Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 20(2008) 231–239
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Biosorption characteristics of unicellular green alga *Chlorella sorokiniana* immobilized in loofa sponge for removal of Cr(III)

AKHTAR Nasreen[1], IQBAL Muhammad[2,*], ZAFAR Saeed Iqbal[3], IQBAL Javed[3]

1. Department of Biology, Government Islamia College for Women, Cooper Road, Lahore-54550, Pakistan. E-mail: Nasreenakhtar_058@hotmail.com
2. Environment Biotechnology Group, Biotechnology and Food Research Centre, PCSIR Laboratories Complex, Lahore-54600, Pakistan. E-mail: Iqbalmdr@brain.net.pk
3. School of Biological Sciences, University of Punjab, Lahore-54570, Pakistan.

Received 27 April 2007; revised 10 July 2007; accepted 16 August 2007

Abstract

Loofa sponge (LS) immobilized biomass of *Chlorella sorokiniana* (LSIBCS), isolated from industrial wastewater, was investigated as a new biosorbent for the removal of Cr(III) from aqueous solution. A comparison of the biosorption of Cr(III) by LSIBCS and free biomass of *C. sorokiniana* (FBCS) from 10–300 mg Cr(III)/L aqueous solutions showed an increase in uptake of 17.79% when the microalgal biomass was immobilized onto loofa sponge. Maximum biosorption capacity for LSIBCS and FBCS was found to be 69.26 and 58.80 mg Cr(III)/g biosorbent, respectively, whereas the amount of Cr(III) ions adsorbed onto naked LS was 4.97 mg/g. The kinetics of Cr(III) biosorption was extremely rapid and equilibrium was established in about 15 and 20 min by LSIBCS and FBCS, respectively. The biosorption equilibrium was well defined by Langmuir adsorption isotherm model. The biosorption kinetics followed the pseudo-second order kinetic model. The biosorption was found to be pH dependent and the maximum sorption occurred at the solution pH 4.0. Desorption studies showed that 98% of the adsorbed Cr(III) could be desorbed with 0.1 mol/L HNO_3, while other desorbing agents were less effective in the order: EDTA > H_2SO_4 > CH_3COOH > HCl. The regenerated LSIBCS retained 92.68% of the initial Cr(III) binding capacity up to five cycles of reuse in continuous flow-fixed bed columns. The study revealed that LSIBCS could be used as an effective biosorbent for the removal of Cr(III) from wastewater.

Key words: biosorption; immobilization; loofa sponge; *Chlorella sorokiniana*; Cr(III); desorption; fixed bed column reactor

Introduction

Cr(III) is an essential dietary mineral in low doses and is required to potentiate insulin and for normal glucose metabolism (ATSDR, 2000). However, its elevated concentrations in the environment are extremely toxic to fauna and flora (Mukherjee, 1998). Cr(III) is released into the environment from a variety of industrial effluents from the textile, leather tanning, electroplating, and metal finishing industries. Current technologies to remove Cr(III) such as precipitation and ion exchange with synthetic resins are expensive and generate toxic sludge that require further disposal (Volesky, 2001). As a result of these shortcomings, the foregoing concerns have led to an interest in the development of new technologies that can reduce Cr(III) concentrations to environmentally acceptable levels at affordable cost (Mohan and Pittman, 2006).

Biosorption, based on the metal binding capacities of various biological materials such as algae (Mehta and Gaur, 2005), fungi (Sag, 2001), bacteria (Chen *et al*., 2005), yeast (Wang and Chen, 2006) and agrowastes (Saeed *et al*., 2005) have gained attention during recent years due to its high efficiency and cost-effectiveness, particularly at low metal concentrations. Among these biomaterials, the heavy metal biosorption capacity of microalgae proved to be better (Mehta and Gaur, 2005) because of their large surface area and complex cell wall, which is composed of fiber-like structure and an amorphous embedding matrix of various polysaccharides (Blumreisinger *et al*., 1983). There are several functional chemical groups such as carboxyl, hydroxyl, amines, phosphates, sulfates, etc., which can attracts and sequester the heavy metal ions (Schiewer and Volesky, 2000). Several microalgal species have been investigated for the sorption of different heavy metals such as Cd(II), Pb(II), Zn(II), Cu(II) and Ni(II), both in single and mixed metal situations (Romera *et al*., 2006), but no work has been reported for the removal of Cr(III) by microalgal biomass. Microalgal biomass, furthermore, consists of small particles with low density, and poor mechanical strength and rigidity, which thus can not be used directly in a standard metal sorption process (Robinson, 1998). This has led to interest in the entrapment of microalgal cells as immobilized preparations

* Corresponding author. Present address: Water and Environmental Research Centre, Institute of Northern Engineering, University of Alaska, Fairbanks, AK 99775-5860, USA. E-mail: iqbalmdr@brain.net.pk.

(Mallick, 2002). Various natural and synthetic gel matrices have been tried for the immobilization of microalgal cells to removal heavy metals (Mallick, 2002; Rangsayatorn *et al*., 2004). Due to their close embedding structures, the immobilization matrices based on these polymeric gels, however, resulted in reduced metal sorbate-biomass sorbent contact and restricted diffusion (Mehta and Gaur, 2005). Their use is further limited by their insufficient mechanical strength and lack of open spaces to accommodate active cell growth resulting in their rupture and cell release into the growth medium (Rangsayatorn *et al*., 2004). These difficulties can be overcome by immobilizing the microbial biomass within the highly porous fibrous network of loofa sponge, papaya wood or petiolar felt-sheath of palm (Iqbal and Zafar, 1997; Akhtar *et al*., 2004; Iqbal and Saeed, 2005).

The present study therefore describes, as a first report, the use of loofa sponge immobilized biomass of microalga *Chlorella sorokiniana* (LSIBCS) as a potential low-cost biosorbent for the removal of Cr(III) from aqueous solution. The study is further unique as there is no existing report for the removal of Cr(III) by any kind of microalgae, both in free or immobilized form. Attempts were also made to characterize the various biosorption process parameters (i.e., pH, equilibrium time, initial metal ions concentration and adsorption isotherm modelling) influencing the metal adsorption-desorption in anticipation of the potential use of this newly developed immobilized biosorption system to large scale metal recovery systems in near future. To highlight the importance of LSIBCS as an innovative biosorbent, a comparison was drawn between the biosorption capacity of LSIBCS, free biomass of *C. sorokiniana* (FBCS) and previously reported Cr(III) adsorbents/biosorbents/ion exchange resins.

1 Materials and methods

1.1 Organism and growth medium

An indigenous strain of unicellular green microalga *C. sorokiniana* isolated from a wastewater body containing effluents from electroplating and leather industries was used in this study. Biomass for inoculum was grown to stationary phase in 100 ml Bold's medium contained in 250-ml Erlenmeyer flasks, shaken in an orbital shaker at 100 r/min at 25±2°C under continuous illumination with cool white light at an intensity of 50 μE/(m^2·s). Similarly prepared biomass was harvested, washed with deionized water, and freeze dried for free biomass Cr(III) biosorption studies.

1.2 Immobilization

Immobilization of *C. sorokiniana* was done on loofa sponge (LS) following the procedure reported earlier (Akhtar *et al*., 2004). The LS was cut into round pieces of approximately 2.5 cm diameter, 2–3 mm thick, soaked in boiling water for 30 min, thoroughly washed under tap water, and left for 24 h in distilled water, changed 3–4 times. The LS pieces were oven dried at 70°C, autoclaved for 20 min and soaked in Bold's medium for 5–10 min under aseptic conditions. Four pre-weighed LS pieces were transferred to 100 ml Bold's medium contained in 250-ml flasks (Fig.1a). Each of these flasks was inoculated with 5 ml, 3 weeks old stationary phase cultures of *C. sorokiniana* and incubated under similar conditions as those for developing inoculum biomass. The LS pieces were removed from the culture flasks, washed thoroughly with fresh culture medium to remove any free algal cells, transferred to 100 ml fresh medium and incubated under the same cultural condition. LSIBCS (Fig.1b) was harvested after 24 d, washed thoroughly with deionized water and freeze dried for further studies on metal biosorption. Quantity of the LSIBCS was determined as the difference between constant dry weights of the LS, before and after immobilization (Figs.1c and 1d).

1.3 Biosorption studies

Desired concentrations of Cr(III) solution were prepared by diluting standard Cr(III) stock solution $Cr(NO_3)_3$ (Merck Ltd., Poole, UK) of concentration 1000±2 mg/L. pH of the solution was adjusted to 4.0, unless otherwise stated using 0.1 mol/L NaOH. Fresh dilutions were used for each biosorption study. The biosorption capacity of both FBCS and LSIBCS was determined by contacting various concentrations (10 to 300 mg/L) of 100 ml Cr(III) solution in 250-ml flasks, with 100±2.6 mg microalgal biomass.

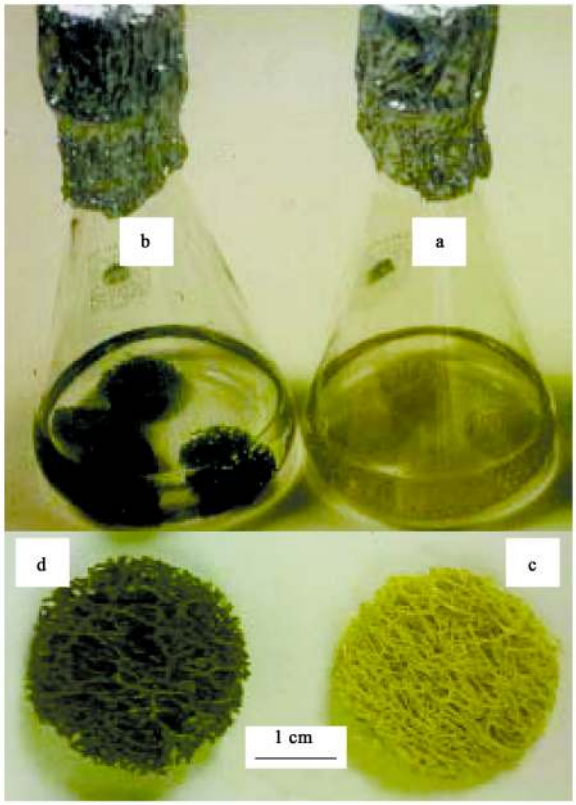

Fig. 1 Immobilization of *Chlorella sorokiniana* onto loofa sponge. (a) loofa sponge pieces in 100 ml growth medium contained in 250-ml Erlenmeyer flask before inoculation of *C. sorokiniana* cells; (b) loofa sponge pieces after 24 d of inoculation of *C. sorokiniana* cells showing growth of immobilized algal cells onto loofa sponge, loofa sponge pieces; (c) before and (d) after immobilization of *C. sorokiniana* cells.

The Cr(III) solutions, so incubated with microalgal biosorbents, were shaken on an orbital shaker at 100 r/min in tightly stoppered flasks at 25±2°C. FBCS was removed from metal solution by centrifugation at 5000 r/min for 5 min, whereas the LSIBCS was separated from the solution by decantation. Residual concentration of Cr(III) in the metal supernatant solutions was determined using an atomic absorption spectrophotometer (UNICAM-969, Unicam, Cambridge, UK). For the determination of rate of metal biosorption by both FBCS and LSIBCS, the supernatant was analysed for residual Cr(III) after the contact period of 5, 10, 15, 20, 30, 60 and 90 min. The effect of pH on Cr(III) sorption by FBCS and LSIBCS was determined by equilibrating the sorption mixture at different pH values of 2, 3, 4, 5 and 6. Metal-free solution and microalgal biomass-free metal solution containing only LS blanks were used as controls.

1.4 Desorption of Cr(III) from Cr(III)-loaded LSIBCS

For desorption studies, analytical grade HCl, H_2SO_4, HNO_3, EDTA and CH_3COOH were used. For batch desorption experiments, a series of 250-ml Erlenmeyer flasks containing 50 ml desorption solution of known concentration was contacted with Cr(III)-loaded biosorbent (100 mg) at room temperature (25±2°C). The mixtures were agitated on orbital shaker at 100 r/min for 30 min. The LSIBCS was removed and the supernatant was analysed for Cr(III) released by atomic absorption spectrophotometer.

1.5 Cr(III) sorption by LSIBCS in a continuous flow fixed-bed column reactor

To demonstrate the Cr(III) removal potential of LSIBCS in continuous flow system, 1.504±0.014 g LSIBCS was packed in glass column (30 cm in height and 2.7 cm in diameter), packing height 25 cm. A Cr(III) solution of 10 mg/L, pH 4.0, was pumped through this fixed bed column in an upward direction at a flow rate of 5 ml/min. Samples were collected at regular intervals (1 h) from the effluents to measure residual Cr(III) concentration. As the bed was saturated at inflow-outflow metal concentration equilibrium, the Cr(III) loading was terminated, and the bed was eluted with 0.1 mol/L HNO_3 solution to recover the Cr(III) ions biosorbed in the fixed bed column. Three such columns were operated separately, under similar conditions, to determine reproducibility. Separate columns, having similar specifications, were packed with the LS pieces, without immobilized algal cells to serve as the control.

1.6 Biosorption-desorption cycles

In order to determine the reusability of the LSIBCS, sorption-desorption cycles were repeated five times in fixed bed columns. For this purpose, 0.1 mol/L solution of HNO_3 was passed through the Cr(III) saturated LSIBCS packed columns in an upward direction at a flow rate of 5 ml/min. Effluent from the column was manually collected after every 20 ml desorbent passed. Each fraction was analyzed for Cr(III) content. The regenerated column bed was washed thoroughly with deionized water before used in the next adsorption cycle.

1.7 Reproducibility and data analysis

Unless indicated otherwise, the data shown are the mean values from three separate experiments. The amount of metal ions adsorbed per unit free and immobilized biomass (mg metal/g dry biosorbent) was determined using the following expression:

$$q = V(C_i - C)/M \tag{1}$$

where, q is the metal uptake (mgCr(III)/g dw of microalgal biomass entrapped within LS), V is the volume of metal solution (ml), C_i the initial concentration of Cr(III) in the solution (mg/L), C is the residual concentration of Cr(III) in the solution at any time, and M is the dry weight of microalgal biomass.

The Langmuir and Fruendlich equilibrium models were used for the evaluation of the adsorption data. Langmuir isotherm assumes monolayer adsorption, and is presented by the following equation:

$$q_{eq} = \frac{q_{max} b C_{eq}}{(1 + bC_{eq})} \tag{2}$$

where, q_{eq} and q_{max} are the equilibrium and maximum metal uptake capacities (mg/g biosorbent); C_{eq} is the equilibrium concentration (mg/L solution); b is the equilibrium constant (L/mg).

The Freundlich model is presented by Eq. (3):

$$q_{eq} = K_F C_{eq}^{1/n} \tag{3}$$

where, K and n are Freundlich constants characteristic of the system.

To examine the controlling mechanism of biosorption process such as mass transfer and chemical reaction, the pseudo-first-order and the pseudo-second-order kinetic models were used to test the experimental data of Cr(III) biosorption by both FBCS and LSIBCS. The first order rate equation of the Lagergren is represented as (Lagergren, 1898):

$$\ln(q_{eq} - q_t) = \ln q_{eq} - K_{1.ad} t \tag{4}$$

where, q_{eq} (mg/g) is the mass of metal adsorbed at equilibrium, q_t (mg/g) is the mass of metal adsorbed at time t and $K_{1.ad}$ (min^{-1}) is the first order reaction rate equilibrium constant. The pseudo-first order considers the rate of occupation of adsorption sites to be proportional to the number of unoccupied sites. A straight line of $\ln(q_{eq}-q_t)$ versus t indicates the application of the first order kinetics model. In a true first order process $\ln q_{eq}$ should be equal to the intercept of a plot of $\ln(q_{eq}-q_t)$ against t. In addition, a pseudo-second order equation (Ho and McKay, 2000) based on adsorption equilibrium capacity may be expressed in the form:

$$t/q_t = (1/K_{2ad} q_{eq}^2) + t/q_{eq} \tag{5}$$

where, K_{2ad} (g/(mg·min)) is the second order reaction rate equilibrium constant. A plot of t/q_t against t should give a linear relationship for the applicability of the second-order kinetics.

2 Results and discussion

2.1 Effect of contact time on Cr(III) biosorption

The effect of contact time on the biosorption of Cr(III) by FBCS, LSIBCS and naked LS is presented in Fig.2. Both FBCS and LSIBCS removed Cr(III) rapidly and a larger amount of Cr(III) was removed in the first 5 min of contact. The equilibrium was established in about 15 and 20 min with maximum Cr(III) uptake of 23.05±0.73 mg/g and 19.34±0.54 mg/g by LSIBCS and FBCS, respectively. Naked LS without immobilized algal biomass adsorbed Cr(III) far less than either FBCS or LSIBCS (Fig.2), indicating little effect of the immobilization matrix on the Cr(III) uptake. It can be noticed that after an equilibrium time of 15 and 20 min, no more Cr(III) was adsorbed suggesting that available sites on the biosorbent are the limiting factor for the biosorption. These results are typical for biosorption of metals involving no energy-mediated reactions, where metal removal from solution is due to purely physicochemical interactions between the biomass and metal in solution. The rapid rate of metal sorption by LSIBCS has a significant advantage over the other currently in use foam, polyvinyl alcohol and gel-immobilized biosorbent systems where a significant decrease in the rate of metal uptake has been reported in comparison with free biomass (Alhakawati and Banks, 2004; Karna *et al*., 1999; Ting and Sun, 2000). The slower metal adsorption rate of these foam/gel-immobilized biosorbents may be attributed to the restrictions encountered by the solute to diffuse through the foam/gel membrane for reaching the functional groups on the biomass surface.

2.2 Effect of pH on Cr (III) biosorption

The effect of pH on Cr(III) biosorption capacity of LSIBCS and FBCS was studied at 25 mg/L Cr(III) solution at 25°C. The biosorption of Cr(III) by both LSIBCS and FBCS showed a marked pH dependency (Fig.3). The uptake of Cr(III) increased significantly as the value of pH increased from 2 to 4. The maximum removal of Cr(III) was noted at pH 4.0 showing 22.94 and 19.54 mg/g biosorbent for LSIBCS and FBCS, respectively. No further increase in the Cr(III) was observed after pH 4.0. The low

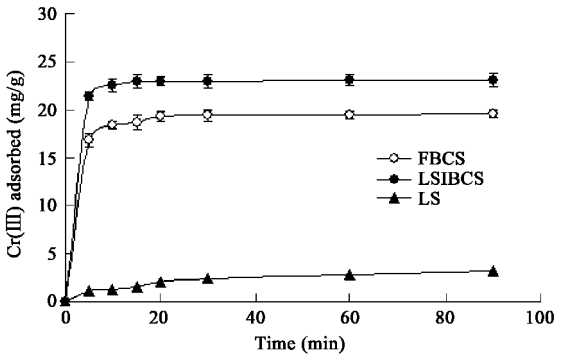

Fig. 2 Biosorption of Cr(III) from 25 mg/L solutions, pH 4.0, by 1 g/L microalgal biomass of *Chlorella sorokiniana* (FBCS) free or immobilized in loofa sponge (LSIBCS) and naked loofa sponge as related to the time of contact during orbital shaking at 100 r/min at 25°C.

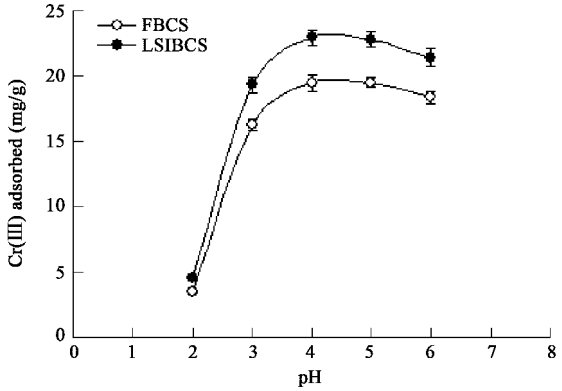

Fig. 3 Biosorption of Cr(III) from solutions of different pH by 100 mg FBCS or LSIBCS mixed in 100 ml 25 mg/L Cr(III) solution contained in 250-ml flasks and incubated on orbital shaker at 100 r/min at 25°C.

Cr(III) biosorption at pH 2 may be explained on the basis of active sites being protonated, resulting in a competition between protons and Cr(III) for occupancy of the binding sites. Similar trends in the sorption data have previously been reported for Cr(III) sorbing onto seaweed biomass of *Sargassum* (Kratochvil *et al*., 1998) and *Ecklonia* sp. (Yun *et al*., 2001). An increase in the biosorption of Cr(III) with the increase of pH can be explained on the basis of decrease in competition between protons and metal cations for the same functional groups and by decrease in the positive surface charge resulting in a lower electrostatic repulsion between the surface and metal ions (Reddad *et al*., 2002). A declining sorption trend was observed when the pH was increased to 6. This may be attributed to the decreased solubility of Cr(III) at high pH (Yun *et al*., 2001). For this reason, metal ion biosorption was not investigated at pH above 6.

2.3 Effect of initial metal concentration on biosorption

For determining the maximum biosorption capacity of Cr(III) by FBCS and LSIBCS, it is necessary to generate the equilibrium sorption data at various metal concentrations. For this purpose 10–300 mg/L of Cr(III) solutions, pH 4, were contacted with 1 g/L FBCS, LSIBCS and naked LS. Cr(III) uptake was noted to increase with the increase in metal ion concentration in the solution until it reached the maximum capacity of 69.26±1.28 and 58.80±1.76 mg/g biosorbent for LSIBCS and FBCS, respectively (Fig.4). This indicates a 17.79% higher Cr(III) removal by LSIBCS than by FBCS. The maximum adsorption of Cr(III) by LSIBCS was also noted to be higher than the other previously reported biosorbents (Table 1). The removal of Cr(III) by LS without immobilized microalgal biomass (naked LS) in the control run was found to be 4.97±0.24 mg/g. Though it is not possible to predict how much of it contributed to the 69.26±1.28 mg/g Cr(III) biosorbed by LSIBCS, yet most of it is likely to have been adsorbed on the expanded surface area of this unique biosorbent provided by microalgal cell biomass immobilized along the outer surface of the individual fibers of LS. From these results, nevertheless, it is clear that the use of LS as an immobilization matrix has significant-

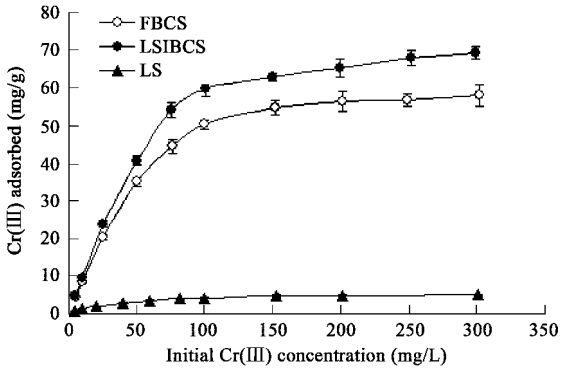

Fig. 4 Effect of initial metal concentration on biosorption of Cr(III) by FBCS, LSIBCS, and naked LS; 100 ml Cr(III) solution (10–300 mg/L, pH 4.0) was mixed with each biosorbent at 100 r/min at 25°C.

ly enhanced the metal biosorption capacity of LSIBCS and had no negative effect on the biosorption process. This is a significant achievement in the development of immobilized biosorbent systems over the currently used gel-immobilized biosorbent systems where a significant decrease in amount of metal sorption has been reported in comparison with free cells (Lopez *et al*., 2002; Rangsayatorn *et al*., 2004). The reduction in the rate of metal uptake by the gel-immobilized biosorbent have been projected to be due to limitations in the movement of metal ions, or the masking of active sites on the biosorbent (Prakasham *et al*., 1999). Moreover, part of the cell surface might be shielded by the gel matrix and thus would not be available for metal binding (Rangsayatorn *et al*., 2004).

2.4 Equilibrium modelling

Analysis of equilibrium data is important for developing an equation that can be used for design purposes. Several isotherm equations have been used for the equilibrium modelling of biosorption systems. Out of these isotherm equations, two have been applied for this study, the Langmuir and Freundlich isotherm models. For each isotherm initial Cr(III) concentrations were varied while the weight of FBCS and LSIBCS in each sample was kept constant. The linearized Langmuir and Freundlich adsorption isotherms of Cr(III) ions obtained for both FBCS and LSIBCS are given in Figs.5a and 5b. The Langmuir and Freundlich constants along with the correlation coefficients (r^2) calculated from Fig.5 are presented in Table 2. The correlation regression coefficients obtained with Langmuir isotherm model show that the adsorption process is better defined by Langmuir than by the Freundlich equation. The maximum capacity q_{max} determined from the Langmuir isotherm defines the total capacity of the biosorbent for Cr(III). The value of q_{max} appears to be higher for LSIBCS (68.51 mg/g) in comparison with the uptakes obtained by FBCS (56.68 mg/g). A large value of b also implied strong bonding of Cr(III) to the LSIBCS.

2.5 Kinetics modelling

To examine the controlling mechanism of biosorption process such as mass transfer and chemical reaction, the pseudo-first-order and the pseudo-second-order kinetic models were used to test the experimental data of Cr(III) biosorption by both FBCS and LSIBCS. The correlation coefficients for the linear plots of t/q_t against t, and experimental and theoretical values estimated from both first order and second order equations are presented in Fig.6 and Table 3. These results suggest that both FBCS and LSIBCS are not described by a first-order reaction, and that the pseudo-second-order kinetic model, based on the assumption that the rate-limiting step may be biosorption involving valence forces through sharing or exchange of electrons between biosorbent and sorbate, provides the best correlation of the data.

Table 1 Comparison of Cr(III) biosorption capacity of LSIBCS with microbial biomass and other biological waste materials; metal removing capacity of commercial ion exchange resins is also included for comparative purpose

Biomass type	Cr(III) uptake (mg/g)	Reference
Bacteria		
Streptomyces noursei	10.6	Mattuschka and Straube, 1993
Rhizobium BJVr 12	16.15	Mamaril *et al*., 1997
Cyanobacteria		
Spirulina sp.	9.62	Chojnacka *et al*., 2005
Fungi		
Aspergillus biomass	15.6	Sekhar *et al*., 1998
Rhizopus arrhizus	31	Tobin *et al*., 1984
Mucor hiemalis	21.4	Pillichshammer *et al*., 1995
Mucor meihi	59.8	Tobin and Roux, 1998
Microalgae		
LSIBCS	69.26	This work
Yeast		
Saccharomyces cerevisiae	8.5	Ferraz *et al*., 2004
Candida guilliermondii	6.7	Kaszycki *et al*., 2004
Biological waste materials		
Canola meal	37.82	Al-Asheh and Duvnjak, 1996
Carrot residues	45.09	Nasernejad *et al*., 2005
Cork powder	3.4	Machado *et al*., 2002
Eggshells	56.50	Chojnacka, 2005
Saw dust	5.52	Li *et al*., 2007
Commercial ion exchange resins		
IR120	67.7	Alguacil *et al*., 2004
IRN77	35.4	Rengaraj *et al*., 2002
1200H	84.04	Rengaraj *et al*., 2003
IRN97H	58.14	Rengaraj *et al*., 2003
SKNI	46.3	Rengaraj *et al*., 2001

2.6 Desorption of Cr(III)

Various types of desorbing agents such as aqueous solutions of mineral acids (hydrochloric, sulphuric and nitric acids), organic acid (acetic acid), and complexing agent (EDTA) were tested for desorption of adsorbed Cr(III) from Cr(III)-laden LSIBCS. Amount of Cr(III)

Table 2 Isotherm model constants and correlation coefficients for biosorption of Cr(III) from aqueous solution

Biosorbent	Langmuir			Freundlich		
	q_{max} (mg/g)	b (L/mg)	r^2	K_F	n	r^2
FBCS	56.68	0.110	0.991	10.67	2.85	0.877
LSIBCS	68.51	0.397	0.996	17.93	3.55	0.896

q_{max} means maximum metal uptake capacity; b means equilibrium constant; r^2 means correlation coefficient; K_F means Freundlich constant; n means Freundlich constant.

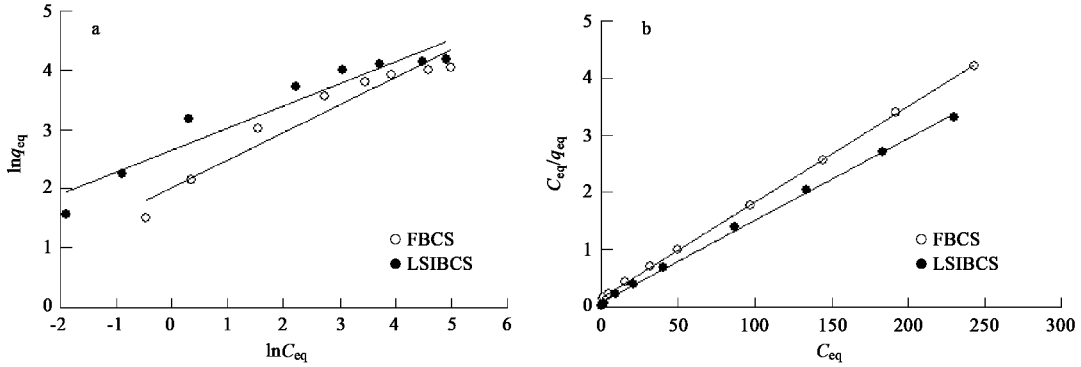

Fig. 5 Freundlich (a) and Langmuir (b) biosorption isotherms for sorption of Cr(III) by FBCS or LSIBCS based on specific quantity (q_{eq}) sorbed and quantity of Cr(III) in solution at equilibrium (C_{eq}).

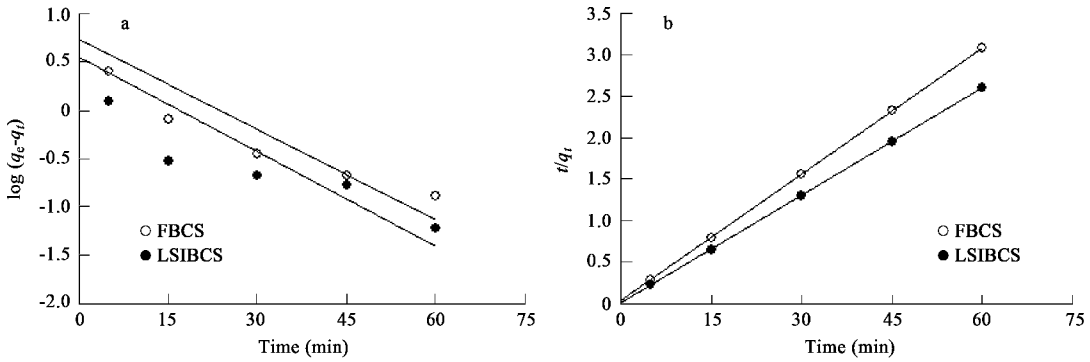

Fig. 6 Pseudo-first (a) and pseudo-second (b) order kinetics models for Cr(III) ions uptake by FBCS and LSIBCS.

Table 3 Theoretically determined constants of first and second order reaction kinetics

Biosorbent	Experimental q_{eq} (mg/g)	First order constants			Second order constants		
		q_{eq} (mg/g)	K_1 (min^{-1})	r^2	q_{eq} (mg/g)	K_2 (g/(mg·min))	r^2
FBCS	19.59	4.23	-0.058	0.88	19.71	0.066	1
LSIBCS	23.15	2.47	-0.055	0.95	23.17	0.152	1

Data are based on the sorption of metals from 25 mg/L Cr(III) solutions, pH 4.0, by 1 g/L FBCS and LSIBCS during shake flask at 100 r/mim for 60 min. q_{eq} means equilibrium metal uptake capacity; K_1 means first order reaction rate equilibrium constant; r^2 means correlation coefficient; K_2 means second order reaction rate equilibrium.

Table 4 Desorption of Cr(III) from Cr(III)-laden LSIBCS by various desorbing agents

Desorption agent (0.1 mol/L)	Biosorption (mg Cr(III)/g biomass)	Desorption (mg Cr(III)/g biomass)	Amount of Cr(III) recovered (%)
HCl	23.67±0.78	16.81±0.66	71.46
H_2SO_4	23.60±0.64	21.73±0.92	92.38
HNO_3	23.72±0.79	23.28±0.76	98.15
CH_3COOH	23.63±0.92	21.50±1.05	91.54
EDTA	23.58±0.52	22.24±0.89	94.32

desorbed by these desorbing agents are presented in Table 4. Sulphuric acid and acetic acid showed approximately equivalent elution efficiency. EDTA in NaOH removed 94.32% of Cr(III) from Cr(III)-laden LSIBCS. Hydrochloric acid (HCl) desorbed only 71.46% of Cr(III) from LSIBCS. Nitric acid (HNO_3) due to its highest Cr(III) desorption activity (98.15%) was selected as desorbing agent for further studies on adsorption-desorption of Cr(III) from Cr(III)-loaded LSIBCS for its reuse. Effect of different concentration of nitric acid on desorption of Cr(III) is presented in Fig.7. The results presented in the Fig.7 indicated that concentration of 0.1 mol/L HNO_3 or higher could remove more than 98% of adsorbed Cr(III). Being the lower concentration, 0.1 mol/L HNO_3 was selected for further studies. These results are also in accordance with previous studies on the biosorption of Cr(III) by other microorganisms (Chojnacka *et al*., 2005; Ferraz *et al*., 2004).

2.7 Performance and reusability of LSIBCS in continuous flow-fixed bed columns for the removal and recovery of Cr(III) from aqueous solution

The column breakthrough curves for Cr(III) adsorption onto LSIBCS are shown in Fig.8a. Breakthrough curves obtained exhibited a typical "S" shape curve of a fixed bed column; an initial zero reading or minimal Cr(III) at column outlet followed by a gradual increase in the effluent Cr(III) concentration that would ultimately reach the initial concentration of feed solution. At this point, the LSIBCS were considered fully saturated with Cr(III). Approximately 100% removal of Cr(III) was obtained in the first

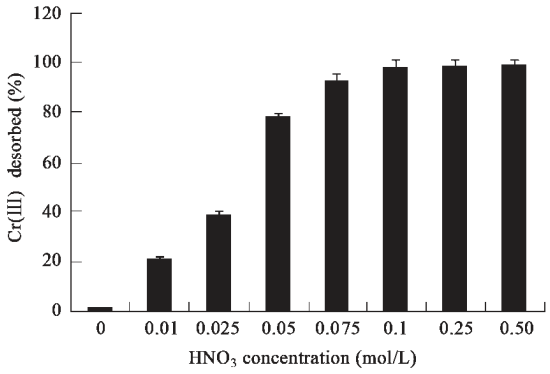

Fig. 7 Effect of HNO_3 concentration on desorption of Cr(III) from Cr(III)-loaded LSIBCS.

8.1±0.3 L, followed by a gradual increase of the Cr(III) concentration in the effluent. Complete breakthrough was detected at about 14.1±0.3 L. The total amount of Cr(III) removed by LSIBCS-packed fixed bed column was found to be 114.43±1.42 mg. This value was obtained by numerical integration of the whole breakthrough curve. Thus, the Cr(III) biosorption capacity of the LSIBCS in column is 76.36±1.54 mg Cr(III)/g LSIBCS, which is even better than the maximum value of 69.26±1.28 mg/g obtained from batch experiments. For the reusability of LSIBCS in continuous flow system, desorption of loaded Cr(III) from LSIBCS packed in column was examined. For the purpose, 450 ml of 0.1 mol/L HNO_3 was passed through the column at a flow rate of 5 ml/min. A complete removal of Cr(III) from the fixed bed column was, however, achieved after passing 300 ml of 0.1 mol/L HNO_3 (Fig.8b). The regenerated LSIBCS were then used for Cr(III) binding as described above for repeated adsorption-desorption cycles and results are shown in Table 5. The decline in Cr(III) uptake efficiency after five cycles was noted to be 7.32%, which shows that the LSIBCS disc biosorbent has good potential to adsorb metal from aqueous solution in fixed bed column bioreactors under continuous liquid flow conditions in repeated cycles.

2.8 Biosorption mechanism approach

Desorption of heavy metals from biosorbents by mineral acids (e.g., HCl, H_2SO_4 and HNO_3) has been interpreted in terms of ion exchange by many workers (Schiewer and Volesky, 2000; Chojnacka *et al*., 2005; Li *et al*., 2006). In each case, the protons (H^+) competes with the bound metal ions for the binding sites and replaces them if the concentration of desorbing agent is high enough. As shown in Table 5, total 98% desorption of Cr(III) from Cr(III)-laden LSIBCS was observed by the addition of HNO_3, with only little change in biosorption/desorption capacity of LSIBCS during five repeated cycles. On the basis of these observations, it may be argued that main Cr(III) binding mechanism is probably ion exchange. The release of Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+} and Fe^{3+} into the reaction solution during biosorption of Cr(III) on LSIBCS observed during the present study (Table 6) also indicates that ion exchange played an important role in biosorption of Cr(III) on LSIBCS. The multi-elemental analysis of solution after biosorption revealed the presence of cations that were initially not present in the solution before biosorption process. When Cr(III) appeared in the solution, cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}) and protons (H^+) from algal biomass were exchanged from the binding sites with Cr(III) ions and process of ion-exchange occurred. Multi-elemental analysis of the LSIBCS before the biosorption of Cr(III) confirmed the presence of these ions in LSIBCS (Table 6). At the same time, decrease in

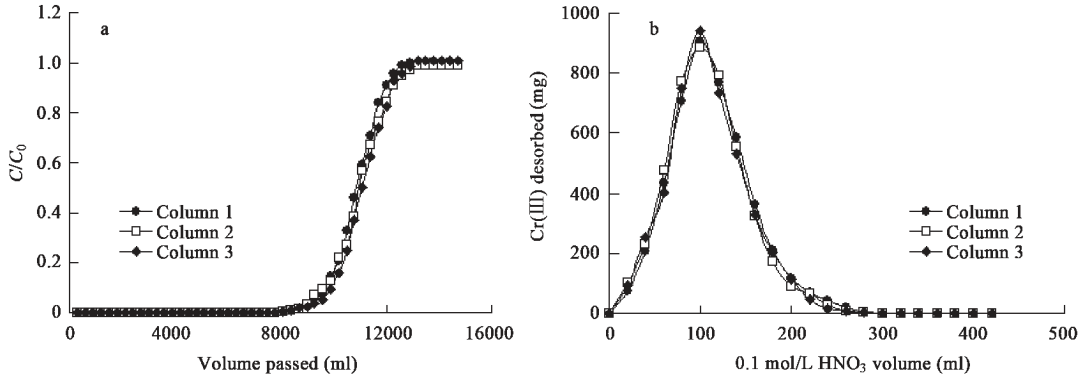

Fig. 8 Biosorption (a) and desorption (b) breakthrough curves for the removal of Cr(III) in a fixed bed column reactors based on LSIBCS as the biosorbent.

Table 5 Adsorption, desorption and desorption/reloading efficiency of Cr(III) in five consecutive cycles by LSIBCS packed in fixed bed column bioreactors

Cycle No.	Adsorption (mg)	Desorption (mg)	Desorption efficiency (%)	Reloading efficiency (%)
1	114.43 ± 1.42	112.32 ± 1.56	98.15	–
2	111.23 ± 1.29	108.90 ± 1.72	97.90	97.20
3	109.28 ± 1.61	107.56 ± 0.59	98.42	95.49
4	107.79 ± 0.98	106.21 ± 2.04	98.53	94.19
5	106.05 ± 1.07	103.52 ± 1.66	97.61	92.68

Table 6 Multi-elemental composition of *Chlorella sorokiniana* before and after Cr(III) uptake

Element	Amount of element (mg/g LSIBCS)		
	Before Cr(III) biosorption	After Cr(III) biosorption	Released in solution after biosorption
Na^+	9.45	1.67	7.78
K^+	12.06	1.11	10.98
Ca^{2+}	0.94	0.08	0.86
Mg^{2+}	2.67	0.48	2.187
Mn^{2+}	0.014	0.005	0.009
Fe^{3+}	0.132	0.028	0.104

solution pH after Cr(III) biosorption was observed, which also suggested the exchange of protons by Cr(III). Other workers while investigating the biosorption of Cr(III) by cyanobacteria (Chojnacka *et al*., 2005; Li *et al*., 2006), have also reported that main Cr(III) sorption mechanism is probably ion exchange because its binding process causes significant release of Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, etc. in sorption solution mixture. Ion exchange was also found to be responsible for Ni(II) by macroalgae (Raize *et al*., 2004) and copper biosorption by fungi *Ganoderma lucidum* (Muraleedharan and Venkobachar, 1990) and *Pycnoporus sanguineus* (Mashitah *et al*., 1999).

3 Conclusions

(1) Loofa sponge immobilized biomass of *Chlorella sorokiniana* (LSIBCS) was shown to be highly effective in removing Cr(III) from aqueous solution. (2) LSIBCS could be regenerated efficiently with 0.1 mol/L HNO_3 and reused for at least five cycles without any significant loss of efficiency. (3) The recovery of sorbed Cr(III) from the LSIBCS was 98%. This enhances its economical use in industrial applications. (4) The study shows that microalgae, which have tended to be ignored for industrial application due the problems of contacting/separation and pressure drop, can now be formulated into viable robust metal biosorbent system after immobilization into low-cost, highly porous and physically strong bio-structural matrix of loofa sponge. (5) The sorption capacity of LSIBCS is higher than most of the reported adsorbent/biosorbents/ion exchange resins for the removal of Cr(III) from water/wastewater (Table 1).

Acknowledgements

Identification of the purified isolate by Dr. Georg Gartner, Institute of Botany, University of Innsbruck, Innsbruck, Austria is acknowledged.

References

Akhtar N, Iqbal J, Iqbal M, 2004. Removal and recovery of nickel(II) from aqueous solution by loofa sponge-immobilized biomass of *Chlorella sorokiniana*: Characterization studies. *J Hazard Mater*, B108: 85–94.

Al-Asheh S, Duvnjak Z, 1996. Biosorption of chromium by canola meal. *Water Qual Res J Canada*, 31: 319–328.

Alguacil F J, Alonso M, Lozano L J, 2004. Chromium(III) recovery from waste acid solution by ion exchange processing using Amberlite IR-120 resin: Batch and continuous ion exchange modelling. *Chemosphere*, 57: 789–793.

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

ATSDR (Agency for Toxic Substances and Disease Registry), 2000. Toxicological profile for chromium: updates. Atlanta: Department of Health and Human Services.

Blumreisinger M, Meindl D, Loos E, 1983. Cell wall composition of *Chlorococcal algae*. *Phytochem*, 22: 1603–1604.

Chen X C, Wang Y P, Lin Q, Shi J Y, Wu W X, Chen Y X, 2005. Biosorption of copper(II) and zinc(II) from aqueous solution by *Pseudomonas putida* CZ1. *Colloids and Surfaces B: Biointerfaces*, 46: 101–107.

Chojnacka K, 2005. Biosorption of Cr(III) ions by eggshells. *J Hazard Mater*, B121: 167–173.

Chojnacka K, Chojnacki A, Gorecka H, 2005. Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue-green algae *Spirulina* sp. *Chemosphere*, 59: 75–84.

Ferraz A I, Tavares T, Teixeira J A, 2004. Cr(III) removal and recovery from *Sacchaomyces cerevisiae*. *Chem Eng J*, 105: 11–20.

Ho Y S, McKay G, 2000. The kinetics of sorption of divalent metal ions onto sphagnum moss peat. *Water Res*, 34: 735–742.

Iqbal M, Saeed A, 2005. Novel method for cell immobilization and its application for production of organic acid. *Lett Appl Microbiol*, 40: 178–182.

Iqbal M, Zafar S I, 1997. Palm petiolar felt-sheath as a new and convenient material for the immobilization of microalgal cells. *J Indust Microbiol Biotechnol*, 19: 139–141.

Karna R R, Uma L, Subramanian G, Mohan P M, 1999. Biosorption of toxic metal ions by alkali-extracted biomass of a marine cyanobacterium, *Phormidium valderianum* BDU 30501. *World J Microbiol Biotechnol*, 15: 729–732.

Kaszycki P, Fedorovych D, Ksheminska H, Babyak L, Wojcik D, Koloczek H, 2004. Chromium accumulation by living yeast at various environmental conditions. *Microbiol Res*, 159: 11–17.

Kratochvil D, Pimentel P, Volesky B, 1998. Removal of trivalent and hexavalent chromium by seaweed biosorbent. *Environ Sci Technol*, 32: 2693–2698.

Lagergren S, 1898. Zur theorie der sogenannten adsorption gelöster stoffe, Kungliga Svenska Vetenskapsakademiens. *Handlingar*, 24: 1–39.

Li Q, Zhai J, Zhang W, Wang M M, Zhou J, 2007. Kinetic studies of adsorption of Pb(II), Cr(III) and Cu(III) from aqueous solution by sawdust and modified peanut husk. *J Hazard Mater*, 141: 163–167.

Li Z Y, Guo S Y, Li L, 2006. Study on the process, thermodynamical isotherm and mechanism of Cr(III) uptake by *Spirulina platensis*. *J Food Eng*, 75: 129–136.

Lopez A, Lazaro N, Morales S, Morales A, 2002. Nickel biosorption by free and immobilized cells of *Pseudomonas fluorescens* 4F39: A comparative study. *Water Air Soil Pollut*, 135: 157–172.

Machado R, Carvalho J R, Correia M J N, 2002. Removal of trivalent chromium(III) from solution by biosorption in cork powder. *J Chem Technol Biotechnol*, 77: 1340–1348.

Mallick K, 2002. Biotechnological potential of immobilized algae for wastewater N, P and metal removal: A review. *Biometals*, 15: 377–390.

Mamaril J C, Paner E T, Alpante B M, 1997. Biosorption and des-

orption studies of chromium (III) by free and immobilized *Rhizobium* (BJVr 12) cell biomass. *Biodegrad*, 8: 275–285.

Mashitah M D, Zulfadhly Z, Bhatia S, 1999. Binding mechanism of heavy metals biosorption by *Pycnoporus sanguineus*. *Artif Cells Blood Substit Immobil Biuotechnol*, 27: 441–445.

Mattuschka B, Straube G, 1993. Biosorption of metals by a waste biomass. *Chem Tech Biotechnol*, 58: 57–63.

Mehta S K, Gaur J P, 2005. Use of algae for removing heavy metal ions from wastewater: Progress and prospects. *Crit Rev Biotechnol*, 25: 113–152.

Mohan D, Pittman C U, 2006. Activated carbons and low cost adsorbents for remediation of tri- and hexavalent chromium from water. *J Hazard Mater*, 137: 762–811.

Mukherjee A B, 1998. Chromium in the environment of Finland. *Sci Total Environ*, 217: 9–19.

Muraleedharan T R, Venkobachar C, 1990. Mechanism of biosorption of copper(II) by *Ganoderma lucidum*. *Biotechnol Bioeng*, 35: 320–325.

Nasernejad B, Zedeh T E, Pour B B, Bygi M E, Zamani A, 2005. Comparison for biosorption modeling of heavy metals Cr(III), Cu(II), Zn(II) adsorption from wastewater by carrot residues. *Process Biochem*, 40: 1319–1322.

Pillichshammer M, Pümpel T, Pöder R, Eller K, Klima J, Schinner F, 1995. Biosorption of chromium to fungi. *Biometals*, 8: 117–121.

Prakasham R S, Merrie J S, Sheela R Saswathi N, Ramakrishna S V, 1999. Biosorption of chromium VI by free and immobilized *Rhizopus arrhizu*s. *Environ Pollut*, 104: 421–427.

Rangsayatorn N, Pokethitiyook P, Upatham E S, Lanza G R, 2004. Cadmium biosorption by cells of *Spirulina platensis* TISTR 8217 immobilized in alginate and silica gel. *Environ Internat*, 30: 57–63.

Raize O, Argaman Y, Yannai S, 2004. Mechanisms of biosorption of different heavy metals by brown marine macroalgae. *Biotechnol Bioeng*, 87: 451–458.

Reddad Z, Gerente C, Andres Y, Cloirec P, 2002. Adsorption of several metal ions onto a low-cost biosorbents: Kinetics and equilibrium studies. *Environ Sci Technol*, 36: 2067–2073.

Rengaraj S, Yeon K H, Kang S Y, Lee J U, Kim K W, Moon S H, 2002. Studies on adsorptive removal of Co(II), Cr(III) and Ni(II) by IRN77 cation-exchange resin. *J Hazard Mater*, 92: 185–198.

Rengaraj S, Joo C K, Kim Y, Yi J, 2003. Kinetics of removal of chromium from water and electronic process wastewater by ion exchange resins: 1200H, 1500H and IRN97H. *J Hazard Mater*, 102: 257–275.

Rengaraj S, Yeon K H, Moon S H, 2001. Removal of chromium from water and wastewater by ion exchange resins. *J Hazard Mater*, 87: 273–287.

Robinson P K, 1998. Immobilized algal technology for wastewater treatment purposes. In: Wastewater Treatment with Algae (Wong Y. S., Tam N. F. Y., eds.). Germany: Springer Verlag and Landes Bioscience, 1–15.

Romera E, Gonzalez F, Ballester A, Blazquez M L, Munoz J A, 2006. Biosorption with algae: A statistical review. *Crit Rev Biotechnol*, 26: 223–235.

Saeed A, Iqbal M, Akhtar M W, 2005. Removal and recovery of lead(II) from single and multimetal (Cd, Cu, Ni, Zn) solution by crop milling waste (black gram husk). *J Hazard Mater*, B117: 64–67.

Sag Y, 2001. Biosorption of heavy metals by fungal biomass and modeling of fungal biosorption: A review. *Sep Purf Methods*, 30: 1–48.

Schiewer S, Volesky B, 2000. Biosorption process for heavy metal removal. In: Environmental Microbe-Metal Interactions (Lovley D. R., ed.). Washington DC: American Society of Microbiology Publication Co., 329–362.

Sekhar K C, Subramanian S, Modak J M, Natarajan K A, 1998. Removal of metal ions using an industrial biomass with reference to environmental control. *Int J Min Process*, 53: 107–120.

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

Tobin J M, Cooper D G, Neufeld R J, 1984. Uptake of metal ions by *Rhizopus arrhizus* biomass. *Appl Environ Microbiol*, 47: 821–824.

Tobin J M, Roux J C, 1998. Mucor biosorbent for chromium removal from tanning effluent. *Wat Res*, 32: 1407–1416.

Volesky B, 2001. Detoxification of metal-bearing effluent: biosorption for the next century. *Hydromet*, 59: 203–216.

Yun Y S, Park D, Park J M, Volesky B, 2001. Biosorption of trivalent chromium on the brown seaweed biomass. *Environ Sci Technol*, 35: 4353–4358.

Wang H L, Chen C, 2006. Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review. *Biotechnol Adv*, 24: 427–451.