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Removal and recovery of zirconium from its aqueous solution by *Candida tropicalis*

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Abstract

Removal and recovery of zirconium from dilute aqueous solutions by *Candida tropicalis* used as biosorbent, was studied by performing biosorption–desorption tests. This biosorbent was selected after screening a range of microbial species. The process was found to be highly dependent on initial pH and concentration of metal solution. At optimized experimental parameters, the maximum zirconium biosorption capacity of *C. tropicalis* was 179 mg Zr g^{-1} dry weight of biosorbent. The adsorption distribution coefficient value of 3968 ml g^{-1} was obtained for zirconium biosorption by *C. tropicalis*. Different theoretical thermodynamic models governing the adsorption behavior of zirconium were also tested. Zirconium biosorption was found to closely follow the Langmuir model. At low biomass concentrations it was found to follow pseudo-first-order kinetics. However when higher biomass concentrations were used kinetics was changed to pseudo-second-order. The zirconium bound to the biomass was stripped out (60.2% at S/L of 1.0 g of zirconium loaded biomass/l of eluent) using sodium bicarbonate and the biomass could be used for multiple sorption–desorption cycles.

Keywords: *Candida tropicalis*; Zirconium biosorption; Langmuir model; Freundlich model; Pseudo-first- and second-order kinetics

1. Introduction

Biological methods for the removal of heavy metals from aqueous streams provide an attractive alternative to physico-chemical processes such as chemical precipitation, chemical oxidation and reduction, ion-exchange, filtration, reverse osmosis, electrochemical measurement and evaporative recovery. Microorganisms are known to accumulate metals by two distinct processes: (i) bioaccumulation, an energy-dependent process and (ii) biosorption, an energy-independent physical adsorption [1–3]. These both processes have been investigated to determine their potential to remove toxic metal ions from polluted waste waters and to concentrate precious metals [4,5]. Various types of microbial cells/species including both heterotrophs (bacteria and fungi) and photoautotrophs (algae and cyanobacteria) are reported to possess metal binding properties and have been studied as potential biosorbents for selected metals [6]. Most biosorption processes use biomaterials, which are abundant in nature such as marine algae, various parts of plants [7], wastes produced by industrial and biological processes such as fermentation [8], activated sludge [9] and activated charcoal [10]. High affinity, rapid rate of metal uptake and maximum loading capacity are some of the important factors for the selecting a biosorbent. Therefore, there is an increased interest in the identification of some new and better biosorbents that show promising uptake of metallic ions.

Zirconium is a significant engineering material for nuclear energy application due to its high transparency to neutrons. Naturally occurring isotopes of zirconium are non radioactive in nature and some isotopes like Zr^{93} and Zr^{95} are produced as a result of uranium fission and dissolution of "Zircaloy" fuel cladding. Due to its long half life (1.5×10^6 years) it has importance in nuclear fuel cycle. Zirconium has become important

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Nomenclature

b	Langmuir constant
C_i	initial metal ion concentration (mg/l)
C_e	equilibrium metal ion concentration (mg/l)
C_f	final metal ion concentration (mg/l)
C_{des}	concentration of metal desorbed into the eluent (mg/l)
E_s	sorption energy (J/mole)
k_1	pseudo-first-order rate constant
k_2	pseudo-second-order rate constant
K_F	Freundlich adsorption capacity
K	adsorption distribution coefficient (mg metal g^{-1} biosorbent mg^{-1} ml^{-1})
L	ligands on the biosorbents
M	metal ions
ML	metal–ligand complex
n	adsorption intensity
q	biosorption capacity (mg/g)
q_e	amount of biosorption at equilibrium (mg/g)
q_{des}	eluted metal contents per gram of the biosorbent (mg/g)
q_{max}	maximum amount of metal ions per unit mass (mg/g)
q_t	amount of biosorption at time 't' (mg/g)
V	volume of metal ion solution (l)
W	weight of biosorbent (g)

as secondary metal for carrying out certain kind of industrial processes such as manufacturing of photoflash bulbs, surgical equipments, and tanning of leather. Despite its ability to be used for many different industrial applications, most of the zirconium produced today is used in water-cooled nuclear reactors. Zirconium has strong corrosion-resistance properties, high melting point as well as the ability to confine fission fragments and neutrons so that thermal or slow neutrons are not absorbed and wasted, thus improving the efficiency of the nuclear reactor. In fact, about 90% of the zirconium produced in 1989 was used in nuclear reactors, either in fuel containers or nuclear products. Pure zirconium metal can be produced by ductile process which is too expensive for general use. Thus once in solution it is of great importance if zirconium can be selectively biosorbed from its solution and can be stripped out in pure form.

In present studies, our approach has been to characterize a number of microbial species with respect to effectiveness in removing zirconium from aqueous solution by resting (washed and resuspended) cells. On the basis of this characterization *Candida tropicalis* (DSM 7524) was chosen for additional experiments to study the effects of different physical and environmental parameters on the rate of zirconium uptake in a well-mixed, single stage-contacting vessel. Although the removal of zirconium has been reported earlier [11,12], but to our knowledge it is the first comprehensive report on its biosorption by *C. tropicalis*

2. Experimental

2.1. Microorganisms used and growth conditions

All fungal and yeast cultures used in screening studies were obtained from NIBGE Culture Collection. Fungi were grown on Vogal's medium, at 28 ± 2 °C in Erlenmeyer flasks and in order to obtain suspended growth having larger surface area, 10–15 glass beads were added to each flask before autoclaving. Cultures were harvested during the stationary growth phase by filtration through a nylon cloth. Yeast biomass was harvested from YPD medium after 3 days of incubation at 28 ± 2 °C (pH 5.5). Harvested biomass was washed thrice with distilled water to remove medium remnants [13].

Wet biomass was determined after blotting the freshly harvested biomass with commercial grade paper towels to remove excess water. This wet biomass was stored in a screw capped bottle at 4 °C. A known weight from this wet biomass was then dried at 80 °C in an oven for 24 h or to a constant weight and factor to calculate dry weight from wet weight was determined. This freshly harvested biomass stored at 4 °C was used throughout the experiments for biosorption studies unless otherwise mentioned.

2.2. Metal solutions

The stock zirconium solution (1000 mg/l) was prepared from analytical grade zirconyl oxychloride ($ZrO_2Cl{\cdot}2H_2O$) in deionized distilled water (DDW). Zirconium solutions of different concentrations were prepared by adequate dilution of the stock solution with DDW. Glassware used was immersed in 10% (v/v) HNO_3 for overnight and rinsed several times with double distilled water. All other chemicals used were also of analytical grades.

2.3. Analytical determinations

A spectrophotometric assay based on the reaction of zirconium with xylenol orange was carried out. A solution of xylenol orange (0.05%) was prepared by dissolving the dry powder in 0.6N HCl. This reagent was added in the ratio of 2:23 (v/v) to sample solution containing up to 50 μg of zirconium/25 ml of final volume. The solutions were mixed and allowed to stand for approximately 10 min and absorbance was measured at 535 nm using 1 cm cell against the reagent blank. The standard calibration curve was drawn by taking standard solutions of 5, 10, 20, 30, 40 and 50 μg in a similar procedure as the sample. Concentration of zirconium present in unknown sample was calculated from this standard curve.

2.4. Biosorption trials

Biosorption experiments were carried out in batches with control samples containing metal ion solution in the absence of biomass to evaluate the effects of age and storage time of culture, pH of metal solution, contact time, temperature, shaking speed, biomass concentration, metal solution concentration, etc. Known amount of biomass was added to the metal solution. The final volume was 100 ml and metal concentrations

were as specified in each experiment. The reaction mixtures were agitated at 100–200 rpm at 28 ± 2 °C in an orbital rotary shaker. Periodically, 1.0 ml sample was taken and analyzed for residual metal concentration determination. Prior to analysis, sample was centrifuged at 9000 × *g* for 15 min and cells were discarded. Residual metal concentration was determined in the supernatant. These experiments were conducted in triplicate and average reading was noted.

The amount of metal ion (mg) biosorbed per g (dry weight) of biomass (q mg/g dry weight) was calculated by the simple concentration difference method [4] using following relationship:

$$q = \frac{(C_i - C_f)V}{W} \quad \text{(I)}$$

where C_i corresponds to the initial metal ion concentration, and C_f final metal ion concentration in the supernatant solution when W (g) the weight of biosorbent was suspended in V (l) volume of the metal solution. From analysis of zirconium solution in control flasks (C_i) losses due to the adsorption to flask walls were found negligible.

2.5. Metal elution and regeneration of biomass

Exhausted biomass samples were washed with distilled water and capabilities of different regenerator solutions including distilled water, HCl, NaOH, Na_2CO_3, $NaHCO_3$, $(NH_4)_2SO_4$, $(NH_4)_2CO_3$ and Na_2EDTA (ethylenediaminetetra-acetic acid disodium salt) to release the zirconium were examined by mixing 0.05 g of metal bearing samples with 30 ml of regenerator solution each having concentration of 0.1 M. The mixture was shaken for 3 h (or mentioned otherwise) at 100 rpm at 28 ± 2 °C. Using efficient desorbents, pulp densities (solid to liquid ratio) were optimized for maximum elution. All experiments were carried out in triplicate.

The regenerated biomass was washed with deionized water for 3–4 times to remove any traces of eluent and again suspended in metal containing solutions for the next adsorption cycle and this sorption–desorption cycle was repeated five times. The q_{des} were calculated from C_{des} into the eluent as follows:

$$q_{des} = \frac{C_{des}V}{W} \quad \text{(II)}$$

The percentage of desorbed metal was established by comparing the metal released to the amount of metal previously biosorbed to the biomass as follows

$$\text{desorption}\,(\%) = \frac{q_{des}}{q} \times 100 \quad \text{(III)}$$

3. Results and discussion

3.1. Screening studies

Twenty-five microbial species belonging to different types were screened for their zirconium biosorption potential at initial pH values of 2, 3 and 4 (beyond pH 4, zirconium precipitated out in solution). The apparent value of q varied from 4.8 to 202 mg/g dry weight of sorbent. The highest value 202 mg/g of sorption capacity was observed for *Curvularia* sp., but it was not due to true biosorption/bioaccumulation but instead was due to precipitation in solution. For all the biosorbents tested, high values of sorption capacities were observed at pH 3 and 4 as compared to those observed at pH 2. In most of the cases, the apparent high values of sorption capacity were due to clearly visible precipitation of zirconium in solution. Incubating zirconium ions solution with *Aspergillus flavis*, *Aspergillus fumigatus*, *Aspergillus japonicus*, *Aspergillus sulphurus*, *Curvularia* sp. and *Rhizopus arrhizus* resulted in precipitation of zirconium hydroxides at pH 3 and 4. However, among all the strains examined *C. tropicalis* exhibited the maximum uptake potential (89.9 mg/g dry weight) without any apparent precipitation in the solution at any pH value tested. Therefore, it was selected for further optimization of process parameters for zirconium biosorption. In literature, only few reports on zirconium bioaccumulation are present; Garnham et al. [11] documented accumulation of zirconium by microalgae and cyanobacteria. While Dhami et al. [12] reported zirconium accumulation from radioactive waste by *Rhizopus arrhizus*.

Comparison of biosorption by viable and non-viable biosorbents, revealed that zirconium biosorption by *C. tropicalis* was not influenced by the physical state of the biosorbent. The percentage decrease in sorption capacity (2.4%) in shifting from wet to dry biomass was not significant to draw definite conclusions. Similar behavior has also been reported while studying biosorption of cadmium by live and dead cyanobacterium *Spirulina* sp. [14]. Therefore for further studies, wet biomass was used.

Zirconium biosorption capacity was found to be significantly influenced by culture age of the biosorbent. The amount of zirconium accumulated by *C. tropicalis* was 80 ± 2, 82 ± 3, 83 ± 3, 88 ± 3, 89 ± 5, 69 ± 2 and 33 ± 3 mg/g dry weight of cells when 16, 20, 24, 40, 48, 72 and 96 h old cultures were used, respectively. An increment of 10.5% in sorption capacity was noticeable when 48 h old culture of *C. tropicalis* were used; while 22.1% decrease in biosorption was observed with older cells (48–72 h) which was followed by a further 52.1% decrease with 96 h old culture. The decrease in sorption capacity with culture age may be due to change in chemical composition of cell wall as well as intracellular components with respect to nature of biopolymers since some biopolymers are reported to be superior in metal uptake than others [13,15,16].

Maximum biomass yield of 4.4 ± 0.3 g/l dry weight was obtained when biomass was harvested after 40 h of incubation. Biomass yields after 16, 20 and 24 h of incubation were 2.7 ± 0.3 3.0 ± 0.3 and 3.4 ± 0.2 g/l, respectively. The biomass yield again decreased to 4.1 ± 0.2 g/l when harvesting was made after 48 h of incubation. Increase in sorption capacity and decrease in biomass yield were 5.9 and 5.7%, respectively, when biomass harvested after 48 h incubation was used instead of biomass harvested after 40 h. Therefore in subsequent experiments, 40–48 h old (stationary phase) culture was used.

The extent of zirconium removal from aqueous solution remained unaffected by the culture storage (4 °C) time up to almost 4 days, and from 4 to 7 days storage, decrease in sorption capacity was about 4.0%. During next 7 days storage, 19.1%

further decrease in sorption capacity was observed. Storage of 60 days resulted in 47.4% overall decrease in sorption capacity. The decrease in biosorption capacity as a result of storage for period longer than 30–60 days may be due to change in functional groups involved in biosorption owing to onset of purification [8].

Zirconium uptake increases with increase in shaking rate. The values of sorption capacities were 28 ± 3 mg/g in the absence of agitation and 61 ± 3, 89 ± 5 and 90 ± 5 mg/g at 50, 100 and 200 rpm, respectively. At a shaking rate of 200 rpm maximum removal from aqueous solution was achieved and biosorption capacity remained constant for agitation rate up to 400 rpm. This may be due to the fact that the increase of agitation rate improved the diffusion of metal ions towards the surface of biosorbent and a shaking rate in the range 100–200 rpm is sufficient for all the binding sites of the biosorbent to be available for bioaccumulation of zirconium. Similar results have also been reported by Antunes et al. [17] for copper biosorption by *Sargassum* sp.

3.2. Effect of pH on zirconium accumulation

The adsorption of zirconium to microbial biomass was strongly affected by initial pH of metal solution; biosorption capacity first increased by increasing the pH from 1 to 3.5, reaching a plateau (91 ± 7 mg/g dry weight) at pH 3.5 and then further increase in pH decreased the biosorptive potential of the biosorbent sharply (24 ± 5 mg/g dry weight). Experiments could not be conducted at pH values above 4 because of visual precipitation of zirconium hydroxides at these pH values which rendered the true sorption studies impossible [18]. Further experiments were conducted at initial solution pH value of 3.5.

3.3. Effect of initial temperature on zirconium accumulation

The values of sorption capacity increased from 20 ± 4 mg/g to a maximum value of 90 ± 3 mg/g by increasing temperature from 10 to 30 °C for zirconium biosorption. The increase was more pronounced between temperatures 10 and 20 °C (73.9%). From 20 to 30 °C percentage increases in biosorption potential was slow (13.5%). Increase in temperature from 30 to 60 °C, resulted in a decrease of sorption capacity to a value of 54 ± 3 mg/g dry weight (39.6% decrease) that almost remained unchanged for further 10 °C rise in temperature (54 ± 1 mg/g dry weight at 70 °C). The increase in uptake at increased temperature may be due to either a higher affinity of sites for the metal or an increase in binding sites on the relevant biomass [19] and decrease in uptake capacity with a decrease in temperature has been suggested due to decrease mobility of potential binding groups/moieties on the biosorbent surface [20].

3.4. Effect of contact time on biosorption capacities

Effect of contact time on zirconium biosorption capacities was investigated using three different biomass concentrations of 0.5, 1.0 and 1.5 g/l of biosorbate (Fig. 1A). For an initial 115 mg/l zirconium ions concentration, the rate of zirconium ions removal was slow, smooth and gradual for first 6 h and from 6 to 24 h it became rapid and 100% removal was achieved

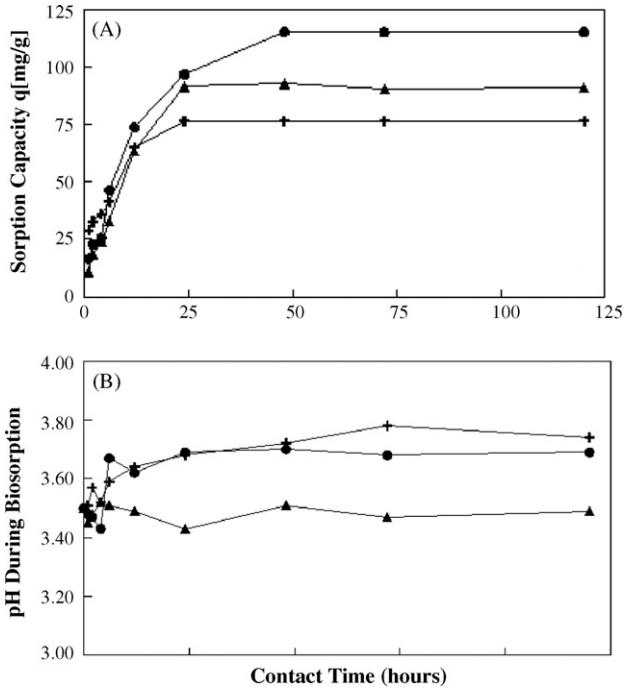

Fig. 1. Time course for zirconium biosorption capacities (A) and pH changes (B) by *C. tropicalis* (freshly harvested biomass corresponding to (▲) 0.05 g, (●) 0.1 g and (+) 0.15 g dry weight was incubated for the indicated times with 100 ml of 115 mg/l zirconium ions solution (pH 3.5) at 100 rpm and 28 ± 2 °C).

after 24 and 48 h for 1.0 and 1.5 g/l of biomass concentration, respectively. With 0.5 g/l biomass concentration, maximum percentage removal achieved was only 40%. The maximum values of sorption capacities for these concentrations were found to be 93 ± 3, 115 ± 1 and 77 ± 2 mg/g dry weight, respectively.

Zirconium biosorption kinetic studies revealed a different trend (Fig. 1A) as zirconium uptake rate was comparatively lower for first 4–6 h as compared to next 18–20 h till equilibrium. A possible explanation for this behavior can be given by considering that in the beginning, biosorption may be only due to cell surface binding. However, with passage of time, the difference in concentration gradient between the solution and the inside of the microbial cell plays its role and the mechanism of uptake is not only due to surface binding but it is shared by metal penetration through the cell wall, thus resulting in a higher metal uptake [21].

Change in pH of bulk solution during course of zirconium biosorption was presented in Fig. 1B. No significant change in solution pH was observed at biomass concentration of 0.5 g/l. The pH values changes from 3.5 to 3.64 and 3.5 to 3.74 at biomass concentration values of 1.0 and 1.5 g/l, respectively.

3.5. Effect of biomass concentration

The effect of *C. tropicalis* cell concentration, on both zirconium biosorption capacity and extent of zirconium removal from aqueous solution containing 116 mg/l zirconium for a period of 24 h incubation is depicted in Fig. 2. The extent of zirconium removal from aqueous solution increased with the increase in cell concentration and almost 100% of zirconium was removed

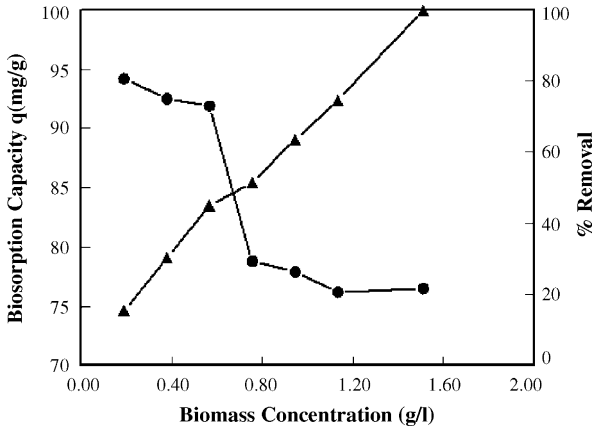

Fig. 2. Zirconium uptake by *C. tropicalis* as a function of biomass concentration (●) sorption capacity mg/g and (▲) % zirconium removal from the aqueous solution (C_i = 116 mg/l, pH 3.5, temperature = 28 ± 2 °C, agitation rate = 100 rpm, contact time = 24 h).

at cell concentration of 1.5 g/l giving sorption capacity value of 77 ± 5 mg/g. In contrast, the values of biosorption capacity decreased with increase in cell concentrations. The maximum value of sorption capacity (94 ± 2 mg/g) was achieved at cell concentration of 0.19 g/l, but at this concentration the extent of zirconium removal was only 15.3%. With an increase in biomass concentration the % removal increases because more biosorbent (binding sites) are available for the same amount of cations while specific uptake of metal ions decreased due to lower metal concentration in solution after a very fast superficial adsorption on to the microbial cells [13,17].

3.6. Effect of initial metal ions concentration on biosorption capacities

Zirconium uptake by *C. tropicalis* cells was also measured after incubating five different concentrations (1.0, 1.5, 2.0, 2.5 and 3.0 g/l) of biomass in media amended with concentrations of zirconium ranging from 50 to 1174 mg/l. It was found that the amount of zirconium taken up by the cells increased with an increase in concentration of zirconium from 50 to 200 mg/l rapidly for all biomass concentrations used (Fig. 3A). Relatively slow increase was observed at concentrations greater than 200 mg/l. The highest concentration of zirconium taken up by *C. tropicalis* was 179 ± 6 mg/g dry weight of yeast biomass at biomass concentration of 1.0 g/l and initial zirconium concentration of 1172 mg/l. The highest values of sorption capacities attained at other biomass concentrations were 143 ± 5, 140 ± 4, 130 ± 7 and 121 ± 4 mg/g for 1.5, 2.0, 2.5 and 3.0 g/l, respectively, at initial zirconium concentration of 1172 mg/l.

The adsorption distribution coefficient (*K*) which describes ratio of the equilibrium concentration in solid and aqueous phase has a unit of ((mg metal g^{-1} biosorbent)/(mg metal ml^{-1}, solution)) or ml g^{-1} biosorbent and is shown in Fig. 3B for zirconium biosorption. A high value of distribution coefficient is the characteristic of a good biosorbent. The value of distribution coefficient was found to be 3968 ml g^{-1} dry weight at C_e of 25 mg Zr l^{-1} that decreased to 180 ml g^{-1} at C_e of 995 mg Zr l^{-1} at biomass

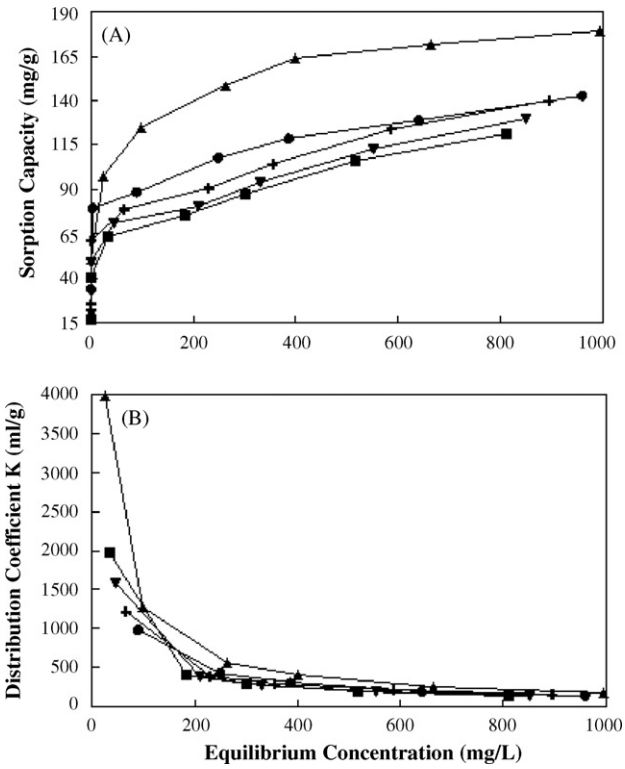

Fig. 3. Effect of initial zirconium ion concentrations on zirconium biosorption capacities (A) and distribution coefficient for zirconium biosorption (B) by *C. tropicalis* (freshly harvested biomass corresponding to (▲) 0.1 g, (●) 0.150, (+) 0.20, (▼) 0.25 and (■) 0.30 g dry weights were incubated for 24 h with 100 ml of zirconium solutions (pH 3.5) having different concentrations at 100 rpm and 28 ± 2 °C).

concentration of 1.0 g l^{-1}. At low initial zirconium ions concentration distribution coefficient approached to infinity due to the complete removal of zirconium from solution, i.e. $C_e = 0$. Similar behavior was also observed at other biomass concentrations. *C. tropicalis* having distribution coefficient value of about 4000 ml g^{-1} was also good for zirconium biosorption, since many industrial separation processes utilized adsorbents with distribution coefficient as small as 10 ml g^{-1} adsorbent [22].

3.7. Data analysis (application of equilibrium and kinetic models of adsorption)

3.7.1. Equilibrium models

To examine the relationship between sorbed (q_e) and aqueous concentrations (C_e) at equilibrium, the Langmuir and the Freundlich adsorption isotherms are widely employed for fitting the data.

For the fitting of experimental data, the linearized form of Langmuir model is as follows:

$$\frac{1}{q_e} = \frac{1}{bq_{max}C_e} + \frac{1}{q_{max}} \quad \text{(IV)}$$

where q_{max} is the maximum amount of metal ions per unit mass of biosorbent to form a complete monolayer on the surface. The q_e represents the practical limiting adsorption capacity when the surface is fully covered with metal ions and allows the compar-

ison of adsorption performance, particularly in the cases where the sorbent did not reach its full saturation in experiments [23]. The plot of $1/q_e$ versus $1/C_e$ was employed to generate the intercept of $1/(bq_{max})$ and the slope of $1/q_{max}$. The metal specific uptake (q_e) for the construction of sorption isotherms is determined as follows:

$$q_e = \frac{C_i - C_e}{M} \quad \text{(V)}$$

The empirical Freundlich equation is

$$q_e = K_F(C_e)^{1/n} \quad \text{(VI)}$$

The above equation can be linearized by taking natural logarithm as follows:

$$\ln\ q_e = \ln\ K_F + \frac{1}{n} \ln\ C_e \quad \text{(VII)}$$

Freundlich constants K_F and n can be calculated from intercept and slope of the straight line (obtained by plotting $\ln q_e$ vs. $\ln C_e$), respectively.

Table 1 compares the values of adsorption capacities (q_{max} and K_F) obtained from Langmuir (Fig. 4A), Freundlich (Fig. 4B) and Dubinin–Radushkevich (Fig. 4C) adsorption isotherms with that of experimental values for zirconium biosorption by *C. tropicalis*. The values of q_{max} obtained from Langmuir adsorption isotherm were close to that of experimental value for biomass concentrations of 1.0 and 1.5 g/l, whereas the values of K_F obtained from Freundlich adsorption isotherms are very low as compared to experimental values for all biomass concentrations used. The values of q_{max} obtained from Dubinin–Radushkevich adsorption isotherms were high as compared to experimental values for all biomass concentrations used. Therefore it is indicated that zirconium biosorption follows Langmuir adsorption isotherms rather than Freundlich and Dubinin–Radushkevich isotherms at low biomass concentrations. Nevertheless failure of any model at higher biomass concentrations demonstrated that zirconium biosorption is a complex phenomenon involving multifaceted, diverse binding sites.

Metal binding according to the Langmuir adsorption isotherm suggests a simple non-interactive monolayer binding to the cell surface. While the values of intensity of adsorption ($1/n$) are <1 suggesting that biosorbents possess heterogeneous surface with identical adsorption energy in all sites and the biosorption of zirconium was limited to monolayer and the adsorbed metal ion interacts only with the active site but not with other.

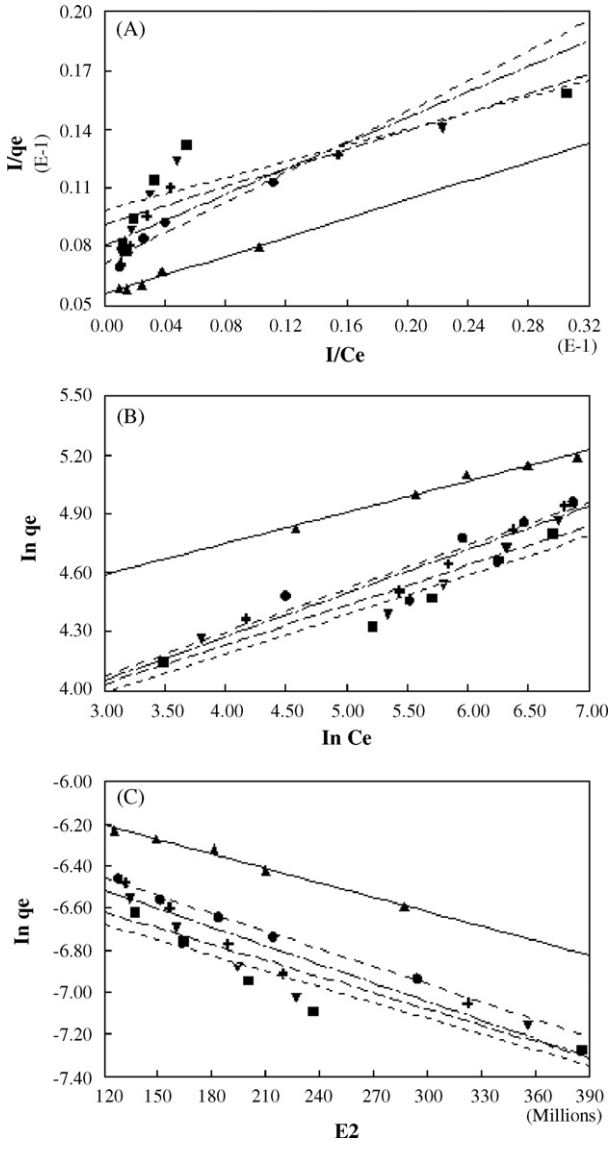

Fig. 4. Langmuir (A), Freundlich (B) and Dubinin-Radushkevich (C) plots of zirconium ions biosorption to *C. tropicalis* at (▲) 1.0 g/l, (●) 1.5 g/l, (+) 2.0 g/l, (▼) 2.5 g/l and (■) 3.0 g/l biomass concentrations.

However, this interpretation should be reviewed with caution as the biosorption isotherm exhibits an irregular pattern due to [24]:

(a) Complex nature of biosorbent.

Table 1
Comparison of q_{max} obtained from Langmuir, Freundlich and Dubinin–Radushkevich adsorption isotherms for zirconium biosorption

Adsorbents concentration (g/l)	q_e (mg/g)	Langmuir		Freundlich		Dubinin–Radushkevich	
		q_{max}	b	K_F	n	q_{max}	$E_s \times 10^4$
1.0	179 ± 6	178.6	0.023	61.16	6.30	241.7	1.47
1.5	143 ± 5	139.3	0.019	30.38	4.53	199.5	1.34
2.0	140 ± 4	124.3	0.025	29.72	4.53	191.8	1.30
2.5	130 ± 7	109.1	0.039	30.83	4.96	165.3	1.40
3.0	121 ± 4	101.3	0.048	29.58	5.00	154.3	1.42

(b) Presence of varied multiple active sites on the biosorbent surface.
(c) Change of metallic compounds chemistry in a solution.

3.7.2. Kinetic models

To understand the controlling mechanism of biosorption, kinetic models pseudo-first-order and pseudo-second-order were used to interpret the experimental data assuming that measured concentrations are equal to cell surface concentrations. Lagergren first-order rate equation [25] is represented as follows.

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad \text{(VIII)}$$

Integration of equation (VIII) applying boundary conditions, $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, resulted in

$$\log\left(\frac{q_e}{q_e - q_t}\right) = \frac{k_1 t}{2.303} \quad \text{(IX)}$$

or

$$\log(q_e - q_t) = \frac{\log q_e - (k_1 t)}{2.303} \quad \text{(X)}$$

Plot of $\log(q_e - q_t)$ versus t should give a straight line.

Pseudo-second-order equation can be expressed as

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad \text{(XI)}$$

Integrating and applying the boundary conditions leads to

$$\left[\frac{1}{q_e - q_t}\right] = \left(\frac{1}{q_e}\right) + k_2 t \quad \text{(XII)}$$

or

$$\left(\frac{t}{q_t}\right) = \frac{1}{k_2 q_e^2} + \left(\frac{1}{q_e}\right) t \quad \text{(XIII)}$$

Plot of t/q_t versus t should give a linear relationship. The values of k_2 and q_e can be obtained from the intercept and slope, respectively.

Table 2 compares the values of q_e obtained from pseudo-first-order (Fig. 5A) and pseudo-second-order (Fig. 5B) plots with that of experimental values for zirconium biosorption. The values of q_e obtained from pseudo-first-order model are close to that of experimental values while values calculated from pseudo-second-order kinetic were high as compared to experimental values for biomass concentration values of 0.5 and 1.0 g/l. At 1.5 g/l biomass concentration value of q_e obtained from pseudo-second-order model was more close to the experimental value as compared to that obtained from pseudo-first-order kinetics. Thus, zirconium biosorption by *C. tropicalis* followed the pseudo-first-order kinetics at low biomass concentration that changes to pseudo-second-order at high concentration of biosorbent.

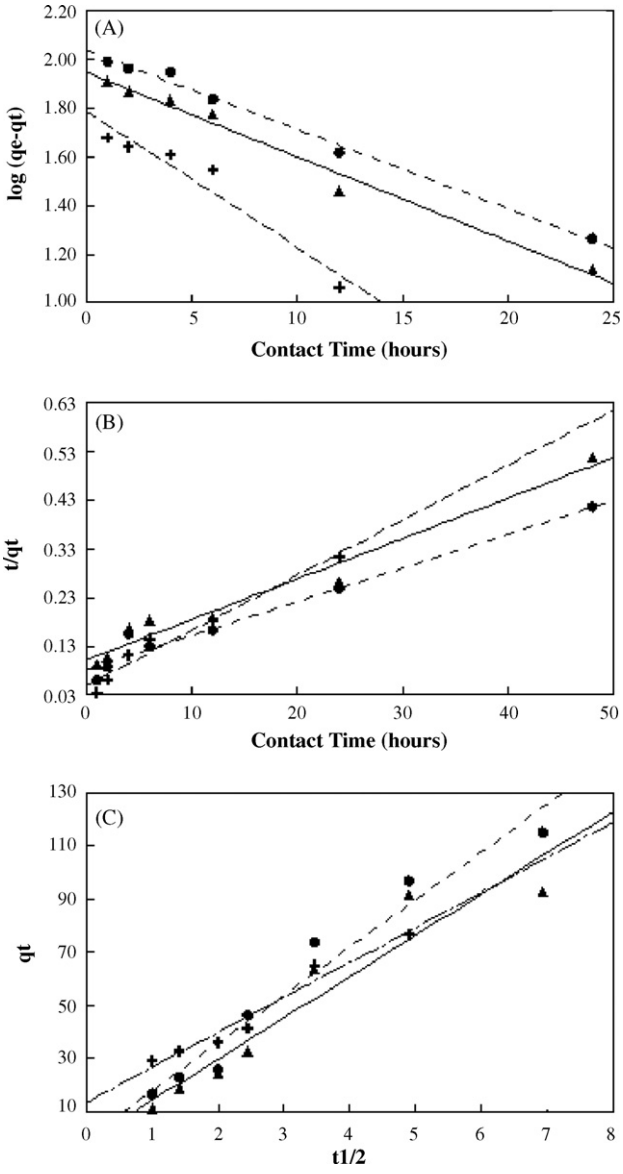

Fig. 5. Pseudo-first-order (A), pseudo-second-order (B) and Weber–Morris (C) plots of zirconium ions biosorption to *C. tropicalis* at biomass concentrations of (▲) 0.5 g/l, (●) 1.0 g/l and (+) 1.5 g/l biomass concentrations.

Table 2
Comparison between adsorption rate constants and q_e estimated to the Lagergren pseudo-first-order and pseudo-second-order kinetic models for zirconium biosorption (C_i = 116 mg/l, pH 3.5, agitation rate = 100 rpm, temperature = 28 ± 2 °C)

Biomass concentration (g/l)	First-order kinetic		Second-order kinetic		$q_{e,exp}$ (mg/g)
	$k_{1,ads}$ (min^{-1})	q_e (mg/g)	$k_{2,ads}$ (g/mg min)	q_e (mg/g)	
0.5	0.080	89.1	0.0007	120.1	93 ± 3
1.0	0.075	109.1	0.0006	143.8	115 ± 1
1.5	0.129	61.3	0.0026	88.1	77 ± .2

Table 3
Desorption of zirconium from loaded biomass by various desorbents

Desorbents	Original pH	% elution after	
		3 h	16 h
Distilled water	05.04	00.00	00.00
Hydrochloric acid	01.46	00.00	00.00
Sodium hydroxide	12.59	00.00	00.00
Sodium carbonate	11.14	04 ± 1	05 ± 1
Sodium bicarbonate	08.55	12 ± 2	43 ± 4
Ammonium sulphate	05.98	00.00	00.00
Ammonium carbonate	08.22	12 ± 4	23 ± 3
Ammonium bicarbonate	08.10	07 ± 3	09 ± 2
NaEDTA	04.68	00.00	00.00

0.05 g of zirconium-loaded biomass was incubated with 30 ml (0.1 M) of eluent at 28 ± 2 °C and 100 rpm.

Application of Weber–Morris equation to kinetic data revealed that zirconium biosorption did not followed this equation (Fig. 5C) although straight lines were obtained but these straight lines were not passing through origin that is prerequisite of this model.

3.8. Metal elution and regeneration of biosorbents

Regeneration of zirconium-loaded biomasses was attempted using various potential eluting agents including acids, alkalies and various salts. Distilled water, HCl, NaOH, $(NH_4)SO_4$, and NaEDTA gave no zirconium elution even after 16 h of incubation at 30 °C and 100 rpm from zirconium-laden biomass (Table 3). Na_2CO_3 eluted only 5 ± 1% of the sorbed zirconium. $(NH_4)_2CO_3$ and NH_4HCO_3 gave 23 ± 3 and 9 ± 2% elution. $NaHCO_3$ showed the maximum eluting efficiency of 43 ± 4% after 24 h of incubation.

The initial pH of eluting agents used varied from 1.5 (HCl) to 12.6 (NaOH). Plot of pH of eluting agents vs. elution efficiency gave a clear relationship between pH and eluting efficiency. Up to pH values of 6, no elution was observed. Elution started at pH value of 8.1, reaching maximum value (43 ± 4%) at 8.6 and drop to 5 ± 1% at pH 11.1. Again at pH 12.6 (NaOH) no elution was observed.

3.8.1. Effect of pulp density on maximum elution efficiency

An important parameter for metal biosorption is the solid-to-liquid ratio (S/L) defined as the mass of metal laden biosorbent to the volume of the elutant [26]. It is desirable to use the smallest possible eluting volume to contain the highest concentration of the metal but at the same time, the volume of the solution should be enough to provide maximum solubility for the metal desorbed [27]. During zirconium desorption, studies were conducted to optimize pulp density (solid/liquid) to obtain maximum elution efficiency by 0.1 M sodium bicarbonate solution (Fig. 6). Each of 0.02, 0.04, 0.05, 0.06 and 0.08 g of zirconium-immobilized biomass was incubated with 50 ml of eluting solution. Analysis of eluting agent after 8, 16 and 24 h of incubation indicated that with increase in incubation time, percentage elution increased with all values of pulp density. The value of percentage elution

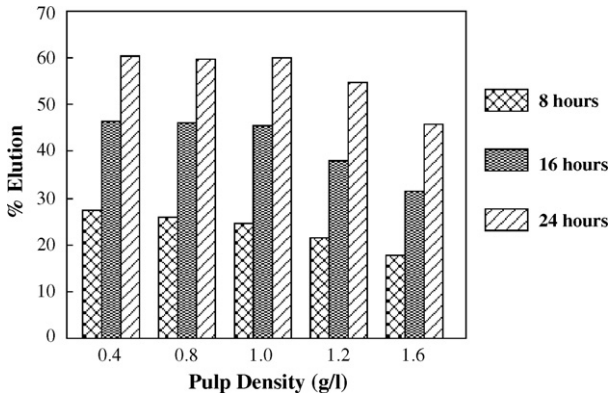

Fig. 6. Effect of pulp density on zirconium elution from *C. tropicalis* by sodium bicarbonate after 8, 16 and 24 h incubation at 100 rpm and 28 ± 2 °C.

decreased with an increased in pulp density for same contact period. Maximum elution efficiency (60 ± 2%) was obtained at solid/liquid of 0.4 g of zirconium loaded biomass/l of eluent after 24 h of incubation and this value was not changed significantly up to 1.0 g/l pulp density. Further increase in pulp density to 1.6 g/l resulted in decrease of percentage elution value to 46 ± 3%.

3.8.2. Sorption/desorption cyclic studies

During zirconium sorption/desorption cyclic studies, biomass at concentration of 0.5 g/l of zirconium solution (concentration 110 mg/l) was used for sorption studies. Elution studies were carried out using 1.0 g of zirconium-immobilized biomass/l of 0.1 M sodium bicarbonate solution. Both sorption capacity and elution efficiency were measured after 24 h of incubation at 28 ± 2 °C and 100 rpm (Fig. 7). In first cycle value of sorption capacity obtained was 91.9 mg/g, and elution efficiency was 60%. In the second cycle sorption capacity decreased to 65.5 mg/g and elution efficiency was more than 95%. Up to five cycles onward no significant change in sorption capacity or elution efficiency was observed.

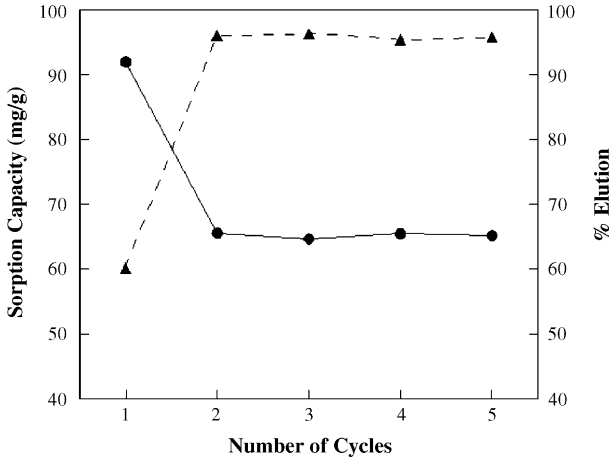

Fig. 7. Batch sorption (●) desorption (▲) cyclic studies for zirconium biosorption by *C. tropicalis* zirconium biosorption: pulp density 0.5 g/l, concentration 110 mg/l, pH 3.5. Flasks were incubated for 24 h at 100 rpm and 28 ± 2 °C. Elution studies: pulp density 1.0 g/l, incubated for 24 h at 100 rpm and 28 ± 2 °C.

Table 4
Amount of metal absorbed and released after digestion of the biomass

Biosorbent	Initial solution concentration (mg/l)	Metal biosorbed (mg/g)	Metal Released after acid digestion (mg/g)
C. tropicalis	50	051 ± 5	048 ± 4
	100	098 ± 3	096 ± 5
	200	125 ± 5	120 ± 3
	400	165 ± 5	160 ± 4
	800	171 ± 2	171 ± 2
	1000	179 ± 4	174 ± 4

Zirconium biosorption: pulp density 1.0 g/l, pH 3.5. Digestion: pulp density 0.05 g/10 ml of acid mixture.

3.9. Mass balance studies

To prove that decrease in concentrations of zirconium ions in solutions during biosorption experiments were really due to biosorption, mass balance studies were conducted. Table 4 summarizes the comparison of metal ions biosorbed and released after digestion. For zirconium biosorption, the differences in metals ions biosorbed and metal ions released were not significant for all initial concentrations used (<5 mg/g of biomass). Also moving from 400 to 1000 mg/l initial concentration, the increase in biosorption capacity was only 14.5 mg/g dry weight.

Zirconium biosorption/bioaccumulation by various microbial biosorbents has been studied previously. Accumulation of Zr by algae and phytoplankton has been described as being due to adsorption [28]. Mann and Fyfe [29] detected no bioaccumulation of Zr in *Ankistrodesmus* sp. Garnham et al. [11] observed Zr bioaccumulation values of $18.2 \pm 0.8\ \mu mol\ g^{-1}$ (*Synechococcus* PCC 6301), $2.1 \pm 0.5\ \mu mol\ g^{-1}$ (*Synechocystiss* PCC 6803), $16.0 \pm 0.1\ \mu mol\ g^{-1}$ (*Plectonema boryanum*),25.6 $\pm 0.7\ \mu mol\ g^{-1}$ (*Chlorella emersonii*), $22.0 \pm 0.6\ \mu mol\ g^{-1}$ (*Scenedesmus obliquus*), 7.50 ± 0.5 (*C. reinhardtii*) $\mu mol\ g^{-1}$ dry weight of biosorbent after 4 h of contact time. In present study, *C. tropicalis* even outperformed anion exchange resin Amberjet 4200 Cl (q_{max} 86 mg Zr g^{-1} of resin) [30] in its Zr biosorption potential (q_{max} 179 ± 6 mg/g).

4. Conclusions

This work describes the removal and recovery of zirconium from its aqueous solutions by *C. tropicalis* under optimized environmental conditions of pH, temperature, and contact time.

The strain *C. tropicalis* was found the best zirconium biosorbent than other fungal and yeast cultures used and gave the highest biosorption capacity of 179 ± 6 mg Zr g^{-1} at biomass concentration of 1.0 g/l of zirconium solution. When equilibrium data in batch mode were fitted to equilibrium models, zirconium biosorption by *C. tropicalis* exhibited simple monolayer biosorption pattern as determined by classical Langmuir model. Moreover, it followed pseudo-first-order kinetics at low biomass concentrations that changed to pseudo-second-order kinetics at high biomass concentrations. Recoveries of sorbed zirconium could be obtained up to 60.2% by desorbing with sodium bicarbonate which was capable of regenerating the biomass which could be used for five cycles. No doubt that few earlier studies exist where removal of zirconium have been described, but this is the first comprehensive report on removal and recovery of this metal by *C. tropicalis*.

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