetrically by reaction with diphenylcarbazide in acid solution and red violet colour was produced. Diphenylcarbazide solution is prepared by dissolving 1,5-Di-phenyl carbazide in 50 ml acetone.

Column studies: Column studies were carried out at various bed heights and various flow rates on the column of 4 cm internal diameters. All the experiments were carried out with synthetic sample of 100 mg l^{-1} at which adsorption efficiency per unit mass of adsorbent is found to be maximum (Taken as reference from the best studies). During column sorption operation, three columns of different bed depths (20, 30 and 40 cm) were considered for study at different loading rates (5, 7.5 and 10 ml min^{-1}). The samples collected from the outlet of column at a time interval of 30 min, were analyzed for residual chromium concentration. The data was used to plot the breakthrough curves of residual Cr (VI) (the concentration of exit Cr (VI) versus time). The breakthrough curves of biosorption were derived as a function of equilibrium time, bed length and flow rate. The metal loaded biosorbent (10 g) was incubated for 1 hr at 28°C with 50 ml of 0.1 M H_2SO_4 in rotary shaker for continous stirring at 150 rpm. The solution was then filtered through whatman filter paper No. 42 and the filtrate was used to determine the amount of metal desorbed. The amount of total desorbed metal was established by comparing the metal released to the amount of metal previously

adsorbed to the biosorbent. All experiments were run in triplicates. The metal stripped biosorbent was rinsed with 50 ml double distilled water for 15 min for two times. The resulting biomass was then reloaded with metal solutions as described above and the desorption treatment was repeated.

FTIR (Fourier transform infrared) analysis: Infra-red spectra of biosorbent, before and after adsorption, were recorded at sophisticated analytical instrumentation facility, Punjab University (Chandigarh) on Nicolet model 6000. FTIR spectrometer equipped with liquid nitrogen cooled detector. The samples for IR examination were prepared in KBr discs. The spectrum was recorded in the range of 4000 cm^{-1} to 400 cm^{-1}.

Results and Discussion

The dynamic removal of metal ion was studied using continuously fed columns packed with immobilized biosorbent beads. During the column sorption operation, an aqueous solution containing 100 mg l^{-1} chromium ions at pH 1.5 was pumped upward through the column at variable flow rates of 5, 7.5 and 10 ml min^{-1} continuously. The samples, collected from the outlet of the column at the preset time intervals of 30 minutes, were analyzed for residual chromium concentration. Three columns of different bed depths were considered for study at different loading rates. The data obtained was used to plot the breakthrough curves of Cr (VI) about C_{out} (the concentration of residual chromium at exit point) versus time, as shown in Fig. 1 (a, b and c).

Effect of bed height and flow rates: The biosorption performance of immobilized biosorbent was investigated with the various bed heights that is 20, 30 and 40 cm, at a different flow rates (5, 7.5 and 10 ml min^{-1}) and 100 mg l^{-1} of effluent metal ion concentration. The pH was kept 1.5 for all the experiments as it was observed to be the optimum pH for removal of Cr (VI) during batch process in earlier study (Chhikara and Dhankhar, 2008).

The column of 20 cm bed height showed a breakthrough time of 330, 180 and 120 minutes respectively at the flow rates of 5, 7.5 and 10 ml min^{-1}. The saturation of column was observed after a time period of 510, 360 and 300 minutes respectively at flow rates of 5, 7.5 and 10 ml min^{-1}. In case of column with 30 cm bed height, the breakthrough time was observed to be 570, 390 and 270 minutes respectively at flow rates of 5, 7.5 and 10 ml min^{-1}. The column saturation was observed after 780, 570 and 420 minutes at flow rates of 5, 7.5 and 10 ml min^{-1}. The 40 cm bed height column was observed to show breakthrough time of 1020, 660 and 540 minutes respectively at the flow rates of 5, 7.5 and 10 ml min^{-1}. The column was observed to become saturated after 1440,1080 and 840 minutes respectively at the flow rates of 5, 7.5 and 10 ml $minute^{-1}$. Maximum value of column capacity of immobilized biosorbent for metal ion was obtained at a bed height of 40 cm. The biosorption yield of metal ions decreased with a decrease in the bed height from 40 to 20 cm. This was due to a relatively small amount of biosorbent in a shorter bed, an increase in the bed height resulted in an increase of equilibrium time of

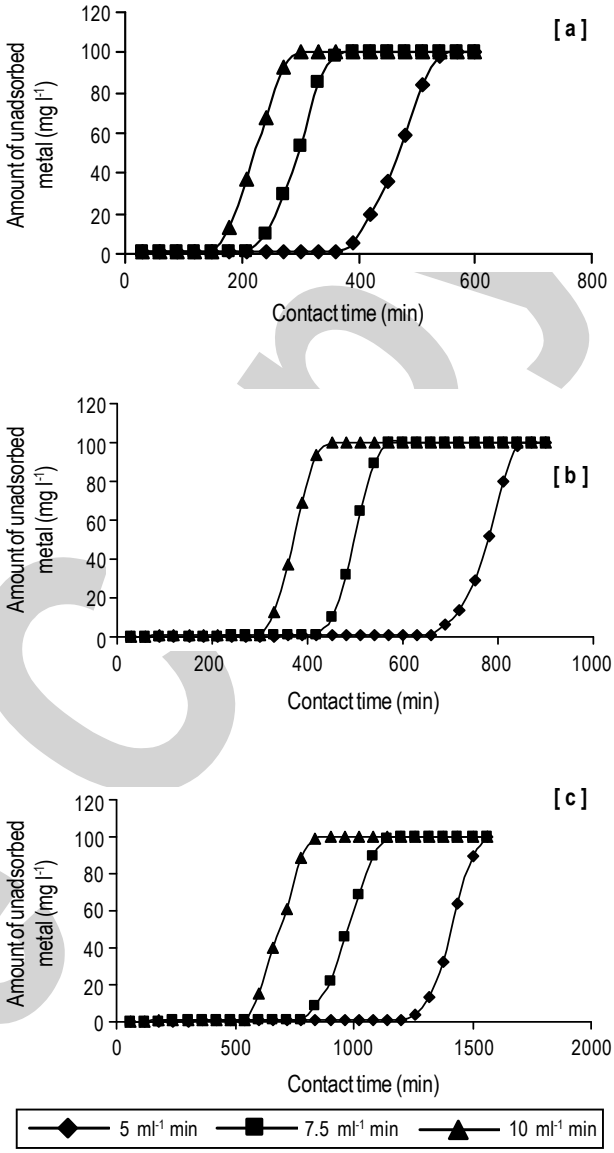

Fig. 1: Breakthrough curves for different flow rates on the amount of biosorption of Cr(VI) by *A. niger* a = bed height 20 cm., b = bed height 30 cm and c = bed height 40 cm

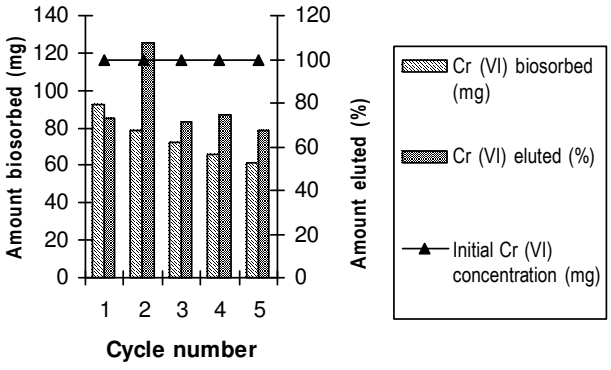

Fig. 2: Adsorption/desorption cycles for Cr (VI) uptake by immobilized acid treated *A. niger*

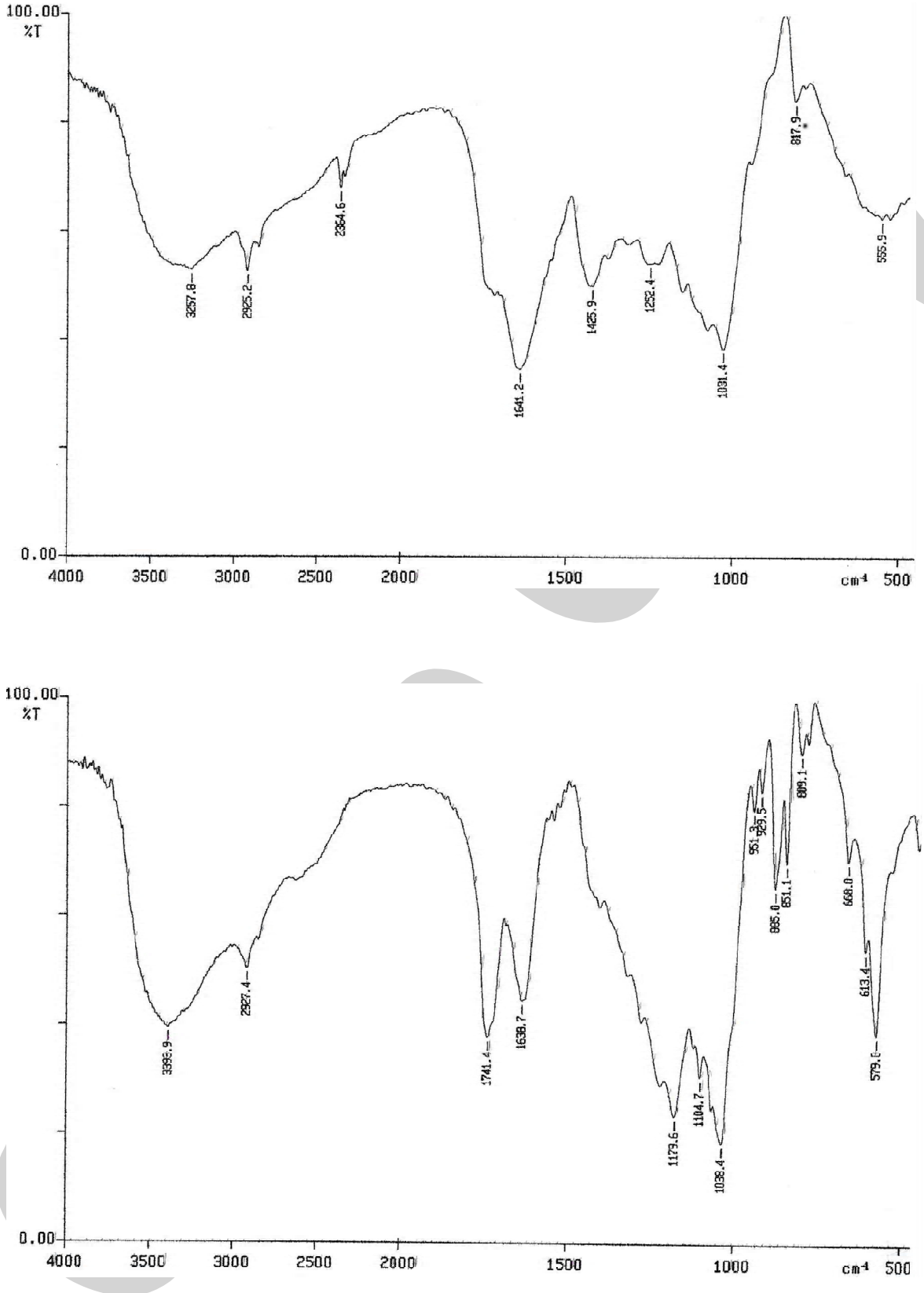

Fig. 3: The FTIR spectrum pattern of acid treated immobilized *A. niger* before (a) and after (b) biosorption

biosorption. The increase in biosorption amount with the amount of biosorbent could be due to an increase in the surface area and the availability of the more binding sites on the biosorbent (Cabuk *et al.,* 2006; Acharya *et al.,* 2008).

These breakthrough curves coincided with the earlier studies (Yan and Viraraghavan, 2001; Matheickal and Yu, 1999). Before the breakthrough point, the concentration of Cr(VI) in effluent was nearly zero as the mass transfer zone was formed in the packed column. As soon as the solution of Cr (VI) was exposed to the fresh layer of the biosorbents, the Cr (VI) was adsorbed onto the biosorbents until all the binding sites were occupied. Additionally, the breakthrough curves increased sharply and reached quickly to the concentration of influent after the breakthrough point, which implies that affinity of the biomass to Cr (VI) was very strong. The correlation co-efficient value for different sets of experiments were found to be higher than 0.985.

Since biosorption depends on the period of contact between the metal ions and biosorbent surface, the flow rate of metal solution through the biosorbent column affects metal removal efficiency as it affects the contact time. At higher flow rates, the contact phase was reduced resulting in early breakthrough and less metal uptake. At lower flow rates large amount of mixing or axial dispersion occurred, thereby, increased the metal uptake. An increase in the height of the column increased metal biosorption because of greater time of contact and more intra particular diffusion.

Desorption of metal ion and reutilization of biosorbent: The total amount of metal biosorbed and desorbed in each subsequent cycle by immobilized biosorbent was calculated as shown in Fig. 2.

The amount of Cr (VI) adsorbed in the 5th cycle was comparable to the first cycle. Also, the amount of metal desorbed after each loading cycle corresponded well to the amount of metal biosorbed , which showed that a complete elution took place. Immobilized biosorbent can be reused for, five consecutive biosorption and desorption cycles without apparent loss of efficiency after its reconditioning.

The FTIR spectrum of unloaded biosorbent showed several distinct and sharp adsorption bands of different wavelength. The FTIR spectra of loaded biomass indicated some shifts in some of characteristic bands. Change in the spectrum depicts change in functional groups of biomass after Cr adsorption. This implied the possibility that biosorption could be taken place through ion-exchange process rather than complexation. The FTIR spectra have been shown in Fig. 3 (a and b).

The very strong adsorption band around 3200-3400 cm^{-1} found in these samples may be due to presence of N-H stretching of amines and amides and polymeric association which was normally found in hydroxyl compounds. The adsorption band around 2900-2850 cm^{-1} correspond to C-H stretching of CH_2 groups. The peak near 2925 cm^{-1} is characteristic of presence of aliphatic (-CH_2) groups in these compounds. The peak appeared near 2300 cm^{-1} was due to stretching vibration of -NH^{2+} as well as –NH^{3+} (Dean and Tobin, 1999). The new peak that appeared near 1740 cm^{-1} were characteristics of the C=O stretching vibrations, which indicates the presence of carbonyl group. The IR analysis of biosorbent specifically the 1650-1620 cm^{-1} band indicated the existence of the amide I band of amide bond in poly-N-acetyl glucosamine (chitin) and the protein peptide bond present in biomass considered to be due to combined effect of double bond stretching vibrations (mainly C=O) and hydrogen bonding (Li *et al*., 2008).

The peak in the proximity of 1400 cm^{-1} in unloaded biomass responded to the symmetric vibrations of the C=O of COO^- at terminal amino acid on biomass (Yee *et al*., 2004). The peak near 1250 cm^{-1} which is related to C-O stretching in COOH group, again strengthens the hypothesis that a carboxyl group was involved in metal biosorption (Yee *et al.*, 2004). The shift of peak from 1030 to 1110 cm^{-1} could be due to the involvement of the C-O of polysaccharides in the biosorption process Han *et al*., 2007. The presence of a new peak at around 950 cm^{-1} in the Cr(VI) treated biomass, was attributed to the presence of Cr(VI)-O bond as suggested by Holman (2002). The strong and complex bands attributed to ether and hydroxyl C-O stretching appeared between 1200 and 940 cm^{-1}. Two weak absorption bands at 929 and 885 cm^{-1} could be attributed to the glycoside linkage in the polysaccharide structure of the biomass. The absorption at 929 cm^{-1} probably corresponded to ring vibrations similar to dioxan (type I) of b-glycosides. The 885 cm^{-1} band corresponded to C_1-H axial hydrogen bending vibrations in b-sugars. The peak at 579 cm^{-1} representing O-C-O scissoring and C=O bending vibrations was due to lipids.

Carboxylic acids are widely distributed in the biopolymers and are most commonly found as side chain constituents of proteins, the uronics, neuraminic acid and related substituted monosaccharides of polysaccharides. Lipoproteins and lipopolysaccharides are also likely to contain phosphodiester bond as part of lipid moiety. Ester sulphate and phosphomono-ester grouping also occur in proteins. Hydroxyl group of serine, threonine and tyrosine are available for specific translational modifications. Anion exchange on biopolymers can take place on a variety of organic-nitrogen based groupings. In proteins, amino (lysil side chain and N-terminal), imidazole (histidyl) and guanidine (arginyl) groupings are common centre of positive charge. Polysaccharides as a group are acidic or neutral macromolecules with basic functional groups being rare and arising as unacetylated amino sugars. So, chitin of fungal cell wall is the notable example where different biomolecules provide centers of positive and negative charge which bind the metal ions to different extents.

The result indicated that the chemical interactions such as Ion-exchange between the hydrogen atoms of carboxyl (-COOH), hydroxyl (-OH) and amine (-NH_2) group of biomass and metal ions are mainly involved in biosorption of Cr(VI) onto *A. niger* surface.

The changes in the functional groups and the surface properties of pretreated fungal biosorbent were confirmed by FTIR spectra.

Thus analyses of functional groups, breakthrough time, desorption and reutilization of biosorbent, can help in carrying out an efficient biosorption process for chromium removal by *A. niger* biosorbent.

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