Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2013.837005

53 (2015) 195–205
January

Taylor & Francis
Taylor & Francis Group

Removal of zirconium from aqueous solution by *Ganoderma lucidum:* biosorption and bioremediation studies

Asma Hanif, Haq Nawaz Bhatti*, Muhammad Asif Hanif

Department of Chemistry and Biochemistry, Environmental Chemistry Laboratory, University of Agriculture, Faisalabad 38040, Pakistan
Tel. +92 41 9200161 69/3319; Fax: +92 41 9200764; email: haq_nawaz@uaf.edu.pk

Received 12 May 2013; Accepted 14 August 2013

ABSTRACT

The development of nuclear science and technology, especially the wide applications of nuclear power, poses a major environmental threat that needs to be remediated. The present study reports the ability of live and dead mycelia of *Ganoderma lucidum* to remove Zr(IV) ions from aqueous solutions in batch system. The biosorption characteristics of *G. lucidum* for Zr(IV) ions were evaluated as a function of medium pH, biomass dosage, contact time, initial zirconium concentration and temperature. Maximum zirconium uptake (142.5 mg/g) was observed at pH 3.5. Increase in biomass dosage did not increase the biosorption capacity. The zirconium biosorption was rapid, with more than 74% of the total biosorption taking place within 15 min and equilibrium was attained after 240 min. The Zr(IV) biosorption process could be well defined by the Langmuir isotherm. The kinetic data fitted the pseudo-second-order kinetic model. A dilute solution of sulphuric acid (0.1 N) was found to be the most effective desorbing agent with a metal recovery up to 91.82%. Bioremediation of zirconium with live mycelia of *G. lucidum* was also investigated as a function of biomass dosage, initial zirconium concentration, contact time and temperature. The results revealed that all the studied parameters affected the bioremediation process. Fourier-Transform Infra-red Spectroscopy analysis of biomass showed the involvement of –OH, $–NH_2$ and –COOH as major functional groups involved in the sequestering of zirconium ions from aqueous solution. The study suggests that *G. lucidum* biomass could be used for treating water containing zirconium ions.

Keywords: Biosorption; Bioremediation; *Ganoderma lucidum*; Immobilization; Kinetics

1. Introduction

Industrial and mining activities are important for economic development, especially in developing countries. The presence of heavy metals in the environment has been a matter of major concern because of their toxicity to human and aquatic life. Toxic heavy metal ions get introduced to the aquatic environment and soils by means of various industrial activities viz. mining, alloy making, electroplating, tanneries, batteries, fertilizers industries, paper industries, pesticides, etc. and pose a serious threat to aquatic environment as well as human beings. Heavy metal remediation of aqueous systems is of special concern due to recalcitrance and persistency of heavy

*Corresponding author.

metal ions in environment [1,2]. Such industries due to low returns constraint themselves from investing in the area of effluent treatments.

Zirconium (Zr) is one the abundant elements which is widely distributed in the earth's crust. Mostly, zirconium is used in the form of different compounds for the ceramic industry, refractories, glazes, enamels and foundry mold. Moreover, it is also used for shielding uranium fuel elements for nuclear energy applications [3]. Naturally occurring isotopes of Zr are not radioactive and some isotopes produced during uranium fission (e.g. Zr^{93} and Zr^{95}) are radioactive and pose threat to environment. Superior mechanical, chemical, electrical, thermal and optical properties are reasons behind recent increase in a wide use of zirconium chemicals in high-technology materials for scientific and multi-industrial applications.

Development of suitable methods for cleaning up contaminated environments continues to be an important topic for environmental restoration and protection. In many developing countries, the removal of heavy metal ions is performed mainly through the use of physicochemical processes, which are very expensive and require large amounts of energy and specialized equipment [4–6]. The overall situation has become even more critical in developing countries, where legislation tends to be weak and water treatment facilities are poor. As a result, it has become critical to search for new techniques to reduce heavy metal concentrations to acceptable environmental levels at manageable costs, in order to protect human health [7,8]. Some micro-organism-based bioremediation techniques have been developed to exploit the potential of certain micro-organisms to degrade and detoxify particular contaminants [9–12]. Various types of biological processes particularly biosorption, bioremediation and bioaccumulation using natural and environmentally safe/compatible materials are gaining importance for the removal of heavy metals from hazardous wastewater streams [13,14]. These biological systems are less affected by environmental extremes than physicochemical methods, and they also have the perceived advantage of being more cost-effective.

Plants and micro-organisms have special focus due to their ability to resist, detoxify and adsorb metals from environments [15–19]. Micro-organisms are preferred sometime over plant biomass as laboratory culture techniques can provide their sufficient quantities all around the year. To decontaminate the natural environments from heavy metals and to stop the further entry of these toxic metals into food chain, the need of the day is the development of efficient biosorbents and effective biosorption/bioremediation systems in an economically feasible way [20]. Systems making use of both dead and living cells are in use.

The objective of the present work is to investigate the bioremediation potential of live and dead mycelia of *Ganoderma lucidum* (white-rot-fungus) in the removal of zirconium from aqueous solution under various conditions of pH, biosorbent dose, initial metal ion concentration, contact time, temperature and co-metal ions.

2. Materials and methods

2.1. Reagents

All the chemicals used in the present study were of analytical grade and mainly purchased from Sigma–Aldrich Chemical Co., USA.

2.2. Micro-organism used and biomass preparation

G. lucidum biomass used this work was collected from Changa Manga Forest-Punjab, Pakistan. Fungal mycelia were collected in test tubes and were carefully transported to the laboratory. Identification of mycelia was carried out in the Mushroom Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. This fungus was maintained for further part of study by subculturing on potato dextrose agar slants. Fungus slants were incubated at 28 ± 1°C for a period of 3 days. The inoculum development was carried out by transferring spores of *G. Lucidum* in 500 ml Erlenmeyer flasks containing 150 ml of the Vogel's medium. The medium was autoclaved for 15 min (15 lbs, 121°C) and cooled before transferring spores. The composition of the inoculum medium is given in Table 1. The pH of the medium was adjusted to 4.5 using 1 M HCl/1 M

Table 1
Composition of inoculum media for *G. lucidum* growth

Ingredients	Quantity/L
Glucose	20 g
Thiamine	0.001 g
10% Tween-80	10 ml
Trace element solution	10 ml
100 mM veratryl alcohol	10 ml
Ammonium tartarate	0.22 g
KH_2PO_4	0.21 g
Chloramphenicol	1 ml
$MgSO_4{\cdot}7H_2O$	0.05 g
$CaCl_2{\cdot}2H_2O$	0.01 g

NaOH. Afterwards, these flasks were placed on a shaking incubator at 120 rpm at 30°C for 3–4 days. Homogenous spore suspensions were obtained after incubation period. Biomass spores were separated from liquid phase using Whatman filter paper No. 42. Harvested biomass was extensively washed using double distilled water (DDW) to remove medium remnants [11]. Live mycelia were used for bioremediation study. For biosorption study, the spores were heat-killed at 60°C for 72 h. The biomass was dried in an oven at 70°C for overnight to a constant weight. This dried biomass was used in the biosorption experiments.

2.3. Immobilization of fungal biomass

The immobilization of *G. lucidum* basidiospores via encapsulation was carried out as follows: 2 g of Na-alginate was dissolved per 100 ml of warm DDW. The solution was heated on an electric plate (if needed) to dissolve remaining Na-alginate. Afterwards, a homogeneous solution was obtained. This solution was then mixed with 10 ml of the fungal spore suspension ($\approx 1 \times 10^9$ basidiospore ml^{-1}). The mixture was taken in a burette with a stopper. The mixture was introduced into a solution containing 0.10 M $CaCl_2{\cdot}2H_2O$ and the solution was stirred to prevent aggregation of the fungal spores-entrapped Ca-alginate beads. The fungal spore-entrapped beads (4 mm) were washed twice with 200 ml sterile distilled water. The obtained beads were washed extensively using sterilized distilled water. For bioremediation studies, the beads (10 g) containing immobilized spores were transferred to the 100 ml of growth medium taken in a 250 ml conical flask. The incubation temperature, shaking speed and incubation periods were 30°C, 150 rpm and 3 days, respectively. The mycelia growth in/on the beads was followed using a microscope during the incubation period. After completion of incubation period, the beads were separated from liquid medium by filtration and washed thrice using DDW. To determine dry weights of the microbial growth after immobilization, beads were dried overnight at 80°C in an electric oven [11].

2.4. Zirconium solutions

The stock zirconium solution (1,000 mg/l) was prepared by dissolving 3.53 g of zirconyl oxychloride ($ZrOCl_2{\cdot}8H_2O$) in 100 ml of deionized distilled water and diluting quantitatively to 1,000 ml using DDW. Zirconium solutions of different concentrations were prepared by sufficient dilution of the stock solution with DDW.

2.5. Analytical measurements

The concentration of zirconium in the solution was determined by colorimetric method based on the reaction of zirconium with xylenol orange. A solution of xylenol orange (0.05%) was prepared by dissolving the dry powder in 0.6 N 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 10–15 min. Then, optical density of the solution was measured at 535 nm using 1 cm cell against the reagent blank. Concentration of zirconium present in unknown sample was calculated from this standard curve [10].

2.6. Batch biosorption studies

For biosorption studies, *G. lucidum* was heat-killed at 70°C for 72 h before its immobilization. Dried biomass was ground into powder form and sieved to obtain a uniform particle size of <0.255 mm. The immobilization of dead biomass was carried out as described in the above section. In all sets of experiments, fixed volume of zirconium solution (100 ml) was thoroughly mixed with known amount of immobilized biosorbent at 30°C with shaking speed of 100 rpm for a definite period. The experiments were conducted with control samples (containing metal ion solution in the absence of biomass) to evaluate the influence of pH (1–4), biosorbent dose (0.05–0.3 g/100 ml), initial metal ion concentration (25–400 mg/l), contact time (0–240 min) and temperature (30–70°C). The flasks were kept on a rotating shaker with constant shaking. At the end of the experiment, the flasks were removed from the shaker and the solutions were separated from the biomass by filtration through filter paper (Whatman No. 40, ashless). The concentration of zirconium in the solutions before and after the equilibrium was determined by colorimetric method as described earlier.

The amount of metal ions adsorbed per unit mass of fungus-immobilized alginate preparations (mg metal ions/g dry beads) was calculated by the simple concentration difference method [21] using the following expression:

$$q = (C_i - C_e)V/W \quad (1)$$

where q is the amount of metal ions adsorbed onto the unit mass of the adsorbent (mg/g); C_i and C_e are the concentrations of the metal ions before and after biosorption (mg/l), respectively; V is the volume of the aqueous phase (L); and W is the amount of the adsorbent (g).

2.7. Bioremediation studies

In case of bioremediation studies, live immobilized mycelia of *G. lucidum* were used. The operational parameters under study were biosorbent dose (0.05–0.3 g/100 ml), initial metal ion concentration (25–400 mg/l), time (0–240 min) and temperature (30–45°C). The pH of the medium was fixed at 4.5 using HCl or NaOH.

2.8. Desorption study

In order to determine the reusability of the alginate beads and immobilized fungal preparations, consecutive adsorption–desorption cycle was conducted by using the same biosorbent. Desorption of metal ions was performed by 0.1 M hydrochloric acid, sulphuric acid, nitric acid, acetic acid and EDTA solutions. The immobilized fungal biomass preparations loaded with metal ions were placed in the desorption medium and stirred at 100 rpm for 24 h at 30°C. The final metal ion concentrations in the aqueous phase were determined by the colorimetric method as described above. Desorption ratio was calculated from the amount of metal ions adsorbed on the immobilized biomass and the final metal ion concentration in the desorption medium. Percent desorption was calculated from the following equation:

$$\% \text{ Desorption} = (\text{Amount of metal ions desorbed}/\text{Amount of metal ions adsorbed}) \times 100$$

2.9. FT-IR analysis

Fourier-transform infra-red spectroscopy (FT-IR) (IR Perkin–Elmer 1600 spectrometer) analysis of selected fungal biomass was carried out to determine chemical functional groups, responsible for the sorption of zirconium ions. IR data were observed over 400–4,000 cm^{-1} by preparing KBr disks of sorbent material and spectrum was recorded.

2.10. Statistical analysis

All data represent the mean of three independent experiments. The results are presented as mean ± SD values. The statistical analysis was done using the Microsoft Excel 2004, Version Office XP.

3. Results and discussion

3.1. Batch biosorption studies

Batch biosorption study for the removal of zirconium from a simulated solution was carried out using immobilized dead mycelia of *G. lucidum*. The effect of medium pH, biosorbent concentration, zirconium concentration and contact time was investigated. The results are discussed as follows:

3.2. Effect of solution pH on zirconium biosorption

The effect of solution pH on the biosorption of Zr(IV) using dead mycelia of *G. lucidum* was investigated by varying the pH of the solution and the results are shown in Fig. 1. The biosorption capacity of fungal biomass was strongly affected by the initial pH of the aqueous metal solution. It was found to increase with pH, exhibiting maxima of 142.5 mg/g at pH 3.5. The higher pH values were not examined because of the precipitation of zirconium at pH > 3.5 [22]. At lower pH, protonation of functional groups takes place resulting in net positive charge on the surface of biosorbent. With the increase in solution pH, de-protonation of functional groups takes place resulting in net negative charge on the surface of biosorbent. The decrease in biosorption capacity at low pH could be due to increase in H^+ and H_3O^+ ions which competes with Zr (IV) ions for binding sites on the surface of fungal biomass. Therefore, further experiments were carried out with an initial pH value of 3.5 since insoluble zirconium hydroxide starts precipitating from solutions at higher pH values, making true sorption studies impossible. The dependency of metal uptake by biosorbent is correlated well in past with solution pH, as the ionization and protonation of functional groups present on or inside cell surface depend upon pH. Secondly, metal solubility is also pH-dependent phenomenon. Akhtar et al. [10] studied the biosorption of zirconium by *Candida tropicalis* and observed maximum biosorption at pH 3.5. Similarly, maximum

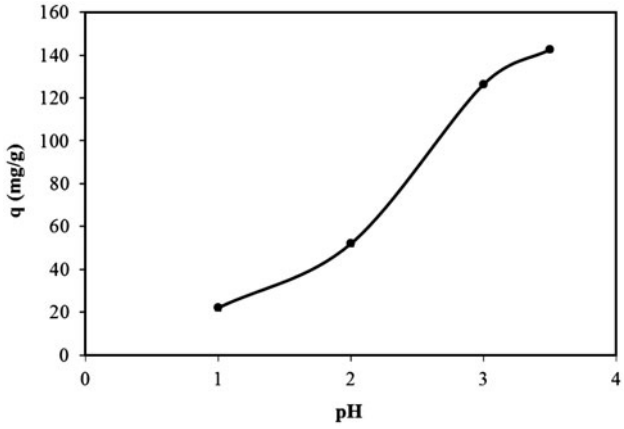

Fig. 1. Effect of pH on the uptake of Zr using *G. lucidum* dead cells.

adsorption of Zr(IV) by *Platanus orientalis* tree leaves was also recorded at pH 3.0 [23].

3.3. Effect of biosorbent dose on zirconium biosorption

Biosorbent dose has a predominant effect on metal uptake capacity of biosorbent. It determines the sorbate–sorbent equilibrium of the system. Moreover, it determines the number of binding sites available for adsorption [2]. Effect of biosorbent dose on zirconium uptake capacity of biomass was checked by increasing biomass dose from 0.025 to 0.3 g/100 ml (Fig. 2). The results reveal that biosorption capacity of fungal biomass first increased and then decreased with an increase in biomass dose. Maximum biosorption capacity (143 mg/g) was observed at biomass concentration of 0.05 g/100 ml. The q for the biomass concentration of 0.1 g/100 ml was 102.3 mg/g. On further increasing the biomass concentration, the decrease in q did not steep. An increase in the biomass concentration generally increases the amount of solute biosorbed due to increased surface area of the biosorbent which in turn increases the number of binding sites [24]. On the other hand, the quantity of biosorbed solute per unit weight of biosorbent decreases with increasing biosorbent concentration which might be due to the interference between binding sites/aggregation of particles of biomass that would result in a low metal ions uptake [25]. Similar trend for q value has been reported during the biosorption of zirconium by *C. tropicalis* biomass [10].

3.4. Effect of contact time and kinetic modelling

The proper selection of equilibrium time for adsorption process is a crucial parameter for practical applications of biosorption studies. The effect of contact time on the uptake of zirconium by immobilized dead biomass of *G. lucidum* was studied from 0 to 300 min at various time intervals. The results show that zirconium biosorption by immobilized dead biomass of *G. lucidum* can be easily divided into two steps: first rapid step up to 15 min followed by a slow adsorption phase until equilibrium point was achieved after 240 min (4 h). Almost 74% adsorption sites of biomass were occupied in the first 15 min. Maximum biosorption capacity (143.9 mg/g) was observed after 240 min. This is due to the fact that in the initial stages of adsorption process, large number of binding sites is available on the surface of biosorbent, which leads to rapid attachment of adsorbate on the adsorbent. With the progress of biosorption process, the binding sites become occupied and lesser sites are available for the adsorption of adsorbate, which results in slow rate of biosorption process at later stages. There are several parameters that determine the adsorption rate such as the agitation speed of the medium, structural properties of the biosorbent, amount of biosorbent, properties of the ion under study, initial concentration of metal ions and, of course, existence of other metal ions, which may compete with the ionic species of interest for the active biosorption sites [26]. The optimum contact time selected was 240 min for subsequent studies. An equilibrium time of 24 and 48 h has been reported during the removal of zirconium by *C. tropicalis* for 1.0 and 1.5 g/l of biomass concentration [10].

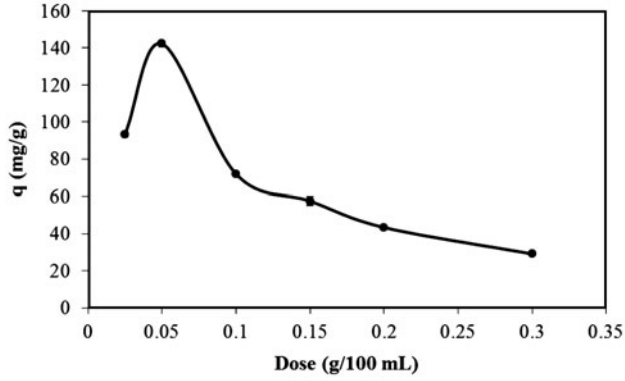

Fig. 2. Effect of biomass concentration on the uptake of Zr using *G. lucidum* dead cells.

Kinetic modelling of the experimental data was done using Lagergren pseudo-first-order (Lagergren, 1898) and pseudo-second-order model [27] kinetic models to interpret the experimental data, assuming that measured concentrations are equal to cell surface concentrations. The pseudo-first-order Lagergren model is generally expressed as:

$$\log\,(q_e - q_t) = \log\, q_e - K_1.t/2.303 \tag{3}$$

where q_e (mg/g) and q are the amounts of adsorbed metal ions on the biosorbent at the equilibrium and at any time t, respectively; and $k_{1,ads}$ is the Lagergren rate constant of the first-order biosorption. Plot of log (q_e−q) vs. t gave a straight line.

The pseudo-second-order model is based on the assumption that biosorption follows a second-order mechanism. So, the rate of occupation of adsorption sites is proportional to the square of the number of unoccupied sites:

$$t/q = 1/K_{2,ads}\, q_e^2 + t/q_e \tag{4}$$

where $k_{2,ads}$ is the rate constant of second-order biosorption (g/mg min). Plot of t/q vs. t gave a linear

relationship. The values of k_2 and q_e were obtained from the intercept and slope, respectively. Table 2 compares the values of q_e obtained from pseudo-first-order and pseudo-second-order plots with the experimental values for zirconium biosorption. The value of q_e obtained from pseudo-second-order model (135.13 mg/g) was close to the experimental value (143.9 mg/g) whereas the value calculated from pseudo-first-order kinetic was low as compared to the experimental value. Thus, zirconium biosorption by immobilized *G. lucidum* dead biomass followed the pseudo-second-order kinetics. Akhtar et al. [10] reported that zirconium biosorption by *C. tropicalis* followed the pseudo-first-order kinetics at low biomass concentration that changes to second-order kinetics at higher concentration of biomass.

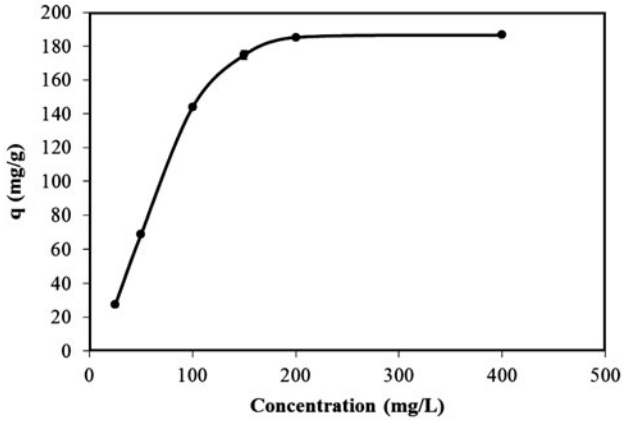

Fig. 3. Effect of initial metal concentration on the uptake of Zr using *G. lucidum* dead cells.

3.5. Effect of initial metal ion concentration and equilibrium modelling

The effect of initial concentration of zirconium on its biosorption by immobilized dead biomass of *G. lucidum* was studied by shaking 0.05 g of biomass with 100 ml of zirconium solution having concentration from 25 to 400 mg/l. The results reveal that the metal uptake capacity of biomass increased with an increase in metal ion concentration from 25 to 200 mg/l. The maximum uptake capacity (186.5 mg/g) was observed when the initial zirconium concentration was 200 mg/l (Fig. 3). This might be due to the saturation of adsorption sites and increase in the number of ions competing for the available vacant sites on the biomass for complexation of zirconium at higher concentration [28]. Once the binding sites present on the biomass got saturated with the zirconium, the availability of binding sites for zirconium decreased. This could explain why the initial phase was fast and then slowed down as the saturation was achieved. The amount of zirconium taken up by *C. tropicalis* biomass also increased with an increase in zirconium concentration from 50 to 200 mg/l [10].

The equilibrium modelling of the data was done using commonly applied Langmuir and Freundlich adsorption isotherms. When an isotherm is plotted using the data at equilibrium of the experimental system, it gives valuable information that is useful for the selection of the biosorbent. Modelling also facilitates the evaluation of feasibility of the adsorption process for a given application. Langmuir parameters were determined for zirconium biosorption from a linearized form of Eq. (4), represented by

$$C_e/q_e = 1/q_{max}K_L + C_e/q_{max} \quad (5)$$

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 biosorption capacity when the surface is fully covered with metal ions and allows the comparison of adsorption performance, particularly in the cases where the adsorbent did not reach its full saturation in experiments [29] and K_L the ratio of adsorption/desorption rates related to energy of adsorption. The plot of C_e/q_e vs. C_e was employed to generate the intercept of $1/(q_{max}K_L)$ and the slope of $1/q_{max}$.

The linear form of the Freundlich isotherm is as follows:

$$\log q_e = 1/n \log C_e + \log K \quad (6)$$

where q_e is the equilibrium metal biosorption capacity (mg/g), C_e is the equilibrium concentration of metal

Table 2
A comparison between pseudo-first-order and pseudo-second-order kinetic models for the removal of zirconium by immobilized dead mycelia of *G. lucidum*

Experimental, q_e (mg/g)	Pseudo-first-order			Pseudo-second-order		
	q_e (mg/g)	$k_{1,ads}$ (min^{-1})	R^2	q_e (mg/g)	$k_{2,ads}$ (g/mg min)	R^2
143.9	40.55	6.90×10^{-3}	0.969	135.1	1.11×10^{-3}	0.998

ion solution (mg/l), K and $1/n$ are constants. Freundlich constant K is a measure of adsorption capacity and $1/n$ the adsorption intensity. These were calculated from intercept and slope of the straight line (obtained by plotting log q_e vs. log C_e), respectively.

The Langmuir and Freundlich biosorption parameters for zirconium biosorption by immobilized *G. lucidum* dead biomass are given in Table 3. The metal loading capacity (q_{max}) was calculated from the slope of the plot C_e/q_e vs. C_e and was found to be 212.76 mg/g. The data fitted the Langmuir isotherm ($R^2 = 0.951$). The value of q_{max} obtained from Langmuir adsorption isotherm was close the experimental value whereas the values of K obtained from Freundlich adsorption isotherms was very high as compared to experimental value. Therefore, it is indicated that zirconium biosorption followed the Langmuir adsorption isotherms rather than Freundlich isotherm. Metal binding according to the Langmuir adsorption isotherm suggests a simple non-interactive monolayer binding to the cell surface. And, 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. Monji et al. [23] studied the selective biosorption of zirconium by rice bran, wheat bran and *P. orientalis* tree leaves, and reported that biosorption of zirconium by rice bran leaf followed the Langmuir adsorption isotherm.

3.6. Effect of temperature

The effect of temperature on the removal of zirconium by immobilized dead biomass of *G. lucidum* was investigated at five different temperatures (30–70°C), and the results are shown in Fig. 4. It is obvious from the figure that the biosorption capacity of G. *lucidum* biomass increased with an increase in temperature from 30 to 40°C. A further increase in temperature from 40 to 70°C exhibited a slight decrease in the uptake of zirconium by the biomass. The results obtained clearly demonstrated that adsorption process was endothermic below 40°C and exothermic above this temperature. The increase in biosorption of zirconium with temperature up to 40°C could be due to two factors. Firstly due to an increase in the number of active surface sites available and secondly due to a decrease in the thickness of the boundary layer surrounding the biosorbent. The decrease in the biosorption of zirconium ions with the rise in temperature may be due to distortion of some sites of the cell surface available for metal biosorption or decreased mobility of binding groups on the biomass surface [30]. The optimum solution temperature was selected as 40°C for further biosorption experiments. The temperature of the biosorption medium could be important for energy-dependent mechanisms in metal ions biosorption by different biosorbents. Generally, adsorption is an exothermic process although some examples of endothermic adsorption have also been reported [2].

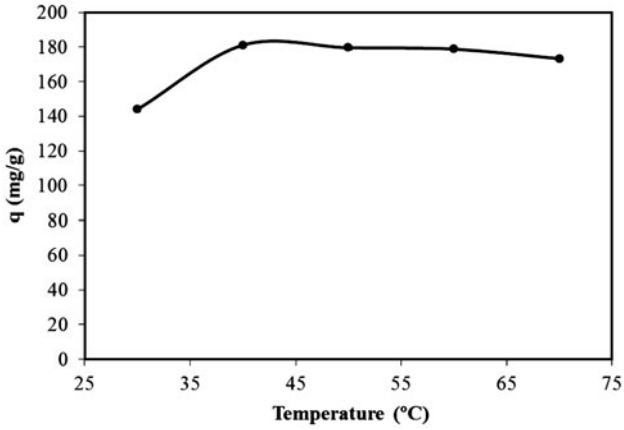

Fig. 4. Effect of temperature on the uptake of Zr using *G. lucidum* dead cells.

3.7. Effect of co-metal ions

Industrial wastewater always contaminated with many co-metal ions. In this regard, the experiments were conducted to evaluate the effect of some metal ions like as K^+, Mg^{2+} and Al^{3+} on the uptake of zirconium by immobilized dead biomass *G. lucidum*. In order to study the effect of metal ions, the concentration of K^+, Mg^{2+} and Al^{3+} ions was varied from 25 to 400 mg/l using a fixed concentration of

Table 3
A comparison between Langmuir and Freundlich adsorption isotherm parameters for the removal of zirconium by immobilized dead mycelia of *G. lucidum*

Experimental, q_{max} (mg/g)	Langmuir model			Freundlich model		
	q_{max} (mg/g)	K_L	R^2	K	n	R^2
186.59	212.76	3×10^{-2}	0.951	280.50	1.97	0.655

zirconium (100 mg/l). The results reveal that all the metal ions decreased the biosorption capacity of fungal biomass. Moreover, this inhibitory effect increased with increasing concentration of these ions. It is a well known fact that a fixed quantity of biosorbent could only offer a finite number of surface-binding sites which would be expected to be saturated by the competing metal ions, especially at relatively high concentration of ions. The presence of more than one metal ion in a single system was found to show a competitive adsorption process. Factors like hydrolysis, hydration and covalent binding of the metallic ions play vital role in competitive processes [31,32]. The extent of inhibition in the zirconium ions uptake in the presence of co-metal ions is in following order $Al^{3+} > Mg^{2+} > K^{+}$. Co-metal ions with higher valence have greater charge in comparison to metal ion with lower valence and, hence, compete more effectively for occupying adsorption sites.

3.8. Desorption studies

Multiple batch use of biosorbent is always desired to reduce process costs [33]. For the recovery zirconium from immobilized dead biomass of *G. lucidum*, five commonly used desorbing agents such as acetic acid, hydrochloric acid, nitric acid, sulphuric acid and EDTA were used in dilute form (0.1 N). The dilute forms of the desorbing agents are recommended to save biosorbents from physical or chemical damage. The results show that all the desorbing agents eluted more than 80% zirconium from the loaded biomass. Maximum recovery of zirconium (91.82%) was observed with sulphuric acid while nitric acid yielded low recovery (88.8%) of zirconium. This might be due to the protonation of biomass under acidic conditions that makes possible desorption of positively charged zirconium ions from the adsorbent surface. The biomass regeneration efficiency was found in the following order: sulphuric acid > EDTA > hydrocholoric acid > acetic acid > nitric acid.

3.9. Bioremediation studies

In bioremediation study, live mycelia of *G. lucidum* were entrapped in calcium alginate beads and grown in nutrient medium for different experiments. The effect of different parameters affecting bioremediation is discussed in the following sections.

3.9.1. Effect of biosorbent dose

Biosorbent dose has a significant effect on the metal uptake process as it affects the sorbent and sorbate equilibrium of the system. Effect of biosorbent dose on zirconium uptake capacity of live immobilized biomass of *G. lucidum* was evaluated by varying dose from 0.025 to 0.3 g/100 ml. The results reveal that biosorption capacity of live mycelia first increased with an increase in mycelia dose and then showed a decreasing trend. Maximum metal uptake capacity (168 mg/g) was recorded with 0.05 g. A decrease in zirconium uptake capacity of fungal biomass was observed with an increase in biomass dose (Fig. 5). This could be the result of poorer biomass utilization at higher biomass concentration. High biomass doses cause the agglomeration of cells as well as reduce the inter-cellular distance. As a result, 'screening effect' is produced among cells due to denser layers which lead to protection of the sites available for binding metal ions. Under such conditions, cells have optimal electrostatic interaction which is a significant factor for metal ions uptake [33,34].

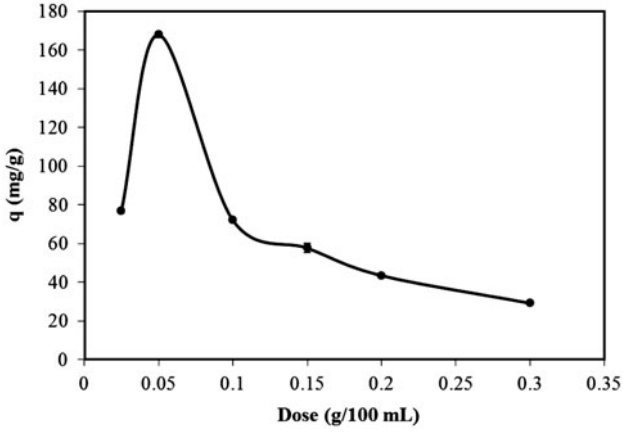

Fig. 5. Effect of biomass dose on the removal of Zr(IV) by live mycelia of *G. lucidum*.

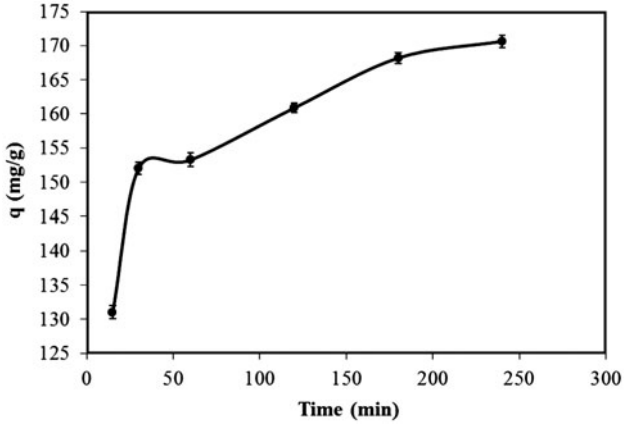

Fig. 6. Effect of contact time on the uptake of Zr using *G. lucidum* living cells.

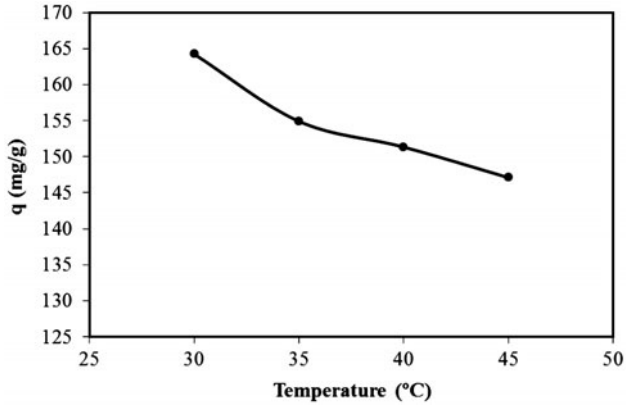

Fig. 7. Effect of temperature on the uptake of Zr using *G. lucidum* living cells.

3.9.2. Effect of initial metal concentration

The initial concentration of a particular metal ion provides an important driving force to overcome all mass transfer resistance of zirconium between the aqueous and solid phases. The bioremediation of zirconium was carried out at different initial concentrations ranging from 25 to 400 mg/l (pH 3.5) to determine the change in metal uptake capacity of immobilized live *G. lucidum* biomass. The results show that bioremediation capacity of the fungus increases with an increase in initial zirconium concentration.

3.9.3. Effect of contact time

The selection of proper contact time is of utmost importance for practical application of a full-scale batch metal removal process using live microbial biomass. The effect of contact time on the uptake of zirconium by immobilized live *G. lucidum* biomass was studied from 0 to 240 min at various intervals and the results are shown in (Fig. 6). As in case of dead biomass, zirconium removal by immobilized live *G. lucidum* biomass can be also be divided into two steps: first rapid step up to 30 min followed by a slow adsorption phase until equilibrium point at 240 min. Almost 84.14% of total metal was accumulated in first 15 min. This value increased to 97.62% after 30 min fallowed by a very slow uptake process until equilibrium. As the most of adsorption occurs in first 15 min, this contact time can be used for removal of Zr(IV) but r maximum possible removal of Zr(VI) ions by *G. lucidum* needs 240 min.

3.9.4. Effect of temperature

Metal–sorbent complex stability is usually influenced by the solution temperature. Living microorganisms are more vulnerable to temperature changes as metabolic and growth temperature exhibit temperature dependency. The temperature of the aqueous

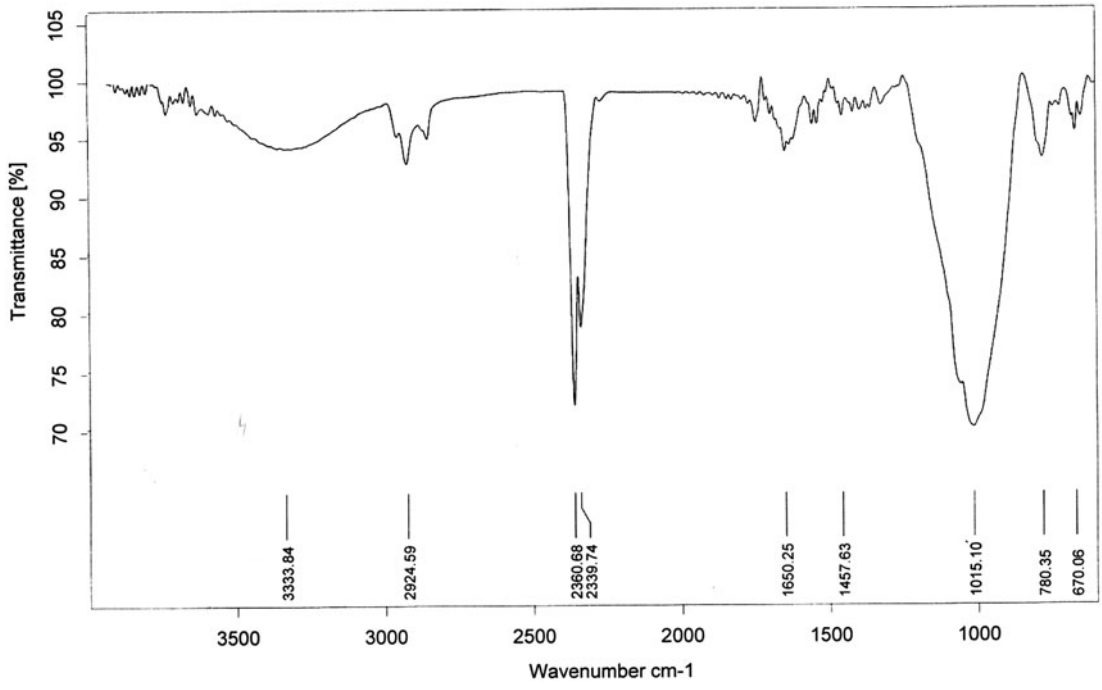

Fig. 8. FT-IR analysis of *G. lucidum* biomass.

solution containing both immobilized microbial cells and zirconium ions was varied from 30 to 45°C and the results are shown in Fig. 7. The figure depicts that the bioremediation capacity of live *G. lucidum* decreases with an increase in the temperature. Maximum uptake of zirconium by immobilized live *G. lucidum* was recorded at 30°C and minimum somewhere between 35 and 40°C.

3.10. FT-IR analysis

The FT-IR is an important spectroscopic technique that identifies some characteristic functional groups responsible for metal ions sequestering from the aqueous solution. The spectrum of *G. lucidum* biomass was studied in the range of 400–4,000 cm^{-1}. The strong asymmetrical stretching band near 3,400 cm^{-1} indicated the presence of –NH functional group, which is further confirmed by the presence of absorptions bands in the range of 1,700–1,500 cm^{-1}. The broad, intense –OH stretching absorption near 3,000 cm^{-1} suggested the presence of –COOH group on cell surface of biomass. Thus, the FT-IR results revealed the presence of –OH, $–NH_2$ and –COOH major functional groups involved in the sequestering of zirconium ions from aqueous solution (Fig. 8).

4. Conclusions

This study indicated that the white rot fungus, *G. lucidum* which is widely available, can be used as an efficient biosorbent material for the removal of Zr(IV) from wastewater. The biosorption process was fast enough, as maximum removal took place with 15 min contact time. This fungus is very effective in view of its adsorption capacity. The value of Zr(IV) uptake found in this work was higher than that reported for other biomaterials. Maximum adsorption capacity of 186.5 mg/g was recorded with an initial Zr(IV) concentration of 200 mg/l. The present investigation of the biosorption and bioremediation using microbial biomass would be an addition to the list of high metal uptake capacity biomaterials. Thus, *G.lucidum* can be used as a potential source for metal remediation.

References

[1] D.G. Sud, G. Mahajan, M.P. Kaur, Agricultural waste material as potential biosorbent for sequestering heavy metal ions from aqueous solutions—A review, Bioresour. Technol. 99 (2008) 6017–6027.
[2] H.N. Bhatti, I.I. Bajwa, M.A. Hanif, I.H. Bukhari, Removal of lead and cobalt using lignocellulosic fiber derived from Citrus reticulata waste biomass, Korean J. Chem. Eng. 27 (2010) 218–227.
[3] A.M. Abdel-Rehim, A new technique for extracting zirconium form Egyptian zircon concentrate, Int. J. Miner. Process. 76 (2005) 234–243.
[4] H.N. Bhatti, M. Amin, Removal of zirconium (IV) from aqueous solutions by *Coriolus versicolor*: Equlibrium and thermodynamic study, Ecol. Eng. 51 (2013) 178–180.
[5] B. Volesky, Detoxification of metal-bearing effluents: Biosorption for the next century, Hydrometallurgy 59 (2001) 203–216.
[6] T.A. Kurniawan, G.Y.S. Chan, W.H. Lo, S. Babel, Physico-chemical treatment techniques for wastewater laden with heavy metals, Chem. Eng. J. 118 (2006) 83–98.
[7] R. Boota, H.N. Bhatti, M.A. Hanif, Removal of Cu(II) and Zn(II) using lignocellulosic fiber derived from *Citrus reticulata* (Kinnow) waste biomass, Sep. Sci. Technol. 44(16) (2009) 4000–4022.
[8] V.L. Colin, L.B. Villegas, C.M. Abate, Indigenous microorganisms as potential bioremediators for environments contaminated with heavy metals, Int. Biodeterior. Biodegrad. 69 (2012) 28–37.
[9] Y.B. Sag, B. Akeael, T. Kutsal, Ternery biosorption equilibria of Cr(VI), Cu(II) and Cd(II) on *Rhizopus arrihzus*, Sep. Sci. Technol. 37 (2002) 279–309.
[10] K. Akhtar, M.W. Akhtar, A.M. Khalid, Removal and recovery of zirconium from its aqueous solution by *Candida tropicalis*, J. Hazard. Mater. 156 (2008) 108–117.
[11] M.A. Hanif, H.N. Bhatti, I.A. Bhatti, M. Asgher, Biosorption of Cr(III) and Cr(VI) by newly isolated white rot fungi: Batch and column studies, Asian J. Chem. 23 (2011) 3375–3385.
[12] G.P. Williams, M. Gnanadesign, S. Ravikumar, Biosorption and bio-kinetic study of halobacterial starins against Ni^{2+}, Al^{3+} and Hg^{2+} metal ions, Bioresour. Technol. 107 (2012) 526–529.
[13] M. Gavrilescu, Removal of heavy metals from the environment by biosorption, Eng. Life Sci. 4 (2004) 219–232.
[14] H.N. Bhatti, M. Amin, Removal of zirconium(II) from aqueous solution by *Coriolus versicolor*: Equilibrium and thermodynamic study, Ecol. Eng. 51 (2013) 178–180.
[15] O.V. Singh, S. Labana, G. Pandey, R. Budhiraja, R.K. Jain, Phytoremediation: An overview of metallic ion decontamination from soil, Appl. Microbiol. Biotechnol. 61 (2003) 405–412.
[16] K.H. Cheung, J.D. Gu, Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: A review, Int. Biodeterior. Biodegrad. 59 (2007) 8–15.
[17] M.A. Hanif, R. Nadeem, M.N. Zafar, H.N. Bhatti, R. Nawaz, Physico-chemical treatment of textile wastewater using natural coagulant *Cassia fistula* (Golden Shower) pod biomass, J. Chem. Soc. Pak. 30(3) (2008) 385.
[18] N. Parveen, M.A. Hanif, S. Noureen, T.M. Ansari, H.N. Bhatti, Phytoremediation of Pb(II) by *Jasminum sambac*, J. Chem. Soc. Pak. 33(4) (2011) 592.
[19] M.A. Hanif, H.N. Bhatti, Bioremediation of nickel from wastewater using immobilized *Phanerochaete chyrsosporium* biomass, Int. J. Chem. Biochem. Sci. 2 (2012) 54–59.
[20] C. Garbisu, I. Alkorta, Phytoextraction: A cost-effective plant-based technology for the removal of metals from the environment, Bioresour. Technol. 77 (2001) 229–236.
[21] B. Volesky, Z.R. Holan, Biosorption of immobilized *Saccharomyces cerevisiae* with successive copper adsorption-desorption cycles, Biotechnol. Lett. 18 (1995) 531–536.
[22] C. Walther, J. Rothe, M. Fuss, S. Bochner, S. Koltsov, T. Bergmann, Investigation of polynuclear Zr(IV) hydroxide complexes by nanoelectrospray mass-spectrometry combined with XAFS, Anal. Bioanal. Chem. 388 (2007) 409–431.
[23] A.B. Monji, S.J. Ahmadi, F. Zolfonoun, Selective biosorption of zirconium and hafnium from acidic aqueous solutions by rice bran, wheat bran and *Platanus orientalis* tree leaves, Sep. Sci. Technol. 43 (2008) 597–608.
[24] A.F. Esposito, F. Pagnanelli, A. Lodi, C. Solisio, F. Veglio, Biosorption of heavy metals by *Sphaerotilus natans*: An equilibrium study at different pH and biomass concentrations, Hydrometallurgy 60 (2001) 129–141.

[25] J. Tangaromsuk, P. Pokethitiyook, M. Kruatrachue, E.S. Upatham, Cadmium biosorption by *Sphingomonas paucimobilis* biomass, Bioresour. Technol. 85 (2002) 103–105.
[26] Y.-G. Liu, T. Fan, G.-M. Zeng, X. Li, Q. Tong, F. Ye, M. Zhou, W.-H. Xu, Y. Huang, Removal of cadmium and zinc ions from aqueous solution by living *Aspergillus niger*, Trans. Nonferrous. Met. Soc. 16 (2006) 681–686.
[27] G. Blanchard, M. Maunaye, G. Martin, Removal of heavy metals from water by means of natural zeolites, Water Res. 18 (1984) 1501–1507.
[28] S.R. Bai, T.E. Abraham, Studies on the Cr(VI) adsorption-desorption using immobilized fungal biomass, Bioresour. Technol. 87 (2003) 17–26.
[29] Z. Aksu, Equilibrium and kinetic modeling of cadmium(II) biosorption by *C. vulgaris* in a batch system: Effect of temperature, Sep. Purif. Technol. 21 (2001) 285–294.
[30] H.N. Bhatti, R. Khalid, M.A. Hanif, Dynamic biosorption of Zn(II), Cu(II) using pretreated *Rosa gruss an teplitz* (red rose) distillation sludge, Chem. Eng. J. 148 (2009) 434–443.
[31] R. Senthilkumar, K. Vijayaraghavan, M. Thilakavathi, P.V.R. Iyer, M. Velan, Seaweeds for the remediation of wastewaters contaminated with zinc(II) ions, J. Hazard. Mater. B136 (2006) 791–799.
[32] C. Yan, G. Li, P. Xue, Wei, Q. Li, Competitive effect of Cu(II) and Zn(II) on the biosorption of lead(II) by *Myriophyllum spicatum*, J. Hazard. Mater. 179 (2010) 721–728.
[33] M.A. Hanif, H.N. Bhatti, M.A. Ali, M. Asgher, I.A. Bhatti, Heavy metals tolerance and biosorption potential of white rot fungi, Asian J. Chem. 22 (2010) 335–345.
[34] N. Saifuddin, A.Z. Raziah, Removal of heavy metals from industrial effluent using *Saccharomyces cerevisiae* (Baker's Yeast) immobilized in chitosan/lignosulphonate matrix, J. Appl. Sci. Res. 3 (2007) 2091–2099.