Journal of Hazardous Materials 170 (2009) 487–494

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Removal of chromium (VI) ions from aqueous solution by adsorption onto two marine isolates of *Yarrowia lipolytica*

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ARTICLE INFO

Article history:
Received 5 December 2008
Received in revised form 8 March 2009
Accepted 19 April 2009
Available online 3 May 2009

Keywords:
Metal removal
Biosorption
Hexavalent chromium
Yarrowia lipolytica
SEM-EDS
FTIR

ABSTRACT

The removal of chromium (VI) ions from aqueous solutions by the biomass of two marine strains of *Yarrowia lipolytica* (NCIM 3589 and 3590) was studied with respect to pH, temperature, biomass, sea salt concentration, agitation speed, contact time and initial concentration of chromium (VI) ions. Maximum biosorption was observed at pH 1.0 and at a temperature of 35 °C. Increase in biomass and sea salts resulted in a decreased metal uptake. With an agitation speed of 130 rpm, equilibrium was attained within 2 h. Under optimum conditions, biosorption was enhanced with increasing concentrations of Cr (VI) ions. NCIM 3589 and 3590 displayed a specific uptake of Cr (VI) ions of 63.73 ± 1.3 mg g^{-1} at a concentration of 950 ppm and 46.09 ± 0.23 mg g^{-1} at 955 ppm, respectively. Scatchard plot analysis revealed a straight line allowing the data to be fitted in the Langmuir model. The adsorption data obtained also fitted well to the Freundlich isotherm. The surface sequestration of Cr (VI) by *Y. lipolytica* was investigated with a scanning electron microscope equipped with an energy dispersive spectrometer (SEM-EDS) as well as with ED-X-ray fluorescence (ED-XRF). Fourier transform infrared (FTIR) spectroscopy revealed the involvement of carboxyl, hydroxyl and amide groups on the cell surfaces in chromium binding.

1. Introduction

Hexavalent chromium ions [Cr (VI)] pose a severe threat to the environment. Cr (VI) ions are released into the surroundings due to an extensive use of this metal in the refectory, metallurgical as well as the tanning industries. Cr (VI) is a stable, non-essential and highly toxic heavy metal. This has deleterious effects on microorganisms, plants, animals and is also carcinogenic to human beings [1,2]. Due to the process of bioaccumulation by aquatic plants and animals, Cr (VI) also persists in marine food chains [3]. The removal of this toxic pollutant from the contaminated environments and waste streams is therefore of prime importance.

The conventional methods for removal of Cr (VI) have certain limitations and are often not economically viable in developing countries [4,5]. In such a scenario, the removal of heavy metals by biosorption or bioaccumulation processes using microorganisms has gained attention of researchers. The former is a potentially attractive technology because of the economic feasibility, effectiveness, shorter operation time, lack of toxic secondary products and reusability of biomass [6]. Biosorption of metals has been reported in bacteria, actinomycetes, yeasts, fungi and algae. Among microorganisms, yeasts are known to play an important role in the removal of toxic heavy metals. The main reason for yeasts being used as inexpensive biosorbents is the availability of their biomass in large quantities [7].

In the recent years, *Yarrowia lipolytica* has emerged as an important non-conventional yeast with significant biological relevance and biotechnological applications [8,9]. This yeast has been used in the remediation of various polluted environments [10–12] and is also applied in the degradation of different wastes [13–16]. *Y. lipolytica* is able to utilize a variety of renewable carbon sources and the biomass of the fungus has been used as single cell protein or as single cell oil [17,18]. *Y. lipolytica* biomass to the best of our knowledge has not been used for the biosorption of Cr (VI) ions although this fungus can survive metal stress and accumulate copper, cobalt, cadmium, nickel, zinc and gold [19,20–22]. This microorganism therefore, displays a potential for the bioremediation of metal polluted environments. In this study, we report the efficient use of two marine isolates of *Y. lipolytica* as biosorbents for removal of Cr (VI) from aqueous solutions and suggest their use in the development of a technology in the future.

2. Materials and methods

2.1. Microorganisms and maintenance

Two strains of *Y. lipolytica*, NCIM 3589 and 3590 were used in the present work. NCIM 3589 has been isolated from oil-polluted

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doi:10.1016/j.jhazmat.2009.04.070

seawater in Mumbai, India [11] and NCIM 3590 (NCYC 789) is a psychrotrophic marine isolate obtained from Scottish seawater [23]. Both the fungi are able to adapt to high salt concentrations and can grow in fresh water, as well as seawater-based media. Stock cultures of the strains were maintained on MGYP slants (malt extract, 3.0; glucose, 10.0; yeast extract, 3.0; peptone, 5.0; agar, 25.0 g l^{-1} of distilled water) and sub-cultured at monthly intervals. The growth temperature for NCIM 3589 was 30 °C while that for NCIM 3590 was 20 °C.

2.2. Preparation of biomass

Both the strains of *Y. lipolytica* were cultivated on YEPD medium (yeast extract, 3.0; peptone, 10.0; dextrose, 10.0 g l^{-1} of distilled water) for 48 h on a shaker at optimum temperatures. The cells were harvested by centrifugation at 6000 × *g* for 10 min at 4 °C and washed thrice with distilled water. Washed cells were suspended in distilled water in a proportion of 1:2 (w/v) and this 'standard yeast suspension' was used for subsequent biosorption studies. For all experiments, the source of Cr (VI) was provided as $K_2Cr_2O_7$ in distilled water. Dry weight of the biomass was determined for each set and this was used during further calculations.

2.3. Effect of pH and temperature on Cr (VI) biosorption

The biosorption assay was carried out in 250 ml Erlenmeyer flasks with 25 ml of metal solution (50 ppm). The pH of metal solutions was adjusted to 7.0, 5.5, 3.0, 2.0 and 1.0 with 1N NaOH or HNO_3. Standard yeast suspensions of NCIM 3589 and 3590 (2.5 ml) were added to each flask. The flasks were incubated on an orbital shaker at 130 rpm. The effect of temperature on biosorption of Cr (VI) was determined with a higher concentration of the metal (200 ppm) at pH 1.0. The reaction mixtures were incubated at 4, 20, 30 and 35 °C. The residual chromium (VI) ion concentration in supernatant was determined after 2 h of incubation.

2.4. Effect of biomass and salt concentration on biosorption of Cr (VI)

Varying volumes of the standard cell suspension (0.5, 1.0, 2.5 and 5 ml) were added to 25 ml of chromium (VI) solutions (200 ppm) pH 1.0, incubated at 35 °C and the biosorption was studied. The effect of sea salts (Sigma–Aldrich) at a concentration of 2.0, 4.0 and 6.0% on biosorption was also studied since both the isolates were of marine origin. Control experiments were carried out without the sea salts. The residual Cr (VI) ion concentration in each flask was determined after 2 h of incubation.

2.5. Effect of contact time, agitation and initial concentration of Cr (VI) on biosorption

The metal biosorption assay was carried out as described above and 2.5 ml of the standard yeast suspension was added to each flask. Samples were withdrawn at different time intervals and the residual Cr (VI) ion concentration of supernatant was determined. Effect of agitation was studied by varying the speed of the shaker from 0 to 130 rpm. To study the effect of initial concentration of the metal ion on biosorption, the concentrations of Cr (VI) were varied from 50 to 955 ppm following the standard conditions described earlier.

2.6. Cr (VI) estimation by diphenylcarbazide method

The concentration of Cr (VI) was determined spectrophotometrically by the diphenyl carbazide method. Cells were harvested by centrifugation for 5 min at 5000 rpm and the Cr (VI) content in supernatant was estimated after adding 6N H_2SO_4, a solution of 1,5-diphenyl carbazide in acetone and measuring the absorbance at 540 nm using a Jasco V-530 spectrophotometer [24]. The method displayed a linear relation with 10–100 μg of Cr (VI) and the calibration graphs used displayed r^2 values of 0.9998.

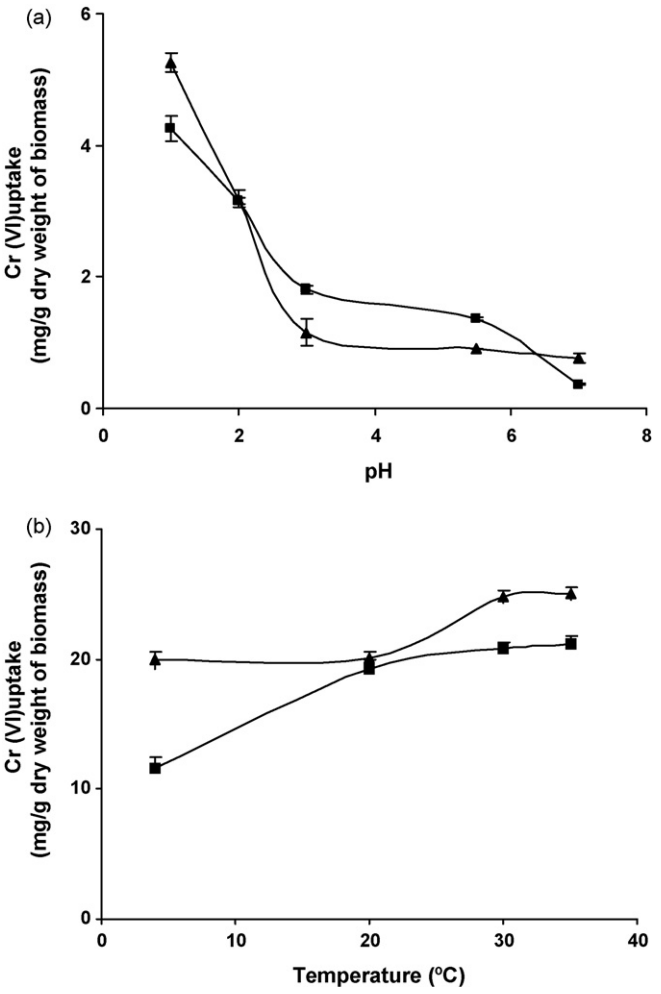

Fig. 1. (a) Effect of initial pH on Cr (VI) biosorption [C_0 = 50 mg l^{-1}, *m* (biomass) = 9.3 g l^{-1}, 130 rpm, *t* (time) = 2 h, for *Y. lipolytica* NCIM 3589 (▲) and NCIM 3590 (■)]. (b) Effect of temperature on chromium (VI) biosorption [C_0 = 200 mg l^{-1}, *m* = 7.42 g l^{-1}, 130 rpm, *t* = 2 h and *T* = 4, 20, 30 and 35 °C for *Y. lipolytica* NCIM 3589 (▲) and NCIM 3590 (■)].

2.7. Adsorption isotherm

The biosorption capacities (q_{eq}) at equilibrium were calculated as follows:

$$q_{eq} = \frac{(C_0 - C_{eq})V}{X} \quad (1)$$

where C_0 was the initial Cr (VI) concentration (mg l^{-1}); C_{eq} the Cr (VI) concentration at equilibrium (mg l^{-1}); *V* the volume of solution used (l); *X* was biosorbent mass (g l^{-1}).

The adsorption yield or efficiency (*Ad*%) was defined as the ratio of metal ion concentration at equilibrium to the initial metal ion concentration and was calculated as follows:

$$Ad\% = \frac{C_0 - C_{eq}}{C_0} \times 100 \quad (2)$$

where C_0 was the initial metal ion concentration (mg l^{-1}) and C_{eq} is the residual metal ion concentration in solution at equilibrium (mg l^{-1}).

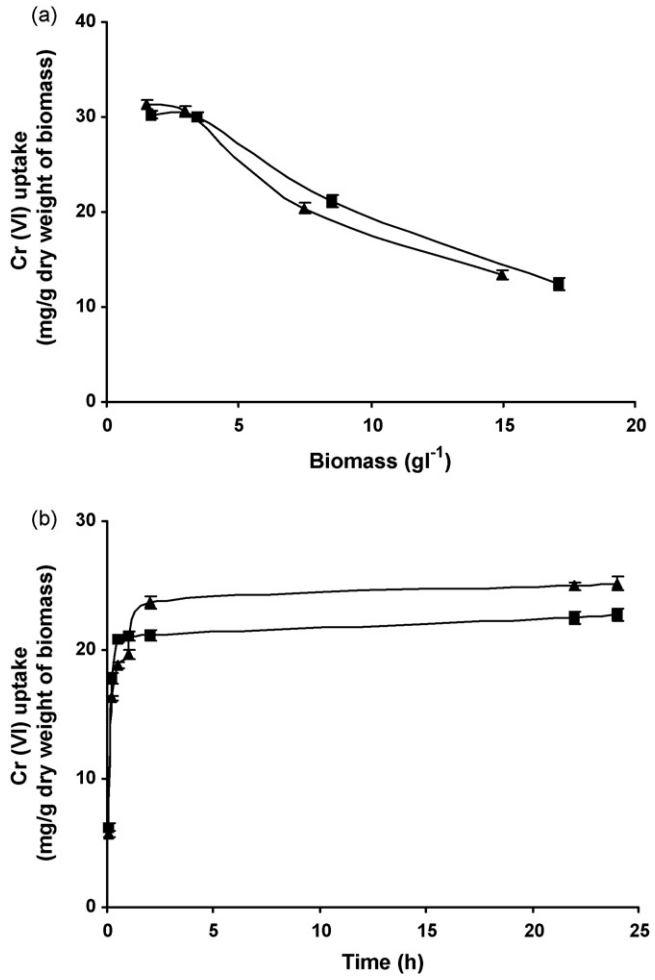

Fig. 2. (a) Effect of cell biomass on chromium (VI) biosorption. [C_0 = 200 mg l^{-1}, 130 rpm, t = 2 h, m = 1.49, 2.98, 7.47 and 14.94 g l^{-1} for *Y. lipolytica* NCIM 3589 (▲) and 1.70, 3.41, 8.54 and 17.08 g l^{-1} for NCIM 3590 (■)]. (b) Effect of contact time on chromium (VI) biosorption [C_0 = 200 mg l^{-1}, m = 7.42 g l^{-1}, 130 rpm, t = 0.083, 0.25, 0.5, 1, 2 and 24 h for *Y. lipolytica* NCIM 3589 (▲) and NCIM 3590 (■)].

The Scatchard plot, Langmuir and Freundlich isotherm models were applied to understand the interaction between Cr (VI) ions and yeast biomass. The Scatchard liberalized form of the Langmuir equation has been used to determine adsorption parameters and primarily understand the type of interaction as well as the affinity of biomass for Cr (VI) ions. The equation was as follows:

$$\frac{q_{eq}}{C_{eq}} = q_m K_b - q_{eq} K_b \quad (3)$$

where q_{eq} is the amount of Cr (VI) adsorbed per unit weight, C_{eq} is the equilibrium concentration of metal ion, K_b and q_m are the adsorption binding constant and maximum biosorption capacity, respectively.

Table 1
Equilibrium adsorbed quantities and adsorption yields of Cr (VI) ion obtained at different initial Cr (VI) ion concentrations for *Yarrowia lipolytica*.

Yarrowia lipolytica NCIM 3589			*Yarrowia lipolytica* NCIM 3590		
C_0 (mg l^{-1})	q_{eq} (mg g^{-1})	*Ad%*	C_0 (mg l^{-1})	q_{eq} (mg g^{-1})	*Ad%*
50	5.25 ± 0.13	95.84	50	4.26 ± 0.12	94.08
280	27.58 ± 0.23	78.88	434	27.50 ± 0.43	57.60
650	52.55 ± 0.53	63.93	674	37.60 ± 1.40	50.74
950	63.73 ± 1.30	46.52	955	46.09 ± 0.24	43.87

Conditions for biosorption studies: temperature: 35 °C; agitation rate: 130 rpm; pH: 1.0; time: 2 h.

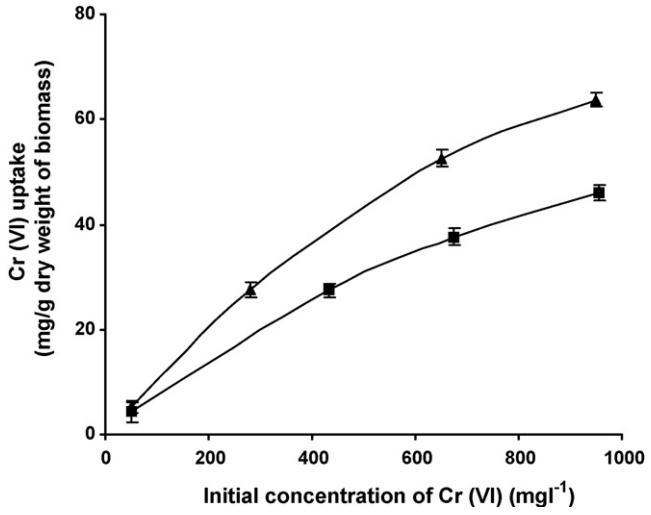

Fig. 3. Effect of initial concentration of Cr (VI) on biosorption [C_0 = 50, 280, 650 and 950 mg l^{-1} for *Y. lipolytica* NCIM 3589 (▲) and 50, 434, 674 and 955 mg l^{-1} for NCIM 3590 (■), m = 7.42 g l^{-1}, 130 rpm and t = 2 h].

The data was also analyzed by the standard Langmuir adsorption isotherm using the following equation:

$$\frac{C_{eq}}{q_{eq}} = \frac{1}{K_b A_s} + \frac{C_{eq}}{A_s} \quad (4)$$

where q_{eq} and C_{eq} are the equilibrium biosorption capacity of the biosorbent and the equilibrium concentration of the metal ion in the aqueous solution, respectively. A_s and K_b are the adsorption isotherm parameters.

In addition, the separation factor values (R_L) that represent whether a sorption system is favorable or unfavorable in batch processes was calculated by using the following equation based on the Langmuir isotherm [25].

$$R_L = \frac{1}{(1 + K_L C_0)} \quad (5)$$

where K_L is the constant from the Langmuir equation and C_0 is the initial concentration of Cr (VI).

Surface coverage (θ), the fraction of yeast biomass surface covered by Cr (VI) was also studied by using the following equation based on the Langmuir isotherm [25].

$$\theta = K_L C_0 (1 - \theta) \quad (6)$$

where K_L is the constant from the Langmuir equation and C_0 is the initial concentration of Cr (VI) ions.

The Freundlich adsorption isotherm equation was as follows:

$$\ln q_{eq} = \ln K_F + \frac{1}{n} \ln C_{eq} \quad (7)$$

where q_{eq} and C_{eq} are the equilibrium biosorption capacity of the biosorbent and the equilibrium metal ion concentration, respectively. K_F and n are the adsorption isotherm parameters [26].

2.8. Statistical analysis

All experiments were run in triplicate and the arithmetic mean of the results was considered during data analysis. Standard deviation and error bars are indicated whenever necessary. Observations on different experimental parameters were tested for significance of difference using ANOVA. For comparison of data between the two strains the unpaired '*t*' test was performed. A probability level of p (0.05) was used throughout the study. All statistical analysis was done by using the GraphPad InStat [DATASET1.ISD] software.

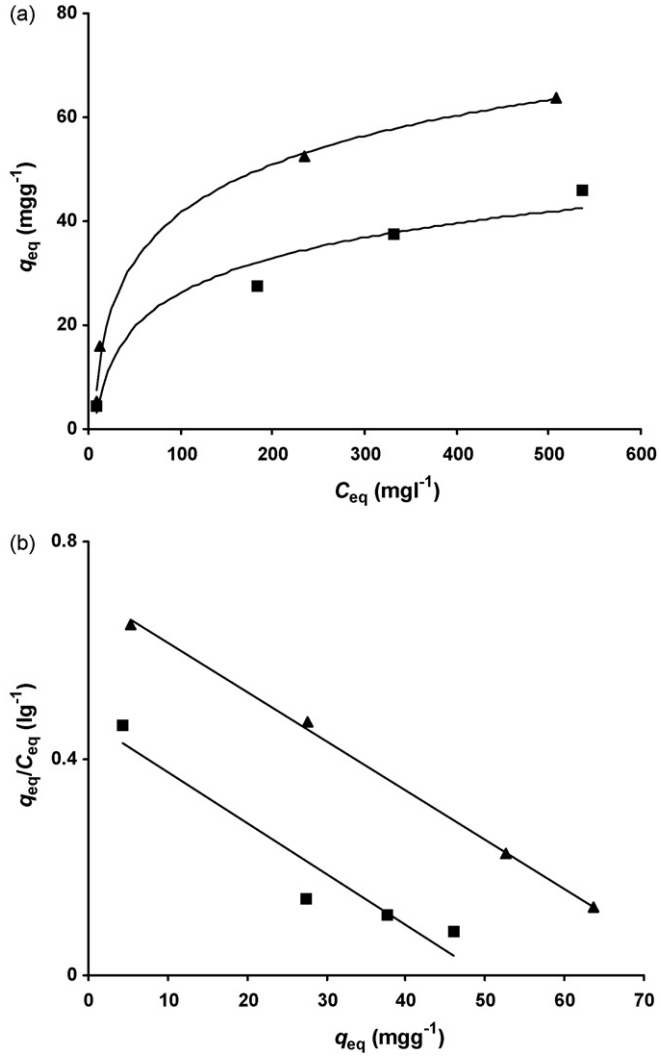

Fig. 4. (a) Non-linear isotherms for the equilibrium binding of Cr (VI) ions biosorption on *Y. lipolytica* NCIM 3589 (▲) and NCIM 3590 (■). (b) Scatchard plots for Cr VI ions biosorption on *Y. lipolytica* NCIM 3589 (▲) and NCIM 3590 (■).

2.9. SEM-EDS, ED-XRF and FTIR analysis

The Cr (VI) loaded biosorbent surface and possible metal–biosorbent interactions were characterized by SEM-EDS, ED-XRF and FTIR analysis. SEM-EDS analyses of air-dried, platinum coated samples were performed by using an analytical scanning electron microscope (JOEL JSM-6360A) equipped with an energy dispersive spectrometer (EDS). Elemental analysis of native and metal loaded biomass was performed by ED-XRF using a Spectro Xepos microanalysis system (Ametek materials analyser division). For FTIR analysis, control and test cells loaded with Cr (VI) were completely dried and blended with KBr to obtain a pellet. The FTIR spectra were collected at resolution of 4 cm^{-1} in the transmission mode (4000–400 cm^{-1}) using a Shimadzu FTIR spectrophotometer (FTIR 8400).

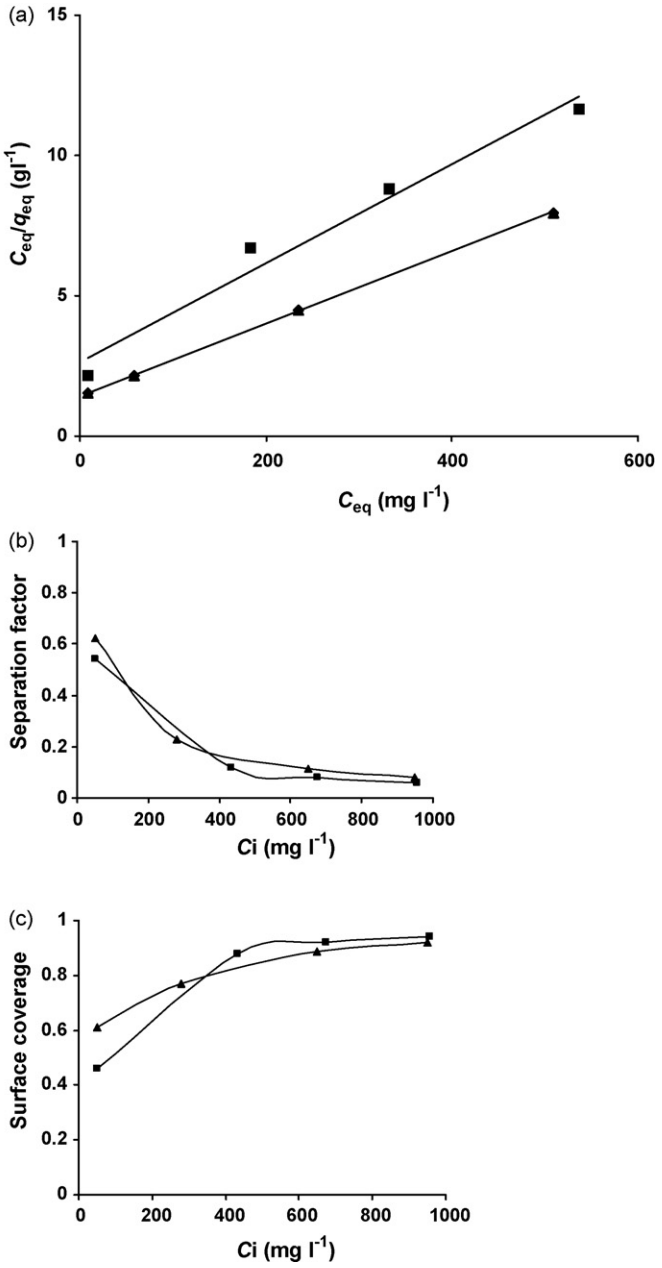

Fig. 5. (a) Langmuir adsorption isotherms of Cr (VI) ions biosorption on *Y. lipolytica* NCIM 3589 (▲) and NCIM 3590 (■). (b) Separation factor profile for biosorption of Cr (VI) as function of initial metal concentration for *Y. lipolytica* NCIM 3589 (▲) and NCIM 3590 (■). (c) A plot of surface coverage (θ) against initial Cr (VI) ion concentration (mg 1^{-1}) for *Y. lipolytica* NCIM 3589 (▲) and NCIM 3590 (■).

3. Results and discussion

3.1. Effect of pH and temperature on biosorption efficiency

To determine the optimum pH for the biosorption for the two cultures, a solution containing 50 ppm of Cr (VI) was used. The

Table 2
Adsorption isotherm parameters for Cr (VI) ions on *Yarrowia lipolytica*.

Isolate	Langmuir isotherm			Scatchard plot analysis			Freundlich isotherm		
	A_s	K_b	r^2	K_b	q_m	r^2	K_F	n	r^2
NCIM 3589	109.75	1.2×10^{-2}	0.99	9.0×10^{-3}	78.09	0.99	0.5376	1.13	0.96
NCIM 3590	150.18	1.7×10^{-2}	0.97	9.4×10^{-3}	49.73	0.91	0.1503	3.96	0.99

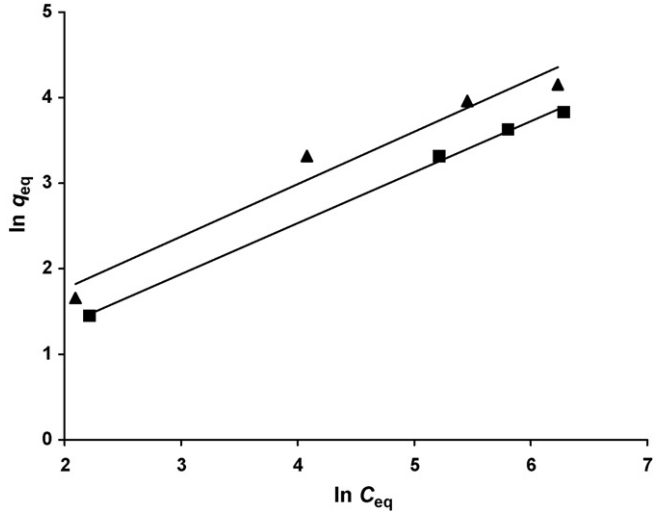

Fig. 6. Freundlich adsorption isotherms of Cr (VI) ions biosorption on *Y. lipolytica* NCIM 3589 (▲) and NCIM 3590 (■).

biosorption capacities for both the strains were greater at pH 1.0 when compared to higher values of pH (Fig. 1a). The differences in metal uptake due to the effect of pH were statistically significant (ANOVA) with $p < 0.05$. All subsequent experiments were therefore carried out at pH 1.0. The results also suggest that active processes displayed by live cells may not be involved in biosorption of Cr (VI). Such observations on enhanced biosorption at acidic pH has been previously reported with other biosorbents such as bacteria [27], fungi [28,29], milled peat [30], cone biomass [31], pods, leaves and bark of an ornamental plant [32] and the husk of Bengal gram [33]. The reason for the enhanced adsorption of Cr (VI) at low pH was that negatively charged $[HCrO_4]^-$, $[Cr_2O_7]^{2-}$, $[Cr_4O_{13}]^{2-}$ and $[Cr_3O_{10}]^{2-}$ ions are the dominant species under such conditions. The surfaces of yeast cell walls at low pH are surrounded by hydronium ions (H_3O^+). The negatively charged ion species are thus effectively adsorbed on the positively charged active sites on the sorbent [28]. With an increase in pH, the binding of ions decreased on account of repulsive forces between the biosorbent (*Y. lipolytica*) and Cr (VI) ions. It is therefore proposed that in a manner similar to *Bacillus thuringiensis*, in *Y. lipolytica* also, the interactions may be primarily electrostatic or coordinative in nature [26].

Fig. 1b shows the biosorption of Cr (VI) at different temperatures for both the cultures. From the figure, it is evident that there was enhanced biosorption at higher temperatures. This difference was also found to be statistically significant ($p < 0.05$). Increased metal uptake at higher temperatures in bacterial biomass (*B. licheniformis*) is reported to be due to a higher affinity of sites for metal or an increase in binding sites on the biomass [27]. Higher removal efficiencies at increased temperatures have also been observed with fungal biomass of *Rhizopus nigricans*. The adsorption of Cr ions to cell wall functional groups in this fungus was an endothermic process, and an increased temperature positively affected metal uptake [34].

3.2. Effect of cell biomass and sea salts on the biosorption of chromium ions

The specific uptake of Cr (VI) decreased with an increase in biomass (Fig. 2a). This is due to the increased electrostatic interactions observed with larger quantities of biomass [35]. Greater biomass offers increased available binding sites for Cr (VI) ions and therefore, the specific metal uptake decreased significantly ($p < 0.05$).

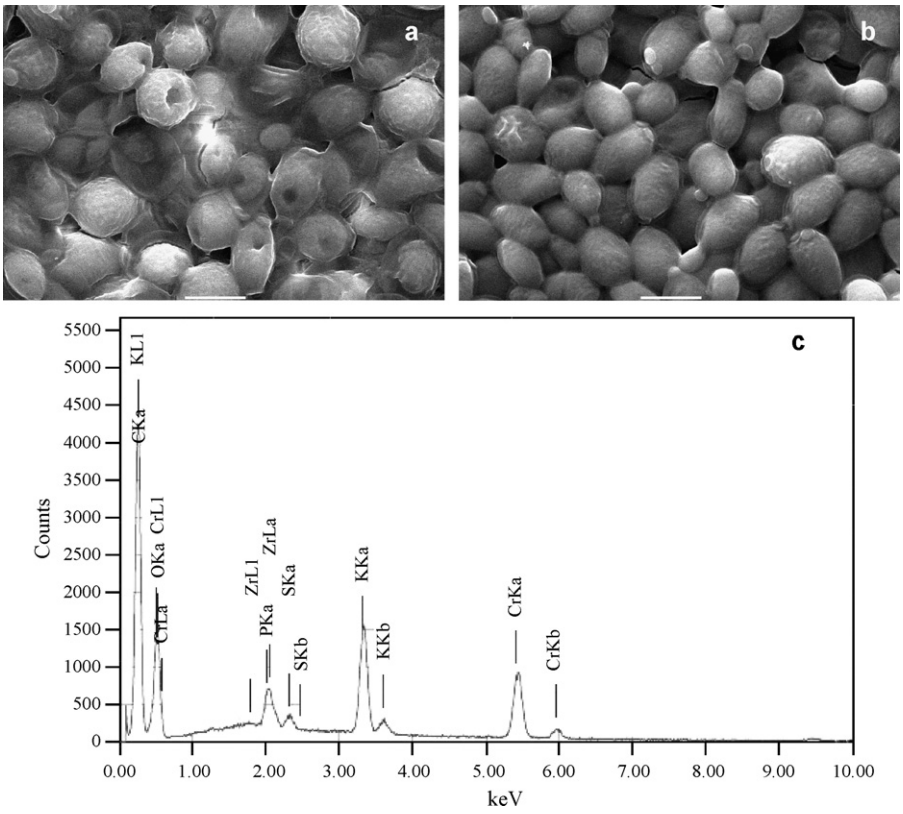

Fig. 7. Scanning electron micrographs of *Y. lipolytica* (a) NCIM 3589 and (b) NCIM 3590 after Cr (VI) biosorption (magnification 3500×; bar at the base centre represents 5 μm). (c) Representative energy dispersive X-ray spectrum of SEM images indicating the presence of Cr.

The effect of sea salts on biosorption was studied. In the absence of sea salts, the Cr (VI) specific uptake was 18.0 ± 0.2 and 17.2 ± 0.2, respectively, for NCIM 3589 and 3590. In the presence of 6% sea salts, this was decreased to 10.59 ± 0.19 for both the cultures. The high ionic strength of seawater is also known to decrease the biosorption process in the micro-alga *Dunaliella* [35]. It is believed that inorganic anions such as chlorides form complexes with heavy metals and affect the biosorption process by preventing the binding of heavy metals to specific sites on the biomass [36].

3.3. Effect of agitation rates and contact time on the biosorption of chromium ions

It is observed that Cr (VI) biosorption was enhanced with an increase in the agitation speed from 0 to 130 rpm. Without agitation, the Cr (VI) specific uptake was 11.960 ± 0.19 and 10.5 ± 0.5, respectively, for NCIM 3589 and 3590. With agitation at 130 rpm, there was a statistically significant increase to 18.0 ± 0.1 and 14.62 ± 0.23 for the two cultures. Increasing agitation enhanced interactions of Cr (VI) with the functional groups on the yeast biomass.

Time of contact between adsorbate and adsorbent is an important parameter for the adsorption process. The effect of contact time on biosorption of Cr (VI) by the biomass of both the *Y. lipolytica* isolates was studied under optimal conditions (Fig. 2b). Biosorption occurred rapidly within 5–15 min. The biosorption process became slower from 15 min to 2 h and thereafter, there was no significant increment until 24 h. Thus, equilibrium was reached within 2 h. Similarly, rapid removal of Cr (VI) ions has been reported for other biosorbents such as bacterial and cone biomass [27,31].

3.4. Effect of initial concentration of Cr (VI)

The initial concentration of Cr (VI) ions greatly influenced the biosorption capacities (q_{eq}) and the adsorption yield (*Ad%*). Table 1 shows that the biosorption capacities for NCIM 3589 increased from 5.25 ± 0.13 to 63.73 ± 1.3 mg g^{-1} with an increasing concentration of Cr (VI) ions (50–950 ppm). With NCIM 3590, this capacity increased from 4.26 ± 0.12 to 46.09 ± 0.24 mg g^{-1} (Fig. 3). Cr (VI) ions adsorbed on the biosorbent thus increased with increasing ion concentration in the solution. This is postulated to be due to two reasons: (i) The initial concentration of Cr (VI) ions may provide

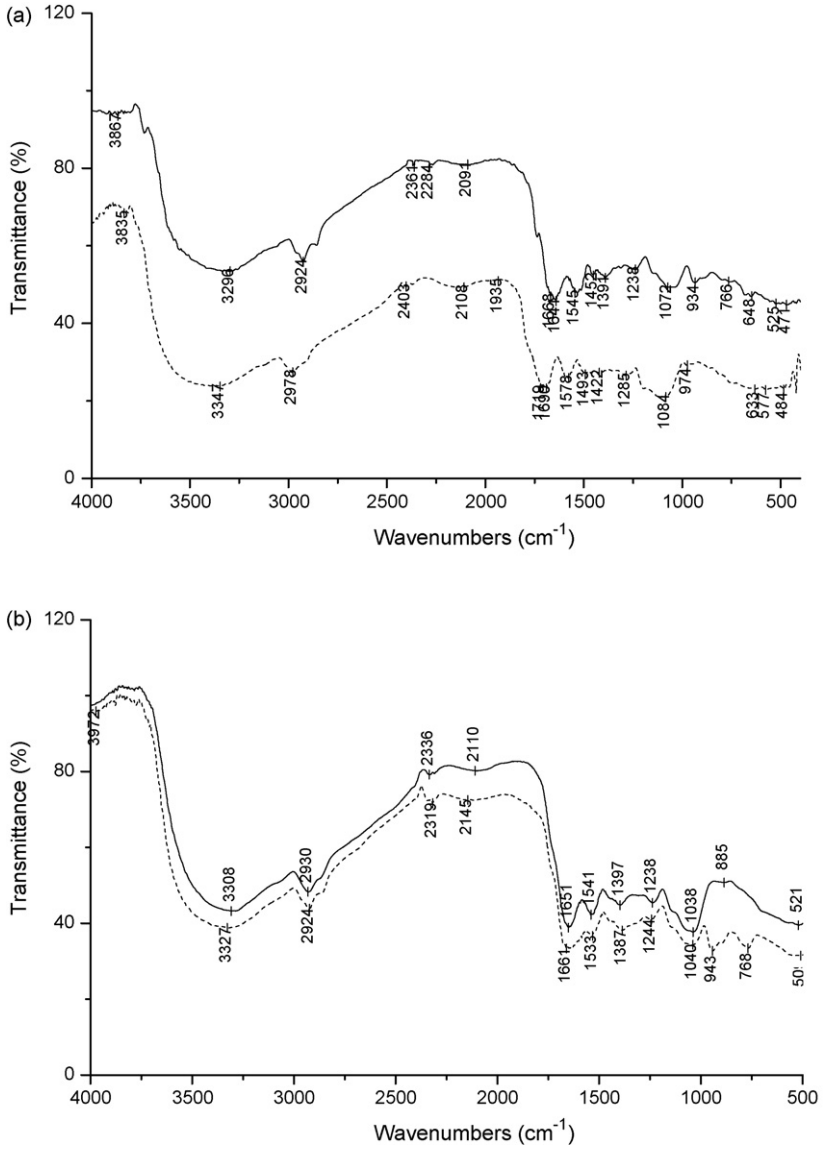

Fig. 8. FTIR spectra of *Y. lipolytica* (a) NCIM 3589 and (b) NCIM 3590 before (—) and after (- - -) Cr (VI) biosorption.

a favorable driving force which enhances adsorption process. (ii) More number of Cr (VI) ions may compete for available binding sites in biomass [27]. The unpaired '*t*' test was used to compare the data obtained for the two strains of *Y. lipolytica*. Such analysis revealed the two-tailed *p* value to be >0.05 indicating that the difference between the two strains was not significant and that both the strains were effective in removing Cr (VI) ions.

Table 1 shows that the adsorption yield (*Ad%*) was higher at lower concentrations of metal ions. For example, at 50 ppm *Ad%* was 95.84 and at 950 ppm this decreased to 46.52. A similar trend was also observed with NCIM 3590 and this decreased from 94.08 to 43.87. These results indicated that at lower concentrations of Cr (VI) ions there was a near complete removal of the toxic Cr (VI) ions. Under these conditions, the ratio of the biosorbent surface to the metal ions was higher and hence most of them reacted with the biosorbent and were removed.

Fig. 4a shows the non-linearized adsorption isotherms (q_{eq} versus C_{eq}) of Cr (VI) ions for NCIM 3589 and NCIM 3590. These isotherms suggest that higher equilibrium concentrations of Cr (VI) ions increased metal adsorption on the yeast surface. The isotherms for both the cultures were steep, indicating that the binding sites on yeast surface had a high affinity for Cr (VI). The bacterium *B. thuringiensis* has also been reported to have a high affinity for Cr (VI) ions [26].

From Fig. 4b it is seen that the Scatchard plots were straight lines indicating that the affinity of Cr (VI) ions for binding sites of yeast biomass did not change over the range of Cr (VI) ions concentration tested. Binding constants and correlation coefficients calculated from the Scatchard analysis are shown in Table 2. The Scatchard plots suggested that adsorption of Cr (VI) ions on biomass of *Y. lipolytica* followed the Langmuir model (Fig. 5a). High correlation coefficients for the Langmuir isotherm were obtained ($r^2 = 0.99$ and $r^2 = 0.97$ for NCIM 3589 and 3590, respectively).

The separation factor values (R_L) were calculated as described in the materials and methods section. Values of R_L were plotted against initial concentrations of the Cr (VI) ions (Fig. 5b). The figure showed that $0 < R_L < 1$, suggesting a favorable isotherm on low as well as high concentrations of Cr (VI) for the biomass of both the yeasts [25]. The surface coverage (θ) with increasing initial concentrations of Cr (VI) was also determined and is shown in Fig. 5c. For the biomass of the two yeasts, the surface coverage increased until the surface was nearly covered with a monolayer. An increase in θ was not observed at concentrations higher than 650 mg l^{-1} and the metal adsorption rate became independent of the Cr (VI) concentration.

For the Freundlich isotherm, r^2 value for NCIM 3589 was 0.96 and for NCIM 3590 this was 0.99. These values indicated that the adsorption isotherms for Cr (VI) followed the Freundlich equation satisfactorily within the range of the Cr (VI) ions concentration used (Fig. 6). High correlation coefficients suggest the possibility of monolayer biosorption and that heterogeneous conditions may co-exist under the experimental conditions employed. The overall biosorption process may involve more than one mechanism such as sorption, ion exchange, surface complexation or electrostatic attraction.

3.5. SEM-EDS, ED-XRF and FTIR analysis

The surface characterization of the cells loaded with Cr (VI) was performed. The scanning electron micrographs of NCIM 3589 and 3590 exposed to Cr (VI) are shown in Fig. 7a and b, respectively. There is a slight difference in the morphology of the two cultures: the cells of NCIM 3589 were rounder and those of NCIM 3590 that were slightly more elongated. These images were subjected to EDS analysis. Fig. 7c shows a representative energy dispersive spectrum of metal loaded biomass of NCIM 3589. With NCIM 3590 also, a similar spectrum was observed. Specific peaks for chromium were observed on surfaces of both the yeasts. In addition, metal loaded cells displayed the presence of chromium in significant amounts when elemental analysis of control cells and test cells were compared using ED-XRF.

In order to determine the functional groups involved in the metal binding, FTIR analysis was carried out. Fig. 8a and b are FTIR profiles (of control and metal loaded cells) of NCIM 3589 and 3590, respectively. The FTIR spectra of the control cells showed a number of peaks reflecting a complex nature of the yeast cell surfaces. There was a change in the intensity of the bands at different regions after interaction with Cr (VI). The stretching vibration of OH or NH group was shifted in NCIM 3589 from 3296 to 3347 cm^{-1} and in NCIM 3590 from 3308 to 3327 cm^{-1}. The peaks related to carboxylic or phenolic groups shifted from 2924 to 2978 cm^{-1} (NCIM 3589) and from 2930 to 2924 cm^{-1} (NCIM 3590). The absorption peak of the amide-I group shifted from 1641 to 1690 cm^{-1} for NCIM 3589 and from 1651 to1661 cm^{-1} for NCIM 3590. The peak representing the amide-II group shifted from 1545 to 1578 cm^{-1} and from 1541 to 1533 cm^{-1} for NCIM 3589 and NCIM 3590, respectively. The vibration due to deformation stretching of the C–H, $-CH_3$ and $>CH_2$ functional groups in NCIM 3589 was shifted from 1452 to 149 cm^{-1} and in NCIM 3590, the shift was from 1397 to1387 cm^{-1}. The peak representing the C–N group shifted from 1072 to 1084 cm^{-1} (NCIM 3589) and from 1038 to 1040 cm^{-1} (NCIM 3590). As reported with other biosorbents [25], the spectra revealed the involvement of carboxyl, carbonyl, hydroxyl and amino groups in metal binding.

4. Conclusions

Experimental evidence suggests that the biomass of the two marine strains of *Y. lipolytica* could be used as efficient biosorbents for the removal of Cr (VI) ions. The biosorption was highest at pH 1.0, with agitation at 130 rpm, within 2 h at 35 °C. Scatchard plot analysis for both the cultures revealed straight lines and the adsorption data fitted well to the Langmuir and the Freundlich isotherms. SEM-EDS and ED-XRF confirmed the presence of Cr (VI) in the bioadsorbed cells and FTIR spectra implicated the role of different functional groups in metal biosorption. Thus, the biomass of this fungus could effectively be applied for the removal of toxic Cr (VI) ions.

Acknowledgements

A.B. wishes to thank Council of Scientific and Industrial Research, India for Junior Research Fellowship and all authors thank University Grants Commission, India, for financial support to Institute of Bioinformatics and Biotechnology.

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