Pak. J. Bot., 44(3): 989-998, 2012.

EVALUATION OF THE BIOSEQUESTERING POTENTIAL OF MICROALGA *KIRCHNERIELLA CONTORTA* IN THE REMOVAL OF HEXAVALENT CHROMIUM FROM AQUEOUS SOLUTION: BATCH AND CONTINUOUS FLOW FIXED-BED COLUMN BIOREACTOR STUDIES

ASMA SAEED[1], MUHAMMAD IQBAL[1,2*], KASHIF IQBAL SAHIBZADA[3] AND SHOUKAT PARVEZ[4]

[1]*Biotechnology and Food Research Centre, PCSIR Laboratories Complex, Ferozepur Road, Lahore, Pakistan*
[2]*Center for Applied Molecular Biology, West Canal Bank Road, Thokar Niaz Baig, Lahore, Pakistan*
[2]*Department of Chemistry, F.C. College University, Lahore, Pakistan*
[4]*PCSIR Head Office, Islamabad, Pakistan*
[*]*E-mail: iqbalmdr@fulbrightmail.org; iqbalmdr@brain.net.pk*

Abstract

In this study, the adsorption capacity of free and loofa sponge-entrapped microalga *Kirchneriella contorta* to remove Cr^{6+} from aqueous solution was investigated. This is the first reported study of biosorption by *K. contorta*. The effects of the experimental conditions, such as pH, initial Cr^{6+} concentration, sorbent-sorbate contact time, and quantity of sorbent mass, on Cr^{6+} removal efficiency were studied. The Cr^{6+} sorption on *K. contorta* was found to be highly pH dependent and the maximum uptake capacity was achieved at pH 1.0. The adsorption isotherms study showed the maximum sorption capacity of the loofa sponge-immobilized biomass of *K. contorta* (LIBKC) of 100.84 mg g^{-1}, which was much higher than 80.61 mg g^{-1} of the free biomass of *K. contorta* (FBKC). The adsorption equilibrium data showed a better fit on the monolayer Langmuir adsorption isotherms model with the regression coefficient (r^2) greater than 0.99 for both FBKC and LIBKC. The rate of Cr^{6+} removal followed the pseudo second-order kinetics equation. The LIBKC on treatment with NaOH resulted in 97% Cr^{6+} recovery and its complete regeneration. The regenerated LIBKC was reused in five repeated cycles without appreciable loss of its metal sorption ability. The potential of LIBKC in a fixed-bed continuous flow column bioreactor for the sorption of Cr^{6+} from the metal contaminated water was also investigated.

Introduction

Technological advancements leading to rapid industrial growth have created numerous environmental problems worldwide. Among the various environmental issues, water pollution related with heavy metals discharged into natural water bodies is of critical significance due to their accumulation in the food chain, their persistent nature in the ecosystem and non-biodegradability (Naiya *et al*., 2009). Several physiological and neurological damages have been associated with them, even when present at low concentrations (Febrianto *et al*., 2009). Hexavalent chromium (Cr^{6+}) is among the most toxic metal ions reported in literature (Arica *et al*., 2005). It is extensively used in several industrial operations such as leather tanning, chromium-based electroplating, metal polishing, nuclear energy plants, chromate chemicals production, and textiles (Murphy *et al*., 2008). Due to its high toxicity and large-volume industrial usage, its maximum limit of discharge into surface water bodies as regulated in the United States, by US-EPA, is below 0.05 mg L^{-1} (EPA, 1990). Traditionally, Cr^{6+} is treated with several conventional methods, including ion-exchange, activated carbon adsorption, chemical precipitation, oxidation-reduction, reverse osmosis, and membrane filtration (Yang & Chen, 2008). These, however, become expensive, non-effective and inefficient at low metal concentrations, while some also cause secondary environmental problems, particularly those necessitating further waste-sludge disposal (Li *et al*., 2008). A method alternative to these conventional technologies is the use of microbial biomass for the remediation of metal ions from contaminated water. Both the living and dead microbial mass have been reported to remove heavy metals from wastewaters efficiently, even at low concentrations, by a phenomenon of passive uptake called 'biosorption' (Preetha & Viruthagiri, 2007). Several kinds of microbial mass, including bacteria (Liu *et al*., 2004), yeasts (Volesky *et al*., 1993), fungi (Iqbal *et al*., 2008), microalgae (Saeed & Iqbal, 2006), and macroalgae (Yang & Chen, 2008) have been successfully used for the sorption of metals. Among these, biosorption capacity of many algae investigated for the purpose has been reported to be greater, which has been attributed to the physico-chemical nature of the algal cell wall, which comprises of a fiber-like structure and an amorphous matrix of various polysaccharides (Bayramo□lu & Arica, 2009). Surface of algal cell walls contains several functional moieties, including amino, imido, sulphate and carboxyl groups that play a role in the sequestering of metal ions from contaminated water (Volesky & Schiewer, 1999). The use of these microbes for biosorption is hindered by numerous problems, which are related principally with their micro-size, low mechanical resistance and easy breakability, difficulties encountered in the biomass removal and separation, and choking of the continuous-flow contact vessels (Vijayaraghavan *et al*., 2008). To overcome these problems, much attention has been focused to entrap these microbial cells into natural and synthetic polymeric structure so as to develop biosorption systems that are easy to operate, efficient, ecofriendly, cost-effective and are reusable by regenerating the biomass (de-Bashan & Bashan, 2010).

Immobilization of algal cells into the fibrous network of naturally occurring biomatrical support of loofa sponge has been successfully reported (Akhtar *et al*., 2003; Iqbal & Zafar, 1993). The sponge biomatrix has several advantages over other biopolymers derived from biological materials, such as gellan gum, alginate, κ-carrageenan, agar, agarose, carboxymethyl cellulose and silica gel, and synthetic polymers, which include polysulfone, polyvinyl alcohol, polyethyleneimine, polyacrylonitril and polyacrylamide (Barbotin *et al*.,

1998; Saeed *et al*., 2009). As reported earlier, loofa sponge consists of multicellular fibers containing small punctuations as interconnections, resulting in extensive open spaces for microbial cell immobilization (Bal, 2004). The immobilized microbial cells thus increase the sorbent surface area and accessibility to sorption sites, providing greater space for the surface-binding of target contaminants. Although several reports have been presented on the remediation of Cr^{6+} from contaminated water using biological materials, the potential use of microalgae in Cr^{6+} treatment is limited to few studies (de-Bashan & Bashan, 2010). The present study, however, is the first of its kind reporting on the behaviour of cell immobilization of a freshwater unicellular green microalga *Kirchneriella contorta*, onto the fibrous network of loofa sponge, to produce a low-cost adsorbent matrix for the sorption of Cr^{6+} from contaminated water. No earlier study has reported the use of *K. contorta* as a biosorbent for the removal of heavy metals including Cr^{6+}. This study explores for the first time, the Cr^{6+} biosorption potential of *K. contorta*. The other noteworthy aspect of the present study was the development of an immobilized microalgal biomass matrix that had significant mechanical strength and stability as a biosorbent system, which was useful in eliminating the difficult step of separation of the solid phase free-suspended biomass from the liquid phase culture medium. The study also reports the reusability of the immobilized microalgal biomass in five repeated cycles of biosorption. Development of a robust, cost-effective and efficient immobilized system, which is reusable in repeated sorption-desorption cycles to remove Cr^{6+} from contaminated water, is of particular interest as the metal ion is extremely toxic to humans and the surrounding ecosystems even at low concentrations.

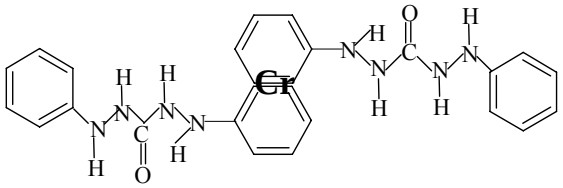
Materials and Methods

Microalga and growth medium: *Kirchneriella contorta* (strain number 11.81), a unicellular green microalga, was obtained from Algal Culture Collection, University of Goettingen, Germany. Axenic culture of *K. contorta* was cultivated to stationary phase of growth in 100 mL Bold's medium (Nicholas & Bold, 1965) at 25±2°C, under continuous cool white light illumination of 50 μE m^{-2} s^{-1} intensity, and constant orbital shaking at 100 rpm.

Immobilization of *K. contorta* onto loofa sponge: The fibrous network of fully ripened and dried fruit of *Luffa cylindrica*, cut into square discs (LS), was used to immobilize *K. contorta* cells. *K. contorta* immobilization, within the LS sponge, was done in accordance with the method described earlier (Saeed & Iqbal, 2006). The LS immobilized biomass of *K. contorta* (LIBKC) was let to grow for 24 days, harvested, washed in deionized water, freeze-dried, and stored for later use in biosorption studies. Immobilized algal biomass was quantitatively determined, after drying to constant weight, as the weight difference of LS with and without immobilized *K. contorta* cells. Free biomass of *K. contorta* (FBKC) grown under similar conditions, but without loofa discs, was harvested at stationary phase, washed in deionized water, freeze-dried, and stored for biosorption studies. SEM of LS, and of free and LS-immobilized cell mass of *K. contorta* was done after their coating with a thin layer of gold, under vacuum using scanning electron microscope (Philips PSEM 501B).

Preparation of solutions: Stock solution of Cr^{6+} (1000 mg L^{-1}) was prepared by dissolving an exact amount of 2.83 g of $K_2Cr_2O_7$ (Merck) in double distilled water. Desired Cr^{6+} concentrations for biosorption studies were made from the stock solution, and pH of the solutions was adjusted with 1.0 M HCl and 1.0 N NaOH. 1,5-diphenylcarbazide (Merck) solution, for Cr^{6+} determination, was prepared by dissolving 250 mg in 50 mL acetone (Merck). All reagents used during the study were of AR grade and prepared by weighing precisely. Double distilled water was used wherever required.

Analysis of hexavalent chromium ion: The quantitative analysis of Cr^{6+} was done spectrophotometrically after complexation of Cr^{6+} with 1,5-diphenylcarbazide and recording of absorbance at 540 nm (UV-1800 spectrophotometer, Shimadzu, Japan). Concentration of the metal ion in solution was calculated using a standardized calibration curve. The structure of coloured Cr-diphenylcarbazide complex formed after complexation is:



Studies on hexavalent chromium ion biosorption: The standard Cr^{6+} stock solution (1000 mg l^{-1}) was diluted to obtain the desired Cr^{6+} concentrations. Double distilled water was used for making the dilutions. The metal solution pH was adjusted at 1.0, or as otherwise required. Stock solution was diluted fresh for each biosorption experiment. The ability of free biomass of *K. contorta* (FBKC) and of loofa immobilized biomass of *K. contorta* (LIBKC) to biosorb Cr^{6+}, was obtained upon contacting 100±2.5 mg microalgal biomass with 100 ml of Cr^{6+} solutions of concentrations ranging between 10 and 300 mg L^{-1}. The Cr^{6+} solutions, of known concentrations, were incubated on an orbital shaker at 100 rpm shaking with FBKC and LIBKC, separately, for different periods of sorbent-sorbate contact. Cr^{6+} solution was centrifuged for 5 min at 5000 rpm to remove FBKC, whereas LIBKC was removed by decantation. The Cr^{6+} remaining unadsorbed in the solution was determined by the standard analytical procedure (Eaton *et al*., 2005), and the quantity (q) of Cr^{6+} ions adsorbed by the algal biomass (calculated as mg Cr^{6+} g^{-1} of algal dry weight) was calculated from the following equation:

$$q = \frac{(C_i - C_f)V}{W} \qquad (1)$$

where V = volume of metal solution; C_i = initial metal concentration; C_f = metal residual concentration; and W = weight of biosorbent.

The rate of Cr^{6+} biosorption by FBKC and LIBKC was determined by analyzing residual concentration of Cr^{6+} in the supernatant after the contact period of 10, 20, 30, 40, 50, 60, 80, 100 and 120 min. The pH-dependence of Cr^{6+} biosorption capacity of FBKC and LIBKC was noted after equilibrating the sorbent-sorbate system at pH values ranging between 1-6. Deionized water and metal solution containing biomass-free loofa sponge were used as controls. All biosorption studies were done in independent triplicate experiments, and Cr^{6+} removal reported as their average value.

Desorption of hexavalent chromium and reusability of LIBKC: Efficiency and reusability of any biosorbent system are important aspects for determining its cost-effectiveness and application potential in the treatment of wastewaters. Various desorbing agents were investigated for determining their efficiency to recover Cr^{6+} from Cr^{6+}-loaded LIBKC. For this purpose, LIBKC was placed in 100 mL solution containing 100 mg Cr^{6+} L^{-1} for biosorption. Cr^{6+} was recovered from the metal-loaded LIBKC by orbital shaking at 100 rpm using several desorbing agents, which included NaOH, KOH, NaCl, KCl, Na_2CO_3, $NaHCO_3$, $CaCl_2.2H_2O$, HCl and HNO_3 at 0.1 M concentration. The repeated regeneration efficiency of the Cr^{6+}-loaded LIBKC for five consecutive adsorption-desorption cycles was determined by contacting 100 mL solution containing 100 mg Cr^{6+} L^{-1} for biosorption, followed by desorption with 0.1 M NaOH for 40 min. The initial and final concentrations (C_i) and (C_f), respectively, of Cr^{6+} in the solution were determined for each cycle. The regenerated LIBKC was washed repeatedly with double distilled water, after the completion of each cycle, and was transferred to fresh Cr^{6+} solution for determining biosorption in the next cycle. Desorption ratios were determined from the following equation:

$$\text{Desorption ratio} = \frac{\text{Amount of } Cr^{6+} \text{ desorbed}}{\text{Amount of } Cr^{6+} \text{ adsorbed}} \times 100 \qquad (2)$$

Biosorption of hexavalent chromium by LIBKC in continuous flow column: LIBKC was further investigated to determine its potential to biosorb Cr^{6+} from solutions of low concentration in a continuous flow column. For this purpose, a glass column (2.7 cm dia and 30 cm length) was made into a fixed-bed with 1.098 g *K. contorta* cell mass immobilized in 40 LS discs packed to 25 cm in height. Cr^{6+} solution (10 mg L^{-1}; pH 1.0) was pumped from bottom of the column upwards to pass through the packed column. The flow rate of pumping was adjusted at 5 ml min^{-1}. The column effluent was collected at intervals to determine residual Cr^{6+} concentration. Cr^{6+} solution loading was stopped when the column fixed-bed was saturated. The bed was washed with 0.1 M NaOH to remove the loaded Cr^{6+} and to regenerate the fixed-bed. The regenerated fixed-bed column was washed with double distilled water for use in the fresh biosorption cycle. The continuous flow column experiments were performed by operating three columns simultaneously in similar conditions. Columns of the same specification packed with only LS discs without immobilized algal cells served as the control.

Results and Discussion

Immobilization of *Kirchneriella contorta* and scanning electron microscopy: *K. contorta* cells were observed to be immobilized on the loofa sponge fibers between 5 and 7 days of incubation. The LS, however, was maintained incubated for 3 more days to allow complete and stabalized entrapment. The LS with entrapped algal cells was then subcultured in fresh medium for further 24 days. The LS was completely covered by the entrapped *K. contorta* cells after this period. The scanning electron microscopic image of naked loofa (Fig. 1a) showed that the surface of the fibers was rough due to the presence of small longitudinal and transverse stripes. The fibers were observed to be interconnected, thus creating void volumes leaving empty spaces that facilitated cell immobilization. Scanning electron microscopic image of a single fiber of loofa sponge, after the immobilization of *K. contorta* cells (LIBKC), revealed a uniformly smooth algal growth filling all depressions and groves (Fig. 1b-c), indicating that immobilized cell mass of *K. contorta* was not limited to a single or few points. This is a significant attribute as uniform distribution of the immobilized biomass is useful for efficient sorption of metal on the entire surface area of the entrapped cell mass. As a result, the entrapment of *K. contorta* cells on LS discs presents several advantages as compared with the free algal cells. The most significant advantage is the increased surface area and consequentially a greater exposure of functional moieties participating in the process of metal binding. In free suspended cultures, on the other hand, algal cells aggregate to form spherical clumps. The clumping of cells creates diffusional restriction and, therefore, provides lesser opportunity to metal ions to approach the adsorption sites containing the functional moieties than is abundantly available on the reticulated honeycomb-like open surface of the LS-entrapped *K. contorta* cell system. The mass of the entrapped *K. contorta* cells in the LS discs was 282.56 mg g^{-1} dry weight of LS discs. This quantity of cell loading was achieved after 24 days of culture. The *K. contorta* biomass did not show any further increase beyond this culture period.

pH-dependence of hexavalent chromium biosorption: Earlier studies have shown that pH of metal solutions is one of the most important parameters that control the sorption process (Saeed *et al*., 2009). This is due to the impact pH exerts on the functional moieties of the sorbent surface and on the ionization status of metal ions in aqueous solution (Basha *et al*., 2008). The adsorption behaviour of Cr^{6+} at different pH values by FBKC and LIBKC at the initial metal concentration of 100 mg L^{-1} is presented in Fig. 2. The biosorption efficiency of Cr^{6+} increased as the pH value was decreased. Maximum adsorption of Cr^{6+} was observed in the range of pH 1-2.

However, when pH was increased from 3-6, adsorption of Cr^{6+} was observed to decrease by both free and immobilized biomass of *K. contorta*. The sorption capacity of FBKC and of LIBKC at pH 1.0 was, respectively, 66.65 and 82.93 mg g^{-1} microalgal biomass. Similar findings that Cr^{6+} sorption was greater as the pH was lowered, and lesser as it was elevated have been reported by other authors (Tewari *et al*., 2005; Bai & Abraham, 2001). Cr^{6+} in aqueous solutions exists as oxy anions, as $[HCrO_4]^-$, $[Cr_2O_7]^{2-}$, $[CrO_4]^{2-}$, $[Cr_4O_{13}]^{2-}$ and $[Cr_3O_{10}]^{2-}$ (Li *et al*., 2008; Rollinson, 1973). The cell wall of microalgae is reported to contain high amounts of polysaccharides, which are associated with proteins and other constituents (Crist *et al*., 1981). These biomolecules on the algal cell wall surface have several functional groups such as amino, carboxyl, thiol, sulfhdryl, phosphate, etc., and are known to participate in metal binding (Volesky & Schiewer, 1999). The active participation of these functional groups depends on the protonation and deprotonation during the metal binding process. At highly acidic pH (1.0-2.0), the active binding sites available on the algal cell wall surface become protonated, resulting in increased Cr^{6+} biosorption capacity. This increased binding of Cr^{6+} at the lower pH values can be explained by the electrostatic binding of positively charged groups, such as NH^+ and $COOH^+$, present on the surface of algal cell walls (Yan & Viraraghavan, 2003). However, as the pH is increased, the concentration of hydroxyl ions in the aqueous phase increases, causing the algal cell wall surface charge to become negative, thus creating hindrance in the removal of such negatively charged chromium ions, such as $[Cr_2O_7]^{2-}$, $[CrO_4]^{2-}$, $[Cr_4O_{13}]^{2-}$and $[Cr_3O_{10}]^{2-}$, resulting in decreased Cr^{6+} biosorption. It is well known that the dominant form of Cr^{6+} at pH 1.0 is $[HCrO_4]^-$, which changes to other forms such as CrO_4^{2-}, $Cr_2O_7^{2-}$ etc., as the pH is increased (Tewari *et al*., 2005). The distribution of Cr^{6+} species and the resulting equilibria developed in the aqueous medium, as determined by spectrophotometric and electrochemical studies, reveals the following (Pehlivan & Altun, 2008):

$$H_2CrO_4 = H^+ + HCrO_4^- \quad (3)$$

$$HCrO_4^- = H^+ + CrO_4^{2-} \quad (4)$$

$$2HCrO_4^- = Cr_2O_7^{2-} + H_2O \quad (5)$$

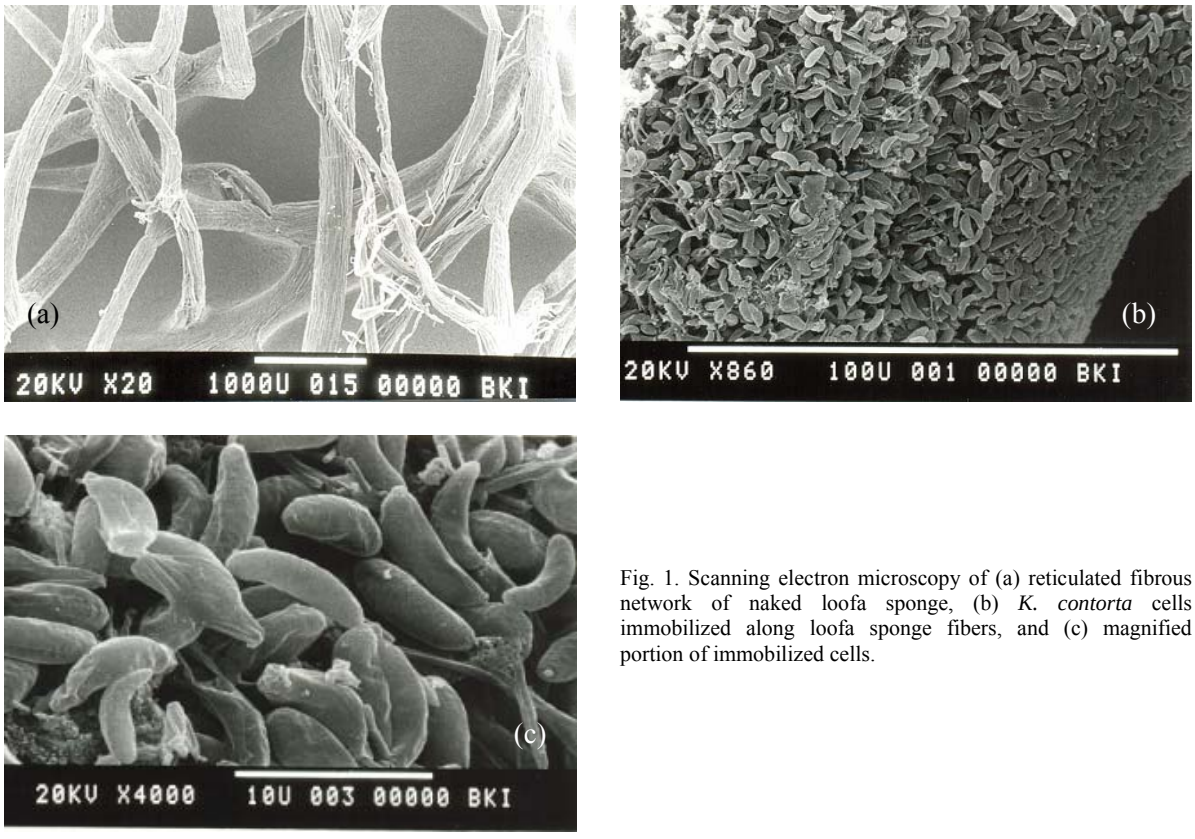


Fig. 1. Scanning electron microscopy of (a) reticulated fibrous network of naked loofa sponge, (b) *K. contorta* cells immobilized along loofa sponge fibers, and (c) magnified portion of immobilized cells.

It may be concluded from Fig. 2, therefore, that the maximum adsorption of Cr^{6+} ions occurs at pH 1.0 with the predonimant Cr^{6+} species as $[HCrO_4]^-$, which decreases gradually at pH 2.0 and progressively at pH 3.0-6.0, in accordance with the chemical reactions noted above.

Effect of the sorbate-sorbent contact time on hexavalent chromium sorption: The effect of sorbate-sorbent contact time on the Cr^{6+} uptake ability of FBKC and LIBKC was determined by contacting these biosorbents with 100 mg Cr^{6+} L^{-1} solution for various periods of time, ranging between 10–120 min at pH 1.0.

The Cr^{6+} sorption by LIBKC was quick, as the maximum metal removed was achieved in 30 min, which amounted to 90.83% sorption (Fig. 3). The sorption slowed down significantly, after this initial quick phase, and the sorption equilibrium was reached in 40 min. The maximum removal of Cr^{6+} by FBKC, on the other hand, was 73.60% in 30 min, and the sorption equilibrium was reached in 60 min. After the sorption equilibrium was reached in 40 and 60 min, respectively, of LIBKC and FBKC, no further Cr^{6+} sorption occurred, indicating saturation of the available active sites, which thus were the limiting factor in further sorption of Cr^{6+} by the algal cell wall surface. It was also noted that at all stages of the sorbate-sorbent contact time interval, the sorption of Cr^{6+} by LIBKC was significantly more than by FBKC, which clearly indicates that LIBKC was better for Cr^{6+} sorption than FBKC. Significantly less sorption of Cr^{6+} ions by FBKC may be due to clumping of algal cells, resulting in reduced surface area and reduced accessibility of Cr^{6+} to active sorption sites of the algal cell walls. On the other hand, higher sorption of Cr^{6+} by LIBKC may be attributed to the greater surface area of the corresponding mass of *K. contorta* cells immobilized on the surface of LS discs and the open spaces present on the characteristic loofa sponge biostructural matrix of LIBKC. These two factors were responsible for enhanced accessibility of Cr^{6+} to the active sorption sites on the biosorbent surface. The fast rate of Cr^{6+} removal by LIBKC is of practical significance for the efficient and economical treatment of wastewaters of small volumes. This attribute is of particularly greater advantage, as compared with other immobilized biosorbent schemes based on foam, gels and synthetic polymers reported by other authors, who have noted reduced rate of metal sorption by the immobilized cells than by free biomass. For example, yeast cells entrapped in polyvinyl alcohol and alginate beads removed metal ions at a slower rate, reaching sorption equilibrium in 12 and 24 h, respectively. This rate was as much as 24 and 48 times slower than the 30 min taken by free mass of yeast cells (Ting & Sun, 2000). Another study reported that sorption equilibrium for the removal Cd^{2+} by microbial cell mass entrapped in Na-alginate beads was achieved in 15 h, which was 100 times greater as compared with the time taken by free microbial cell mass (Gourdon *et al.,* 1990). This slower rate of Cd^{2+} sorption by the microbial cell mass entrapped in Na-alginate beads was attributed by these authors to the diffusional restriction exerted by the gel matrix on the movement of Cd^{2+} ions. Similar slower rate of Cu^{2+} sorption by cell mass of *Ascophyllum nodosum* entrapped in hydrophilic polyurethane foam, which was 320 min compared with 90 min taken by free cell mass, has been also reported (Alhakawasti & Banks, 2004). This was suggested to be due to the additional time needed for the diffusion of Cu^{2+} through the entrapment matrix to reach the active sorption sites on the biosorbent surface. The LIBKC, however, did not present such a restriction as the microalgal cells were immobilized on the open-faced surface of the LS discs and easy solid-liquid phase contact was possible through the highly porous biostructural matrix of loofa sponge.

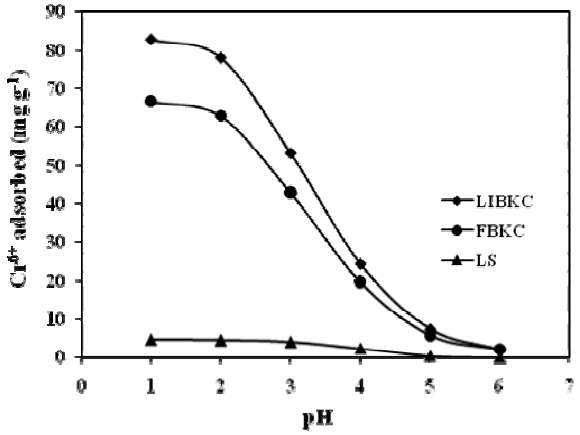


Fig. 2. Effect of pH on the sorption capacities of naked loofa sponge (LS), free biomass of *Kirchneriella contorta* (FBKC) and loofa immobilized biomass of *K. contorta* (LIBKC) on the removal of Cr^{6+} ions from 100 mL of 100 mg L^{-1} metal solution, during orbital shaking at 100 rpm for 60 min at ambient temperature.

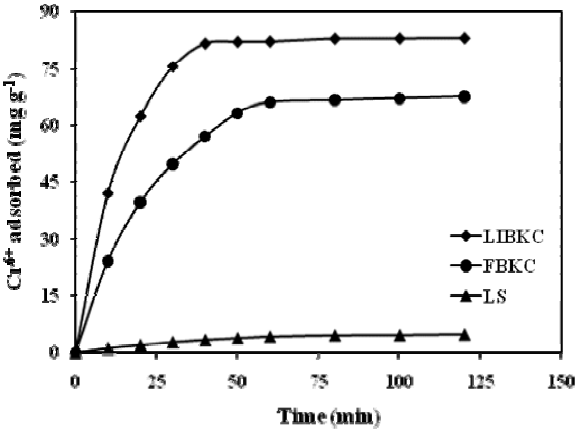


Fig. 3. Effect of contact time on the biosorption capacities of naked loofa sponge (LS), free biomass of *Kirchneriella contorta* (FBKC) and loofa immobilized biomass of *K. contorta* (LIBKC) on the removal of Cr^{6+} ions from 100 mL of 100 mg L^{-1} metal solution, pH 1.0, on orbital shaking at 100 rpm at ambient temperature.

Adsorption isotherms and maximum hexavalent chromium sorption capacity of LIBKC: The maximum Cr^{6+} biosorption capacity of LIBKC and FBKC was, respectively, 100.84 mg g^{-1} and 80.61 mg g^{-1} dry weight algal cell mass (Fig. 4). This shows that LIBKC had 25.09% higher Cr^{6+} sorption capacity than FBKC. Cr^{6+} sorption by the LS discs, without immobilized *K. contorta* cells (the naked LS control) was only 4.73 mg g^{-1} loofa sponge. Though it cannot be predicted as to what quantity of it was contributed to the 100.84 mg Cr^{6+} g^{-1} sorption by the LIBKC biosorbent system, yet it is likely to be insignificant as the quantity sorbed by the naked LS was too small anyway. It may thus be concluded that sorption by LIBKC was due to its expanded surface area, which was a result of the immobilization of *K. contorta* biomass along the surface of the fibrous structure of LS, which appeared completely covered by the algal cells (Fig. 1b), that would have nevertheless appreciably masked the

sorption sites present on the naked loofa sponge. From these findings it can be concluded, therefore, that the use of LS discs as a biostructural tool to immobilize *K. contorta* cells can significantly enhance the sorption capacity of LIBKC without any negative influence on the Cr^{6+} removal process. This is an important achievement as most of the biosorbents based on immobilized microbial cells, used previously, were close-bodied gels or beads made from natural or synthetic polymers, which have been reported to result in decreased metal sorption capacity as compared with free cells. For example, heavy metals biosorption by immobilized algal cells was reduced by 40% when silica gel was used as the entrapment matrix in comparison with the capacity of free algal cells (Mahan & Holcombe, 1992). Similarly, when steepness of the decrease in Cr^{6+} uptake by various immobilizing agents, against free biomass of *R. nigricans* was investigated, the decreasing trend in biosorption was observed in the order of free biomass > polysulfone > polyisoprene > PVA > alginate > polyacrylamide, as 97.67 > 89.61 > 89.98 > 81.78 > 70.07 > 41.76 % Cr^{6+} adsorbed, respectively (Bai & Abraham, 2001). Another study reported a decrease of 10.44% in Cr^{6+} adsorption when *R. arrhizus* was entrapped in alginate beads in comparison with free mycelia mass of the fungus (Prakasham *et al*., 1999). These reductions have been attributed to the restrictions exerted on the movement of metal adsorbate, or due to masking of the functional group sites of the biosorbent (Viyayaraghavan *et al*., 2007). Further, surface of the immobilized cells may get partly shielded by the gel matrix, which would then become unavailable for adsorbate binding (Gourdon *et al*., 1990). In the present study, surface immobilization of *K. contorta* on the LS discs enabled a direct contact of the algal cells to Cr^{6+} ions, which as a result increased cell surface area for the exposure of functional active moieties. This open-faced system is better for removal of the metal sorbate than the enclosed or beaded biomass entrapment systems based on polymeric gels.

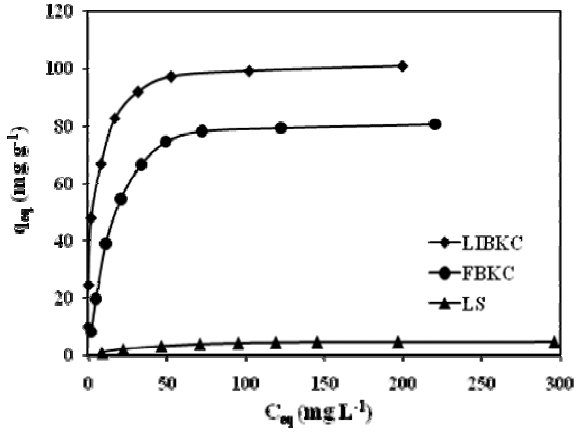


Fig. 4. Effect of initial metal ion concentration on biosorption of Cr^{6+} by naked loofa sponge (LS), free biomass of *Kirchneriella contorta* (FBKC) and loofa immobilized biomass of *K. contorta* (LIBKC) on the removal of Cr^{6+} ions from 100 ml of metal solution (10-300 mg L^{-1}), pH 1.0, on orbital shaking at 100 rpm at ambient temperature, where q_{eq} is the amount of Cr^{6+} sorbed at equilibrium (mg Cr^{6+} adsorbed g^{-1} LIBKC) and *Ceq* is the equilibrium concentration (mg L^{-1}).

Several isotherms models have been used for the analysis of biosorption data at equilibrium. During the present study, Langmuir and Freundlich isotherms equations were used for finding fit of the biosorption data. According to Langmuir isotherms equation, adsorption is monolayer, which is expressed as:

$$q_{eq} = \frac{q_{\max} b C_{eq}}{\left(1 + bC_{eq}\right)} \qquad (6)$$

where q_{eq} = amount of Cr^{6+} sorbed at equilibrium (mg g^{-1}); q_{max} = maximum Cr^{6+} uptake capacity (mg g^{-1}); C_{eq} = equilibrium metal concentration (mg L^{-1}); and b (L^{-1} mg) = equilibrium constant

On the other hand, the Freundlich isotherms model is an empirical equation that is based on the sorption of a sorbate on a heterogeneous surface of a sorbent as below:

$$q_{eq} = K_F C_{eq}^{1/n} \qquad (7)$$

where K_F and n = Freundlich empirical constants, respectively, indicative of sorption capacity and sorption intensity

Both isotherms models consider q_{eq} as a function of the C_{eq}, corresponding to the equilibrium distribution of ions between aqueous and solid phases as the C_i increases. For each isotherms model, the C_i of Cr^{6+} was varied between 10 and 300 mg L^{-1}, however, the weight of LIBKC was kept the same. The linearised Langmuir and Freundlich isotherms plots of Cr^{6+} biosorption for both FBKC and LIBKC are presented in Fig. 5a and 5b. The constants of the two adsorption isotherms, alongwith their respective regression correlation coefficients (r^2) were calculated from these plots (Table 1). As may be observed from the data given in the table, very high r^2 values of 0.997 and 1.0 were obtained for Langmuir isotherms model for FBKC and LIBKC, respectively. Accordingly, Langmuir model appeared to be suitable for describing Cr^{6+} biosorption by *K. contorta* cells at equilibrium. On the other hand, r^2 values for Freundlich isotherms model of were 0.856 and 0.912, respectively, for FBKC and LIBKC, were relatively low.

Biosorption kinetics modelling of hexavalent chromium: In order to analyse the biosorption kinetics of Cr^{6+} ions, pseudo first-order (Lagergren, 1989) and pseudo second-order (Ho & Mackay, 1999) equations were used to analyse the sorption equilibrium time data. The pseudo first-order kinetics equation postulates that the rate of occupation of active sorption sites is proportional to the number of unoccupied sites at the given time. The following equation is used to express the pseudo first-order kinetics:

$$\frac{dq_t}{dt} = k_{1.ads}\left(q_{eq} - q_t\right) \qquad (8)$$

where q_{eq} = amount of Cr^{6+} adsorbed at equilibrium (mg g^{-1}); q_t = Cr^{6+} adsorbed at any given time t (mg g^{-1}); and $k_{1.ads}$ = rate constant of adsorption (min^{-1})

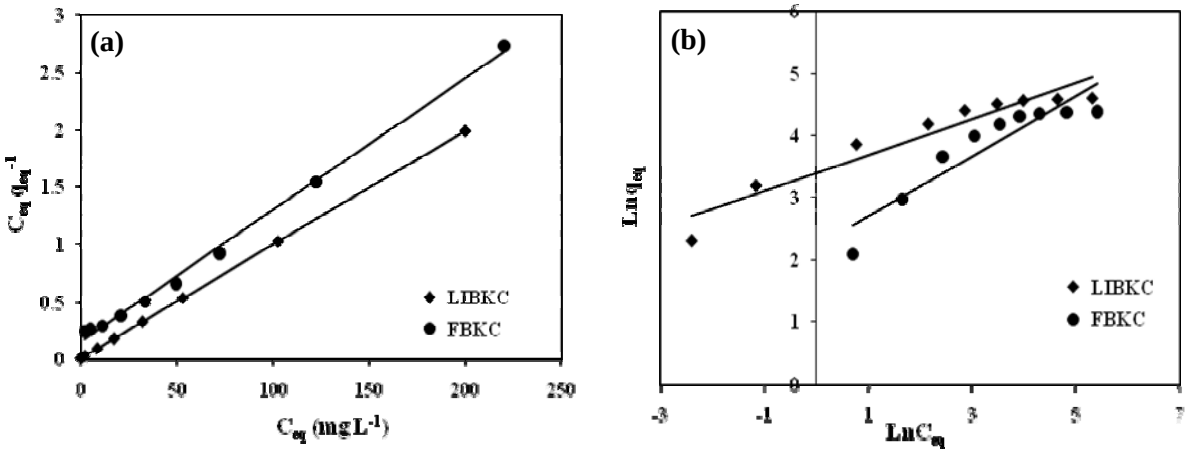


Fig. 5. The linearized (a) Langmuir and (b) Freundlich adsorption isotherms for the sorption of Cr^{6+} by free biomass of *Kirchneriella contorta* (FBKC) and loofa immobilized biomass of *K. contorta* (LIBKC), where q_{eq} is the amount of Cr^{6+} sorbed at equilibrium (mg g^{-1}) and C_{eq} is the equilibrium concentration (mg L^{-1}).

Table 1. Isotherms models and correlation coefficients (r^2) for the biosorption of Cr^{6+} ions from aqueous solution by free biomass of *Kirchneriella contorta* (FBKC) and loofa immobilized biomass of *K. contorta* (LIBKC), where q_{max} is maximum Cr^{6+} uptake (mg g^{-1} biosorbent), b is the equilibrium constant, and K_F and n are the Freundlich constants.

Biosorbents	Experimental	Langmuir			Freundlich		
	q_{eq} (mg g^{-1})	q_{max} (mg g^{-1})	b (l mg^{-1})	r^2	K_F	n	r^2
FBKC	80.61	79.49	0.055	0.99	9.18	2.06	0.856
LIBKC	100.84	100.43	0.768	1.00	30.46	3.46	0.912

On application of certain boundary conditions, we get:

$$q_t = 0 \text{ at } t = 0;\ q_t = q_t \text{ at } t = t$$

when equation (8) is integrated, we get:

$$\text{Ln}(q_{eq} - q_t) = \text{Ln}q_{eq} - k_{1.ads}t \qquad (9)$$

The rate constant ($k_{1.ads}$) and the quantity of Cr^{6+} adsorbed (q_{eq}) at equilibrium were, respectively, determined from the slope and intercept point of the graph of Ln(q_{eq} - q_t) versus time (Fig. 6a; Table 2). The graph of Ln(q_{eq} - q_t) versus t indicates the status of applicability of pseudo first order kinetics model (Ho & Mackay, 1999). However, as the data did not yield a straight line (Fig. 6a), it is concluded that the data did not fit the Lagergren pseudo first-order model. Whereas pseudo first-order model gives a good description of the sorption data, it is reported not to be applicable in several cases, for which the application of pseudo second-order equation has been recommended (Aksu, 2001; Ho *et al*., 1996). The pseudo second-order model describes the sorption capacity at equilibrium, for which the following equation is used:

$$\frac{dq_t}{dt} = k_{2.ads}\left(q_{eq} - q_t\right)^2 \qquad (10)$$

where $k_{2.ads}$ = pseudo second-order rate constant (g mg^{-1} min^{-1})

Table 2. Theoretically determined constants of pseudo first-order and pseudo second-order kinetics models based on the sorption from 100 mg L^{-1} of Cr^{6+} solutions, pH 1.0, by free biomass of *Kirchneriella contorta* (FBKC) and loofa immobilized biomass of *K. contorta* (LIBKC).

Biosorbents	Experimental	Pseudo first-order constants			Pseudo second-order constants		
	q_{eq} (mg g^{-1})	q_{eq} (mg g^{-1})	$k_{1.ads}$ (min^{-1})	r^2	q_{eq} (mg g^{-1})	$k_{2.ads}$ (g mg^{-1} min^{-1})	r^2
FBKC	66.07	101.54	-0.0659	0.975	67.91	0.0005	0.993
LIBKC	82.17	63.15	-0.0729	0.973	82.30	0.0016	0.996

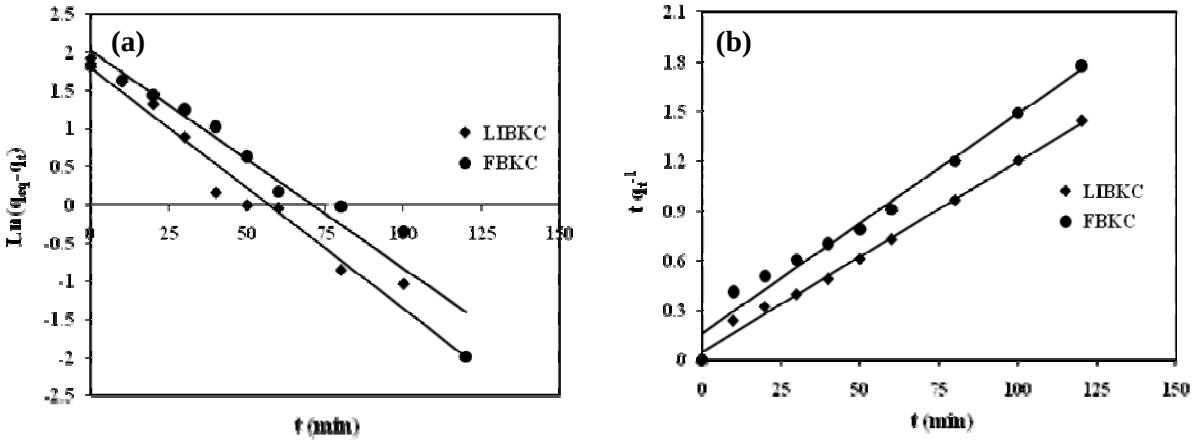


Fig. 6. Linearised pseudo first-order (a) and pseudo second-order (b) kinetic models for Cr^{6+} ions uptake by loofa immobilized biomass of *Kirchneriella contorta* (LIBKC) and free biomass of *K. contorta* (FBKC).

Linearizing equation (10), after applying boundary conditions ($q_t = 0$ at $t = 0$; $q_t = q_t$ at $t = t$), we get:

$$\frac{t}{q_t} = \frac{1}{k_{2.ads} q_{eq}^2} + \frac{1}{q_{eq}} t \qquad (11)$$

The graph of t/q_t against t resulted in a straight line (Fig. 6b), having r^2 value greater than 0.99 for pseudo second-order kinetics equation for both FBKC and LIBKC for the contact time of 60 min. The experimental q_{eq} and theoretical q_{eq} values for both FBKC and LIBKC were very close when pseudo second-order equation was applied (Table 2). These observations suggest that the LIBKC biosorbent system was not described by the pseudo first-order model, whereas the data fit well to the pseudo second-order model, based on the assumption that the rate limiting step may be biosorption involving valence forces through sharing or exchange of electrons between the active binding sites of the sorbent and the sorbate (Ho & Mackay, 1999). It may be noted that a fit to the pseudo second-order equation is significantly useful on the following accounts: (a) the data can be used for the determination of time needed to achieve sorption equilibrium; (b) the rate of sorption can be used for the development of predictive models; and (c) the model can be used for understanding the variables that influence the adsorption process (Singh *et al*., 2001; Ho *et al*., 1996).

Regeneration and reusability of LIBKC: The regeneration capacity of a biosorbent is likely to be the key factor for assessing its potential for any large-scale application. A series of desorbing agents were, therefore, used to recover Cr^{6+} ions from the metal loaded-LIBKC, for subsequent application of the Cr^{6+} desorbed LIBKC for next adsorption cycle. Percentage recovery of Cr^{6+} ions by various agents from the Cr^{6+}-loaded LIBKC is given in Fig. 7. Efficiency of Cr^{6+} ions recovery of different desorbing agents used in the study was in the descending order of NaOH > Na_2CO_3 > $NaHCO_3$ > KCl > KOH > $CaCl_2.2H2O$ > NaCl > HCl > HNO_3. It was observed that 96.81% of the loaded-Cr^{6+} onto LIBKC was recovered with 0.1 M NaOH, which was used in subsequent studies for consecutive five sorption-desorption cycles (Table 3). The data so obtained clearly show the successful repeated reusability potential of LIBKC for five sorption-desorption cycles of removal of Cr^{6+} ions from metal contaminated water. A decrease of only 4.08% in the adsorption capacity of LIBKC was noted during these five sorption-desorption cycles. These findings show that the LIBKC biosorbent system has a good potential to adsorb Cr^{6+} ions from aqueous solution and can be used repeatedly, for the removal of hexavalent chromium, after desorption with 0.1 M NaOH.

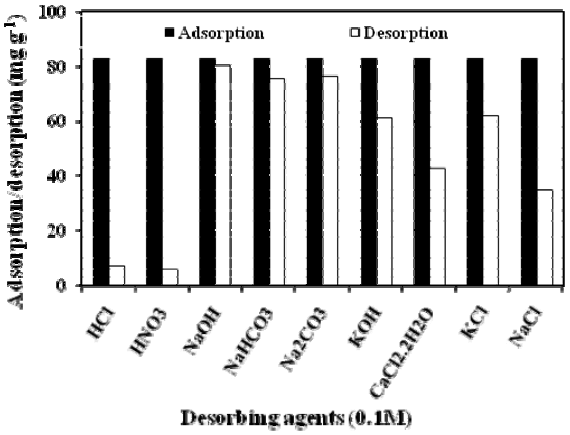


Fig. 7. Desorption efficiencies of Cr^{6+} from the metal-loaded loofa immobilized biomass of *Kirchneriella contorta* (LIBKC) using different desorbing agents at 0.1 M concentration.

Evaluation of LIBKC in continuous flow columns for sorption-desorption of hexavalent chromium: The potential of LIBKC for the sorption of Cr^{6+} in continuous flow columns was determined by packing LIBKC as fixed-bed columns. The breakthrough curve for Cr^{6+} adsorption onto LIBKC is shown in Fig. 8a. The Cr^{6+}-loading curve shows a clear zone of 99.9% removal of Cr^{6+} before the breakthrough point was reached. It was observed that the column packed with 1.098 g of immobilized biomass of *K. contorta* as LIBKC had the capacity to treat 6.5 L of 10 mg Cr^{6+} L^{-1} contaminated water before reaching the breakthrough point (Fig. 8a).

During loading of the loofa sponge packed fixed-bed column, a total of 112.60 mg of Cr^{6+} was accumulated in the column at saturation point, which was achieved after passing 14.8 L of the metal solution contaminated with 10 mg Cr^{6+} L^{-1}. The value of 112.60 mg Cr^{6+} loading was obtained on numerical integration of the breakthrough curve between the breakthrough and saturation points. Thus, Cr^{6+} sorption capacity of the LIBKC packed column was 103.31 mg g^{-1} of immobilized biomass of *K. contorta*, which agrees well with the experimental q_{max} of 100.84 mg g^{-1} obtained during batch studies (Table 3).

Table 3. Biosorption and desorption of Cr^{6+} from 100 mg L^{-1} metal solution by loofa immobilized biomass of *Kirchneriella contorta* (LIBKC) in five consecutive cycle, using 0.1 M NaOH as the desorbent.

Cycle no.	Cr^{6+} adsorbed (mg g^{-1}LIBKC)	Decrease in adsorption capacity (%)	Cr^{6+} desorbed (mg g^{-1}LIBKC)	Efficiency of desorption (%)
1	82.91		80.34	96.9
2	81.88	1.24	79.83	97.5
3	81.23	0.8	77.57	96.2
4	80.46	0.95	77.88	96.8
5	79.58	1.09	76.20	95.8

Reusability of LIBKC in a continuous flow column was determined by the desorption of Cr^{6+} loaded onto the LIBKC packed column. This was done by passing 0.1 M NaOH through the column at the flow rate of 5 ml min^{-1}. Complete desorption of Cr^{6+} from the fixed-bed column was achieved on the passage of 300 ml 0.1 M NaOH (Fig. 8b). The Cr^{6+} desorbed by the passage of 300 ml NaOH was 109.47 mg, which amounts to 97.22% of the 112.60 mg total Cr^{6+} adsorbed in the fixed-bed column. The volume of Cr^{6+} solution treated for adsorption was 14.8 L, whereas only 0.3 L of 0.1 M NaOH was used to elute 109.47 mg Cr^{6+} during desorption. The Cr^{6+} solution thus obtained in the small NaOH volume of 0.3 L was highly concentrated, which was desirable as the eventual recovery of the contaminant metal was rendered more feasible.

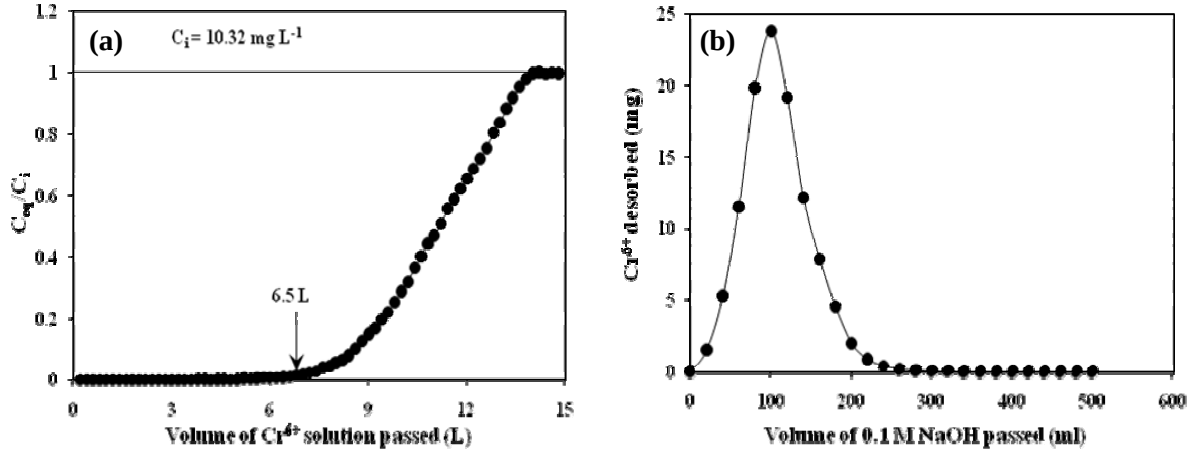


Fig. 8. Biosorption (a), and desorption (b) breakthrough curves for the removal of Cr^{6+} in a fixed-bed column reactor packed with loofa immobilized biomass of *Kirchneriella contorta* (LIBKC).

Acknowledgements

The supply of *Kirchneriella contorta* culture by Prof. Dr. Peter Pohl, University of Kiel, Germany is gratefully acknowledged. Also the funding provided by International Foundation for Science (IFS), Sweden and COMSTECH, Pakistan, through project grant W/3781-2 for the purchase of spectrophotometer. Thanks are also due to Prof. Dr. Naeem Rashid, School of Biological Sciences, Punjab University, Lahore, Pakistan for making available some chemicals.

References

Akhtar, N., A. Saeed and M. Iqbal. 2003. *Chlorella sorokiniana* immobilized on the biomatrix of vegetable sponge of *Luffa cylindrica*: a new system to remove cadmium from contaminated aqueous medium. *Bioresour. Technol*., 88: 163-165.

Aksu, Z. 2001. Equilibrium and kinetic modeling of cadmium(II) biosorption by *C. vulgaris* in batch system: effect of temperature. *Separ. Purif. Technol*., 21: 285-294.

Alhakawasti, M.S. and C.J. Banks. 2004. Removal of copper from aqueous solution by *Ascophyllum nodosum* immobilised in hydrophilic polyurethane foam. *J. Environ. Manage*., 72: 195-204.

Arica, M.Y., İ. Tüzün, E. Yalçin, . İnce and G. Bayramo□lu. 2005. Utilization of native, heat and acid-treated microalga *Chlamydomonas reinhardtii* preparations for biosorption of Cr(VI) ions. *Process Biochem*., 40: 2351-2358.

Bai, R.S. and T.E. Abraham. 2001. Biosorption of chromium(VI) from aqueous solution by *Rhizopus nigricans*. *Bioresour. Technol*., 79: 73-81.

Bal K.E., Y. Bal and A. Lallam. 2004. Gross morphology and absorption capacity of cell fibers from fibrous vascular system of loofah (*Luffa cylindrica*). *Text. Res. J*., 74: 241-247.

Barbotin, J.N. and J.E.N. Sauedo. 1998. Bioencapsulation of living cells by entrapment in polysaccharide gels. In: *Polysaccharides: Structural Diversity and Functional Versatility* (Eds.): S. Dumitriu. Marcel Dekker, New York, pp. 749-774.

Basha, S., Z.V.P. Murthy and B. Jha. 2008. Biosorption of hexavalent chromium by chemically modified seaweed, *Cystoseria indic. Chem. Eng. J*., 137: 480-488.

Bayramo□lu, G. and M.Y. Arica. 2009. Construction of a hybrid biosorbent using *Scenedesmus quadricauda* and Ca-alginate for biosorption of Cu(II), Zn(II) and Ni(II): kinetics and equilibrium studies. *Bioresour. Technol*., 100: 186-193.

Crist R.H., K. Oberholser, N. Shank and M. Nguyen. 1981. Nature of bonding between metallic ions and algal cell walls. *Environ. Sci. Technol*., 15: 1212-1217.

de-Bashan, L.E. and Y. Bashan. 2010. Immobilized microalgae for removing pollutants: Review of practical aspects. *Bioresour. Technol*., 101: 1611-1627.

Gottler, R.A. and M.D. Piwoni. 2005. Metals. In: *Standard Methods for the Examination of Water and Wastewater* (Eds.): Eaton A.D., L.S. Clesceri, E.W. Rice and A.E. Greenberg. Washington, DC, pp. 3: 1-3.107.

EPA (Environmental Protection Agency). 1990. Environmental Pollution Control Alternatives EPA/625/5-90/025, EPA/625/4-89/023, Cincinnati, USA.

Febrianto, J. A.N. Kosasih, J. Sunarso and Y.-H. Ju. 2009. Equilibrium and kinetic studies in adsorption of heavy metals using biosorbent: a summary of recent studies. *J. Hazard. Mat*., 162: 616-645.

Gourdon, R., E. Rus, S. Bhende and S. Sofer. 1990. A comparative study of cadmium uptake by free and immobilized cells from activated sludge. *J. Environ. Sci. Health*, A25: 1019-1036.

Ho Y.-S. and G. Mackay. 1999. Pseudo second-order model for sorption process. *Process Biochem*., 34: 451-465.

Ho Y.-S., D.A.J. Wase and C.F. Forster. 1996. Kinetic studies of competitive heavy metal adsorption by *Sphagnum* peat moss. *Environ. Technol*., 17: 71-77.

Iqbal M., A. Saeed and R.G.J. Edyvean. 2008. Biosorption of lead(II) by free and immobilized fungal biomass of *Phanerochaete chrysosporium*: a comparative study. *Inter. J. Environ. Poll*., 34: 353-363.

Iqbal, M. and S. I. Zafar. 1993. "The use of fibrous network of matured dried fruit of *Luffa aegyptiaca* as immobilizing agent. *Biotechnol. Tech*., 7: 15-18.

Lagergren S. 1989. Zur Theories der sogenannten Adsorption gelöster Stoffe, Kungliga Svenska Vetenskapsakademiens. *Handlinger*, 24: 1-39.

Li S., X. Jin-lan, H. Huan and N. Zhen-yuan. 2008. Comparative study on biosorption of Pb(II) and Cr(VI) by *Synechococcus* sp. *Trans. Nonferr. Metals Soc. China*, 18: 1336-1342.

Li, H., T. Liu, Z. Li and L. Deng. 2008. Low-cost supports used to immobilize fungi and reliable technique for removal hexavalent chromium in wastewater. *Bioresour. Technol*., 99: 2234-2241.

Liu H.-L.L., B.-L.L. Chen and Y.-W.W. Lan. 2004. Biosorption of Zn(II) and Cu(II) by the indigenous *Thiobacillus thiooxidan. Chem. Eng. J*., 97: 195-201.

Mahan C.A. and J.A. Holcombe. 1992. Immobilization of algae cells on silica gel and their characterization for trace metal preconcentration. *Analyt. Chem*., 64: 1933-1939.

Murphy V., H. Hughes and P. McLoughlin. 2009. Comparative study of chromium biosorption by red, green and brown seaweed biomass. *Chemosphere*, 70: 1128-1134.

Naiya T.K., P. Chowdhury, A.K. Bhattacharya and S.K. Das. 2009. Sawdust and neembark as low-cost natural biosorbent for adsorptive removal of Zn(II) and Cd(II) ions from aqueous solutions. *Chem. Eng. J*., 148: 68-79.

Nicholas, H.W. and H.C. Bold. 1965. *Trichosarina polymorpha* gen. et. sp. Nov. *J. Phycol*., 1: 34-38.

Pehlivan, E. and T. Altun. 2008. Biosorption of chromium(VI) ion from aqueous solutions using walnut, hazelnut and almond shell. *J. Hazard. Mat*., 155: 378-384.

Prakasham, R.S., J.S. Merrie, R. Sheela, N. Saswathi and S.V. Ramakrishna. 1999. Biosorption of chromium VI by free and immobilized *Rhizopus arrhizus*", *Environ. Poll*., 104: 421-427.

Preetha, B. and T. Viruthagiri. 2007. Batch and continuous biosorption of chromium (VI) by *Rhizopus arrhizu. Sep. Puri. Technol*., 57: 126-133.

Rollinson, C.L. 1973. Chromium, molybdenum and tungsten. In: *Comprehensive Organic Chemistry* (Ed.): T. Dickenson. 3rd Edition. Pergamon Press, Oxford, UK, pp. 691-694.

Saeed, A. and M. Iqbal. 2006. Immobilization of blue green microalgae on loofa sponge to biosorb cadmium in repeated shake flask batch and continuous flow fixed bed column reactor system. *World J. Microbiol. Biotechnol*., 22: 775-782.

Saeed, A., M. Iqbal and S.I. Zafar. 2009. Immobilization of *Trichoderma viride* for enhanced Methylene Blue biosorption: batch and column studies. *J. Hazard. Mat*., 168: 406-415.

Singh, S., B.N. Rai and L.C. Rai. 2001. Ni(II) and Cr(VI) sorption kinetics by *Microcystis* in single and multimetallic system. *Process Biochem*., 36: 1205-1213.

Tewari, N., P. Vasudevan and B.K. Guha. 2005. Study of biosorption of Cr(VI) by *Mucor hiemalis*", *Biochem. Eng. J*., 23: 185-192.

Ting, Y. and G. Sun. 2000. Use of polyvinyle alcohol as a cell immobilization matrix for copper biosorption by yeast cells. *J. Chem. Technol. Biotechnol*., 75: 541-546.

Yan, G. and T. Viraraghavan. 2003. Heavy metal removal from aqueous solution by fungus *Mucor rouxii*. *Water Res*., 37: 4486-4496.

Yang, L. and J.P. Chen. 2008. Biosorption of hexavalent chromium onto raw and chemically modified *Sargassum* sp. *Bioresour. Technol*., 99: 297-307.

Viyayaraghavan, K., M.H. Han, S.C. Choi and Y.-S. Yun. 2007. Biosorption of Reactive Black 5 by *Corynebacterium glutamicum* biomass immobilized in alginate and polysulfone matrices. *Chemosphere*, 68: 1838-1845.

Vijayaraghavan, K., J. Mao and Y.-S. Yun. 2008. Biosorption of Methylene Blue from aqueous solution using free and polysulfone-immobilized *Corynebacterium glutamicum*: batch and column studies. *Bioresour. Technol*., 99: 2864-2871.

Volesky, B., H. May and Z.R. Holan. 1993. Cadmium biosorption by *Saccharomyces cerevisiae. Biotechnol. Bioeng*., 41: 826-829.

Volesky, B., S. Schiewer, M.C. Flickinger and S.W. Drew (Eds.): 1999. Biosorption of Metals", "*Encylopedia of Bioprocess Engineering*", John Wiely & Sons, New York, pp. 433-453.

(Received for publication 4 November 2010)