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Studies on accumulation of uranium by fungus *Lentinus sajor-caju*

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Abstract

The untreated, heat- and alkali-treated *Lentinus sajor-caju* mycelia were used for the recovery of uranium from aqueous solutions. The effect of pH, temperature, initial concentration of UO_2^{2+} ions and contact time parameters were investigated in a batch system. The particles sizes of the fungal mycelia were ranging from 100 to 200 μm. Biosorption equilibriums were established in about 30 min and the correlation regression coefficients show that the adsorption process can be well defined by the Freundlich equation. The alkali treated form had a high biosorption capacity (378 mg/g) than those of the untreated (268 mg/g) and heat-treated fungal mycelia (342 mg/g). Optimum biosorption was observed at pH 4.5 for all the tested fungal preparations and was independent of temperature (5–35 °C). In addition, the polarity and surface energy of the fungal biomass film preparations were determined by contact angle measurement. The fungal biomass could be regenerated using 10 mM sodium carbonate, with up to 93% recovery. The biosorbents were used in six biosorption–desorption cycles and no considerable loss in the biosorption capacity was observed.

Keywords: White-rot fungus; *Lentinus sajor-caju*; Biosorption; Uranium(VI); Chemical and physical treatment; Contact angle; Surface energy

1. Introduction

Uranium is one of the most important heavy metals because of its chemical toxicity and radioactivity. Excessive amounts of uranium have found their ways into the environment through activities associated with the nuclear industry. The average uranium concentration in the earth's crust is about 3 mg/kg. Uranium forms more than 160 mineral species and accounts for 5% of all known mineral [1]. Uranium biosorption by various microorganisms and related biopolymers are reported in the literature [1–6]. These studies document that various biomass from fungi, yeast, algae, and unicellular bacteria are capable of uptake or binding of uranium greater than 15% of biomass and dry weight [1]. A metal loading capacity of greater than 15% of biomass and dry weight has been defined as an economic threshold for practical applications of biosorption compared to alternative methods such as traditional adsorption, ion exchange, chemical precipitation, solvent extraction, and reverse osmosis [1,8].

Fungi cell walls contain large quantity of polysaccharides and proteins, which offer many functional groups (such as carboxyl, hydroxyl, sulphate, phosphate and amino groups) for binding metal ions [9–16]. Several metal biosorption studies using various strains of white-rot fungus have already identified these groups of fungus as potential bioaccumulator of various metals. Live or physically and chemically inactivated microbial cells can be used to remove heavy metal ions, but maintaining the survivability of the microbial cells during biosorption process is difficult, because they require a continuous supply of nutrients and metal toxicity might take place for microbial cells [17]. Several researchers have also shown that non-living biomass is also able to bind heavy metals effectively. For example, heat inactivated biomass can produce additional binding sites via denaturation of proteins on cell wall structures. Alkali treatment can cause hydrolysis of protein and also deacetylation of chitin into chitosan. It should be that the performance of a microbial biomass depends on its surface properties [18,19]. The chemical structures, the hydrophobic and polar characters of the microbial cells surface are the most important [20]. The latter two can be determined by contact angles measurements.

Lentinus sajor-caju is a white-rot fungus and its biomass may be a good source for removal of toxic heavy metal ions from aqueous solutions. *L. sajor-caju* grows readily on an easily

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acquired carbon sources (i.e., cellulose) and has several extracellular enzymes for bioremediation of various xenobiotics. The purpose of this research was to study the enhancement of the adsorptive capacity of the *L. sajor-caju* biomass for the removal of UO_2^{2+} ions from aqueous solution. For this purpose, *L. sajor-caju* biomass was modified via heat and alkali treatment. The effects of contact time, initial concentration, and pH on the biosorption of UO_2^{2+} ions have been investigated. The changes on the surface properties of *L. sajor-caju* biomass before and after treatments were characterized by contact angle measurements and, the surface free energy parameters of the untreated and treated fungal preparations were calculated from the measured contact angles values using the acid–base method of van Oss [21].

2. Materials and methods

2.1. Microorganism and media

Pure cultures of *L. sajor-caju* (MAFF 430306) were obtained from MAFF Gene Bank, Culture Collection, Kannondai, Tsukuba, Ibaraki, Japan. The growth medium and growth conditions were previously described elsewhere [7]. Inoculates were obtained from 7 days old agar slant culture. The fungal mat (about 0.3 g) was removed and macerated aseptically in 5.0 ml sterile medium using a blender. It was used to inoculate 50 ml of medium in 250 ml flask, and the flasks were incubated on a shaker (150 rpm) for 3 days at 30 °C. After this period, the mycelia harvested by filtration from the growth medium, washed several times with sterile saline solution (0.85%, w/v) and stored at 4 °C until use.

2.2. Treatment of fungal biomass

L. sajor-caju mycelia was inactivated in physiologic saline solution by heating at 90 °C for 20 min and after heat treatment referred as heat-treated biomass. For alkali treatment of fungal biomass 0.1 M NaOH was used at ambient temperature for 6.0 h while continuous stirring at 200 rpm, and hereafter they called alkali-treated fungal biomass. Heat and alkali-treated fungal biomass preparations were then filtered, washed with sterile saline solution, dried in a vacuum oven at 50 °C and powdered to particles of size ranging from 100 to 200 μm.

2.3. Preparation of experimental solutions

Uranium acetate ($UO_2(OCOCH_3)_2{\cdot}2H_2O$) was used for the preparation of stock solution containing 1000 mg uranium/L. Sodium salicylate ($C_6H_4(OH){\cdot}COONa$), analytical grade, was used for the preparation of 10% (w/w) solution. The water used in the following experiments was purified using a Barnstead (Dubuque, IA, USA) ROpure LP reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANO pure organic/colloid removal and ion exchange packed-bed system.

2.4. Biosorption studies

Biosorption of UO_2^{2+} ions from aqueous solutions were studied in batch systems. Effects of pH and temperature of the medium on the biosorption rate and capacity were studied. The effects of the medium pH and temperature on the biosorption capacity of the fungal preparations were investigated at an UO_2^{2+} ions concentration 200 mg/L, in the pH range 2.0–7.0 (which was adjusted with HCl or NaOH at the beginning of the experiment and not controlled afterwards) at 25 °C and at four different temperatures (i.e., 5, 15, 25 and 35 °C). To determine the adsorption capacities of the untreated, heat- and alkali-treated fungal preparations, the initial concentration of UO_2^{2+} ions was changed between 25 and 750 mg/L. After 60 min incubation period, the aqueous phases were separated from the fungal biomass preparations and the concentration of the UO_2^{2+} ions in these phases was determined.

A simple and sensitive spectrophotometric method based on colored complexes with sodium salicylate in aqueous medium was calibrated using UO_2^{2+} standards. A measured volume of UO_2^{2+} solution containing UO_2^{2+} in the 9.25×10^{-3} to 27×10^{-3} mmol range was placed into 50 ml volumetric flasks, followed by 1.0 ml 10% (w/w) sodium salicylate and water to reach up the mark. The absorbance of the solution was measured at 468 nm using an UV/Visible spectrophotometer (Shimadzu Model 1601, Japan). The instrument response was periodically checked with the UO_2^{2+} standard solutions. For each set of data reported, standard statistical methods were used to determine the mean values and standard deviations.

The amount of adsorbed UO_2^{2+} ions per unit fungal biomass (mg metal ions/g dry fungal biomass) was obtained by using the following expression:

$$q = [(C_0 - C)V]/M \quad (1)$$

where q is the amount of UO_2^{2+} adsorbed onto the unit amount of the biomass (mg/g), C_0 and C the concentrations of the UO_2^{2+} ions in the initial solution (mg/L) before and after biosorption, respectively, V the volume of the aqueous phase (L) and M is the amount of the biomass (g). All experiments were repeated three times and results given are the average values. Controls (without biomass) were run simultaneously along with the experimental glasses for all the experiments.

2.5. Desorption/reuse

In order to determine the reusability of the fungal biomass preparations, consecutive adsorption–desorption cycles were repeated six times by using the same biosorbent. Desorption of UO_2^{2+} ions were performed by 10 mM Na_2CO_3 solution. The fungal biomass preparations loaded with UO_2^{2+} ions were placed in the desorption medium and stirred at 200 rpm for 60 min at 25 °C. The final UO_2^{2+} ions concentration in the aqueous phase was determined as described above. Desorption ratio was calculated from the amount of UO_2^{2+} ions adsorbed on the fungal preparations and the final UO_2^{2+} ions concentration in the desorption medium.

2.6. Characterization studies

The surface areas of the untreated and modified fungal biomass samples were measured the Brunauer, Emmett and Teller (BET) method using a surface area apparatus. The dried fungal biomass was coated with gold under reduced pressure and their scanning electron micrographs were obtained using a JEOL (JSM 5600) scanning electron microscope. FT-IR spectra of powdered untreated, heat- and alkali-treated fungal biomass were obtained by using a FT-IR spectrophotometer (Mattson 1000 FT-IR, England). The dry fungal biomass (about 0.1 g) mixed with KBr (0.1 g) and pressed into a tablet form. The FT-IR spectrum was then recorded.

2.7. Contact angle measurements and calculation of surface energy

Contact angles to different test liquids (i.e., water, glycerol and DIM) of all the investigated mycelia film preparations were measured by sessile drop method at 25 °C by using a digital optical contact angle meter CAM 200 (KSV Instruments Ltd., Helsinki, Finland). Both the left and right contact angles and drop dimension parameters were automatically calculated from the digitalized image using CAM 200 software operated under Windows 98. The contact angles for both sites of each drop were measured between the liquid and mycelia film on the glass slide. The measurements were the average of nine contact angles at least operated on three fungal biomass film samples.

The surface free energy parameters of untreated, heat- and alkali-treated and UO_2^{2+}- covered fungal biomass films were calculated using the contact angle data of the probe test liquids. The results were analyzed according to van Oss et al.'s acid–base method [21]. The relevant equations are summarized below.

The total surface free energy, γ^{TOT}, can be divided two components where,

$$\gamma^{TOT} = \gamma^{LW} + \gamma^{AB} \quad (2)$$

γ^{LW} and γ^{AB} are the dispersive and acid–base components of the free surface energy, respectively.

Substituting the appropriate expressions, Eq. (3) is obtained,

$$(1 + \cos\theta)\gamma_l = 2[(\gamma_s^{LW}\gamma_l^{LW})^{1/2} + (\gamma_s^{+}\gamma_l^{-})^{1/2} + (\gamma_s^{-}\gamma_l^{+})^{1/2}] \quad (3)$$

where γ^+ and γ^- refer to proton and electron donating character of acid–base component, respectively.

The known parameter values of three liquids and their contact angles on mycelia sample were used in Eq. (3) and the method equation was solved using CAM 200 software package.

3. Results and discussion

3.1. Characterization of biosorbent systems

The mechanism of UO_2^{2+} biosorption by untreated, heat- and alkali-treated *L. sajor-caju* biomass was elucidated on the basis of biomass treatment; FT-IR, BET, SEM and contact angle studies. Heat treatment can produce additional binding sites via denaturation of proteins on the cell wall structures. Alkali treatment can cause hydrolysis of protein and also deacetylation of chitin. All those changes control adsorption capacity of modified mycelia increased.

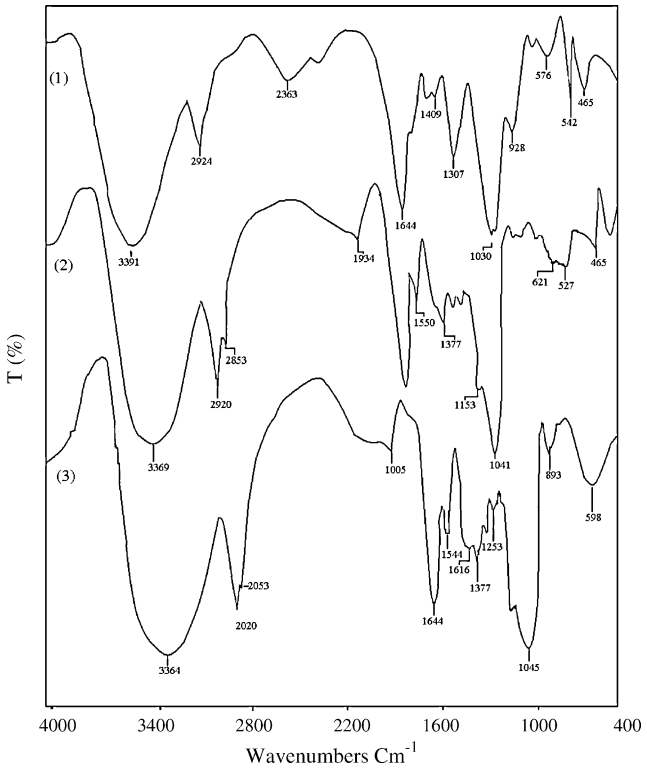

Fig. 1. FTIR spectra: (1) untreated; (2) heat-treated; (3) alkali-treated fungal biomass.

The changes in the functional groups and surface properties before and after physical and chemical treatment are confirmed by FT-IR spectra (Fig. 1) of the fungal preparations. FT-IR-spectra of untreated, heat and alkali treated *L. sajor-caju* confirm the biosorbents heterogeneity and evidence the presence of different characteristics peaks in agreement with the possible presence of amino, carboxylic, hydroxyl and carbonyl groups are presented in Fig. 1. In general, the FT-IR spectra of all the fungal preparations have intense peaks at a frequency level of 3400–3200 cm^{-1} representing –OH stretching of carboxylic groups and also representing stretching of –NH groups. The strong peaks at around 1650 cm^{-1} are caused by the bending of N–H of both chitin and chitosan on the cell wall structure of fungal mycelia. The peaks at around 1900 cm^{-1} are observed in the fingerprint region representing aromatic ring substitution overtones. The peaks at 2920, 1550, 1370 and 1040 cm^{-1} representing C–H stretching vibrations, N–H bending (scissoring), $–CH_3$ wagging (umbrella deformation) and C–OH stretching vibrations, respectively, are due to the several functional groups present on the fungal cell walls. On the other hand, the peaks of N–H stretching vibrations at 1000 cm^{-1} are also masked with the broad band of C–O stretching and the peak at 576 and 542 cm^{-1} representing O–C–O scissoring and C=O bending vibrations are only observed for the untreated fungus and these peaks were not seen for the treated preparations are due to resulted of the

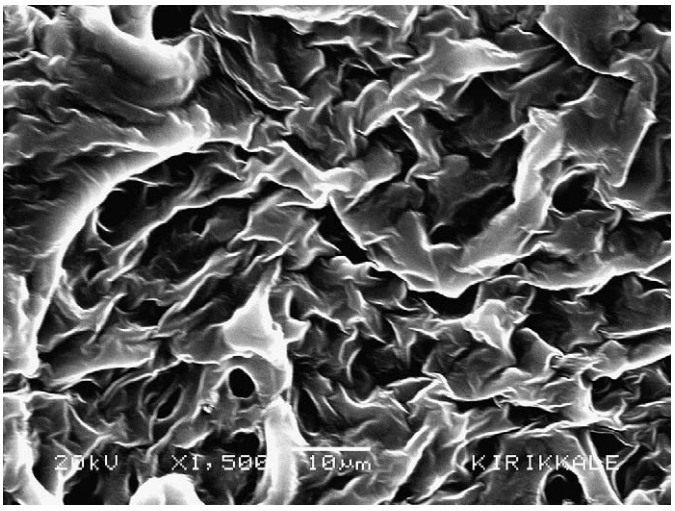

Fig. 2. SEM micrograph of untreated fungal biomass.

removal of lipid compounds after physical and/or chemical treatments. The band between at 510 and 480 cm^{-1} representing C–N–C scissoring is found in polypeptide structure.

The surface areas of the untreated, heat- and alkali-treated fungal biomass preparations were measured by BET method and were found to be 0.545, 0.875 and 0.765 m^2/g fungal biomass, respectively. The surface areas of the fungal biomass were increased in varying extent after physical and chemical treatments compared to untreated counterpart. It should be noted that physical and chemical treatments appear to provide more surface area for the biosorbents would favour higher adsorption capacity for UO_2^{2+} ions due to the increase in the surface area of the modified fungal mycelia.

The surface morphology and bulk structure of untreated *L. sajor-caju* mycelia is exemplified by the scanning electron micrograph in Fig. 2. As clearly seen here, the fungal mycelia have rough and porous surface. This surface property should be considered as a factor providing an increase in the total surface area. In addition, the micropores on the fungal biomass could reduce the diffusional resistance and facilitate mass transfer because of their high internal surface area.

The contact angle values for water, glycerol and diiodomethane on the untreated, heat- and alkali-treated fungal preparations are presented in Table 1. The untreated, heat- and alkali-treated fungal preparations gave quite different contact angle values depending on the surface properties. The variation of the contact angle values after heat and alkali treatment and UO_2^{2+} sorbed shows that the hydrophilicity of the fungal preparations was increased with respect to untreated form. As seen from the table, the untreated mycelium film is hydrophobic $\theta > 90^\circ$ as shown by contact angle measurement, whereas has a lower UO_2^{2+} biosorption capacity than those of the treated fungal preparations. As mentioned earlier, untreated form has more hydrophobic entities on the surface of the cell wall structures and most of them were removed after physical and/or chemical treatments. As a result, the treated fungal preparations donated more available adsorptive side on the surfaces as presented in Table 1, because of they lost their hydrophobic entities during treatment processes. As expected, biosorption capacities of all the treated fungal preparations were increased compared to untreated form.

Physical and chemical treatments of the fungal mycelia alter the surface properties compared to untreated form. Such changes cause contact angles (Table 1) and later surface energy changes too (Table 2). The determined overall surface free energy (γ^{TOT}) calculated using acid–base method of the van Oss' consisting of the sum of the Lifshitz–van der Waals (γ^{LW}) and the acid–base components (γ^{AB}) applies for all investigated fungal preparations at different values. As can be seen from Table 2, all investigated fungal preparations show different acid–base components (γ^{AB}) of the surface energy (γ^{TOT}) due to different chemical structures of the untreated and modified fungal cell surfaces. For all the tested fungal preparations, the γ^{LW} component is highly larger than the acid–base component. It should be noted that, the same trend was observed for the base component (γ^-) was considerably higher than the acid component (γ^+) of the fungal preparations. Heat or base treatments of fungal biomass resulted in increase in the surface polarity and in the polar component of the surface free energy. These parameters were further increased for the uranium-sorbed fungal biomass. As expected, the charge–charge interaction increased between the treated biomass and UO_2^{2+} ions after removal of hydrophobic entities (as shown by contact angle data and calculated surface free energy of the fungal preparations) via physical and chemical methods, and all the treated fungal preparations yielded higher adsorption capacities than that of the untreated form.

3.2. Biosorption rate

Uranium biosorption rate was obtained by following the decrease of the concentration of UO_2^{2+} ions within the adsorption medium with time (Fig. 3). As can be seen from the figure,

Table 1
Contact angles of various test liquids for the tested fungal preparations

Biomass form	Contact angle, θ (°)		
	Water (γ_{erg} = 71.3)	Glycerol (γ_{erg} = 64.0)	DIM (γ_{erg} = 50.8)
Untreated	100.6 ± 0.7	95.8 ± 0.5	57.6 ± 0.8
Heat-treated	82.2 ± 1.1	85.3 ± 1.1	33.1 ± 0.4
Alkali-treated	81.8 ± 0.2	79.3 ± 1.9	31.0 ± 0.3
UO_2^{2+}-biosorbed	66.9 ± 0.7	76.7 ± 1.4	38.7 ± 0.5

γ_{erg}: surface tension of test liquid.

Table 2
Surface free energy parameters (mN/m^2) of the untreated and heat-, acid- and alkali-treated mycelium according to the van Oss et al.

Biomass form	γ^{LW} (mN/m^2)	γ^+ (mN/m^2)	γ^- (mN/m^2)	γ^{AB} (mN/m^2)	γ^{TOT} (mN/m^2)	Polarity[a] (%)
Untreated	29.94	0.81	1.50	2.43	32.37	7.5
Heat-treated	42.99	1.16	3.17	7.33	50.31	14.5
Alkali-treated	44.63	0.98	3.32	7.10	51.73	13.7
UO_2^{2+}-sorbed	40.58	1.14	5.50	12.55	53.40	23.5

[a] Polarity (%) = $(\gamma^{AB} \times 100)/\gamma^{TOT}$.

the UO_2^{2+} removal rate was high at the beginning of biosorption and equilibrium was completely established at about 30 min. UO_2^{2+} ions biosorption increased with time during the first 30 min and remained nearly constant after this period. After equilibrium, the amount of adsorbed UO_2^{2+} ions did not significantly change with time. This trend in binding of UO_2^{2+} ions suggests that the binding may be through interactions with functional groups located on the surface of the biosorbents. Contact time 60 min was maintained for other batch experiments. Note that there are several parameters, which determine the biosorption rate such as stirring rate of the aqueous phase, structural properties both of the metal ions and the biosorbent. For example, the biosorption equilibrium time of UO_2^{2+} on the *Pseudomonas strain* [22] and *Aspergillus fumigatus* [3] was about 60 min.

3.3. Influence of pH and temperature

Most microbial cells such as algae, fungi and bacteria have cell walls composed mainly of polysaccharides and carbohydrate, e.g., cellulose, chitin, chitosan xylan and mannans. These cell wall constituents contain many other side groups, ligands such as amino, carboxyl, carbonyl, alcohol, phosphate and sulphide groups. These groups provide the microbial cell wall with an overall negative charge. The ionic forms of the metal in solution and the electrical charge of the fungal biomass depend on the solution pH [1,10,23]. The variation of equilibrium biosorption for uranium ions at different initial pH values for untreated, heat- and alkali-treated fungal preparations are presented in Fig. 4. As seen from the figure, uranium biosorption by the fungal preparations increased with increasing pH from 2.0 to 4.5 then reached a plateau value at pH between 4.5 and 5.5. Increasing the pH thereafter caused a decrease in biosorption. This can be explained by the charge of the uranyl ions state in solution, which depends on the uranium concentration and on the solution pH. As previously reported, the hydroxo complexes are determined by the following equilibria [5,24]:

$$UO_2^{2+} + 2H_2O \leftrightarrow UO_2(OH)^+ + H_3O^+ \quad (pK = 5.80) \quad (4)$$

$$2UO_2^{2+} + 4H_2O \leftrightarrow (UO_2)_2(OH)_2^{2+} + 2H_3O^+ \quad (pK = 5.62) \quad (5)$$

$$3UO_2^{2+} + 10H_2O \leftrightarrow (UO_2)_3(OH)_5^{2+} + 5H_3O^+ \quad (pK = 15.63) \quad (6)$$

As seen in Fig. 4, at the plateau pH values (i.e., 4.5–5.5) the predominant species of uranium ions in the adsorption medium

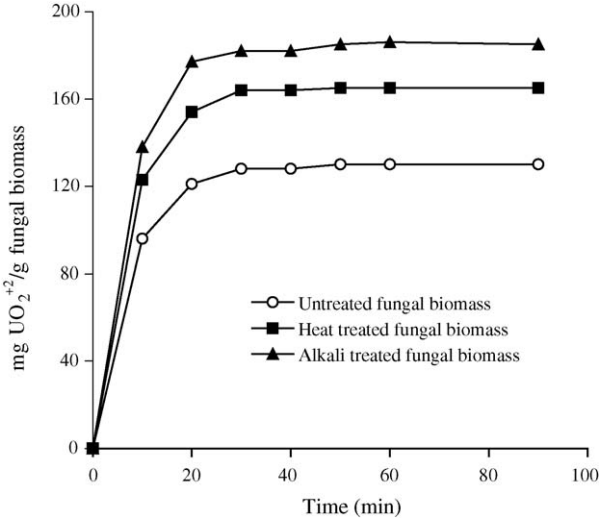

Fig. 3. Biosorption rates of UO_2^{2+} on the untreated, heat- and alkali-treated fungal biomass preparations: biosorption conditions—initial concentration of UO_2^{2+}, 200 mg/L; pH 4.5; temperature, 25 °C; amount of biomass, 1.0 g/L.

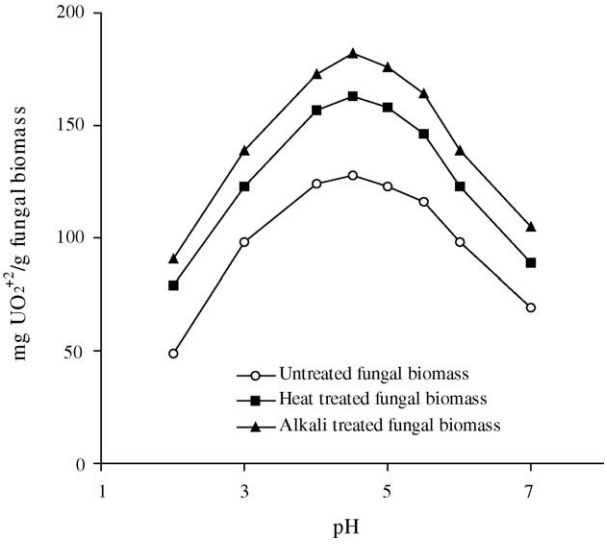

Fig. 4. Effect of pH on the biosorption capacities of the untreated, heat- and alkali-treated fungal biomass preparations for UO_2^{2+}: biosorption conditions—initial concentration of UO_2^{2+}, 200 mg/L; amount of biomass, 1.0 g/L; temperature, 25 °C.

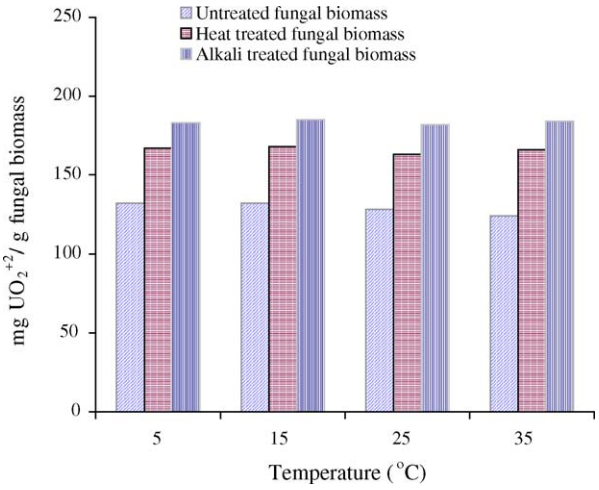

Fig. 5. Effect of temperature on the biosorption capacities of the untreated, heat- and alkali-treated fungal preparations for UO_2^{2+}: biosorption conditions—initial concentration of UO_2^{2+}, 200 mg/L; amount of biomass, 1.0 g/L; pH 4.5.

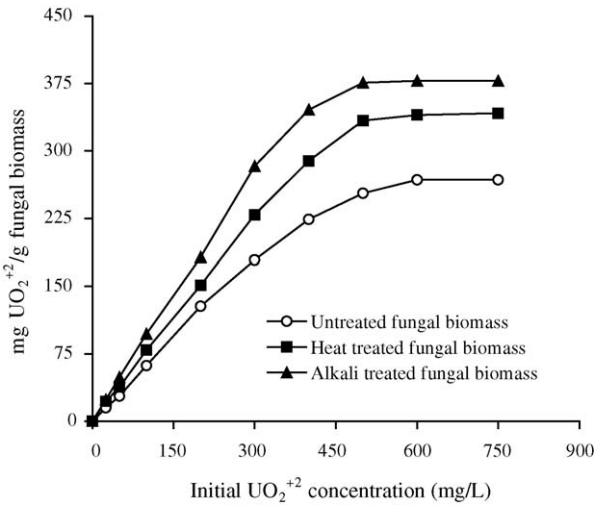

Fig. 6. Biosorption capacities of untreated, heat- and alkali-treated fungal biomass preparations for UO_2^{2+}: biosorption conditions—initial concentration of UO_2^{2+}, 25–750 mg/L; amount of biomass, 1.0 g/L; pH 4.5; temperature, 25 °C.

are UO_2^{2+}, $UO_2(OH)^+$ and $(UO_2)_2(OH)_2^{2+}$. Since solution pH influences fungal biomass surface metal binding sites and as uranium ions are anionic, the interaction of these ions with fungal biomass should be primarily coordinative or electrostatic in nature [2]. The amounts of adsorbed uranium on the dry bases of the untreated, heat- and alkali-treated fungal biomass preparations at pH 4.5 were found to be 128, 163, and 182 mg/g biosorbent, respectively. The best pH for uranium biosorption on the fungal biomass is reported to be between 4.0 and 6.0. For example, the optimal pH for biosorption of uranium by *Rhizopus arrhizus* was in the pH range 4.0–5.0 [6], 5.6–6.0 for *A. niger* [25] and at pH 5.0 for *A. fumigatus* [3]. All of these acidic optimal pH conditions give clue about the responsible ligands (i.e., carboxyl, phosphate and amino groups) could be major complexing sites for uranium ions. These ligands have pK_a values in the pH range from 3.0 to 6.0.

The temperature of the adsorption medium could be important for energy dependent mechanisms in metal biosorption by microorganisms. Energy-independent mechanisms are less likely to be affected by temperature since the process responsible for biosorption is largely physico-chemical in nature. As seen in Fig. 5, the biosorption of uranium ions species by the fungal biomass preparations appears to be temperature independent over the temperature range tested (5–35 °C). Similar results have been reported by other researcher [5,11,26].

3.4. Effect of initial uranium ions concentration on biosorption

The uranium ions biosorption capacities of the untreated, heat- and alkali-treated fungal biomass preparations were presented as a function of the equilibrium concentration of UO_2^{2+} ions within the aqueous medium (Fig. 6). Biosorption capacities of the all tested biosorbents increased with increasing initial concentration of uranium ions in the medium and reached a saturated value. Physical and chemical treatment methods have usually shown an increase in the metal sorption capacity for a variety of microbial species, and several treatment techniques have been used to increase the biosorption capacity of the biosorbent such as heat, acid or alkali treatment [1,27]. As seen from Fig. 6, the amount of biosorbed UO_2^{2+} ions on the untreated, heat- and alkali-treated fungal biomass were 268, 342 and 378 mg/g dry fungal biomass, respectively. The higher UO_2^{2+} ions biosorption capacity was obtained with the alkali-treated biomass may be explained by the increase in the availability of the binding sites (i.e., $-NH_2$ group) by deacetylation of cell wall components chitin into chitosan after treatment with NaOH. It should be noted that the biosorption capacity of the alkali-treated biomass to UO_2^{2+} ions was increased about 41% compared to the untreated fungal biomass. The alkali treatment was observed to be effective in increasing the biosorptive capacity of the fungal biomass. The alkali treatment causes deacetylation of cell wall components chitin to chitosan. It was reported that chitosan–glucan complexes have a high affinity for metal ions. As expected, the biosorption capacity of the heat-treated fungal biomass was also favourably affected and increased about 28% compared to untreated fungal biomass. The biosorption capacity order of the fungal biomass preparations was observed as follows: alkali-treated > heat-treated > untreated fungal biomass preparations. The biosorption capacity (q_{eqex}) defines the equilibrium capacity of fungal biomass for UO_2^{2+} ions under given experimental conditions. The magnitude of the q_{eqex} was found to span to a range of values (268–378 mg/g dry fungal biomass) comparable to other types of biomass earlier reported. For example, maximum biosorption capacity for immobilised heat inactivated fungal biomass of *Phanerochaete chrysosporium* and *Trametes versicolor* was reported as 158 and 309 mg U(VI)/g dry biomass, respectively [2]. *Penicilium chrysogenum* was used for uranium biosorption, and the adsorption capacity of the biosorbent was found to be 388 mg U(VI)/g dry biomass [8]. Maximum biosorption capacity of *R. arrhizus* biomass for U(VI) ions was 146 mg U(VI)/g biomass [6].

Table 3
Langmuir and Freundlich constants and correlation coefficients for biosorption of UO_2^{2+} on the fungal biomass

Biomass form	AA	Langmuir constants			Freundlich constants		
	q_{exp} (mg/g)	q_m (mg/g)	K_d ($\times 10^3$ L/mg)	R^2	n	K_F	R^2
Untreated	268	433	4.48	0.937	1.26	2.99	0.970
Heat-treated	342	420	14.0	0.983	1.59	11.76	0.963
Alkali-treated	378	394	88.2	0.999	2.16	41.26	0.932

3.5. Adsorption isotherms

The adsorption isotherm models used to characterize the interaction of UO_2^{2+} with the fungal biomass preparations. The Langmuir and Freundlich equations are commonly used for describing adsorption equilibrium for water and wastewater treatment applications. The Langmuir model is based on the assumption that maximum adsorption occurs when a saturated monolayer of solute molecules is present on the adsorbent surface, and the energy of adsorption is constant and there is no migration of absorbate molecules in the surface plane. The mathematical description of this model is

$$q = q_m C/(K_d + C) \quad (7)$$

where C and q also show the residual metal concentration and the amount of metal adsorbed on the adsorbent at equilibrium, respectively, $K_d = k_2/k_1$ is the Langmuir constant of the system.

The Freundlich equation is the empirical relationship whereby it is assumed that the adsorption energy of a metal binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. This empirical equation takes the form

$$q = K_F(C)^{1/n} \quad (8)$$

where K_F and n are the Freundlich constants, the characteristics of the system. K_F and n are the indicator of the adsorption capacity and adsorption intensity, respectively. The ability of the Freundlich model to fit the experimental data was examined. For this case, the plot of log C versus log q was employed to generate the intercept value of K_F and the slope of n.

The Langmuir and Freundlich constants calculated from the isotherms with the correlation coefficients are presented in Table 3. The corresponding Scatchard plots gave a non-linear plot for biosorption of UO_2^{2+} ions on the fungal preparations. In other words, a non-linear Scatchard plot indicates the adsorption heterogeneity. Since the adsorption of UO_2^{2+} ions onto the fungal preparations cannot be described in terms of the Langmuir model. The magnitudes of K_F and n (Freundlich constants) show easy separation of UO_2^{2+} ions from aqueous medium and indicate favourable adsorption. The intercept K_F value is an indication of the adsorption capacity of the adsorbent; the slope $1/n$ indicates the effect of concentration on the adsorption capacity and represent adsorption intensity [10,28]. As seen from Table 3 for all experimentally tested untreated, heat- and alkali-treated fungal biomass, n values were found high enough for separation.

3.6. Biosorption kinetics modeling

The kinetics of UO_2^{2+} biosorption on the fungal biomass preparations was determined with two different kinetic models, i.e., the first and second order. The first-order rate equation of Lagergren is one of the most widely used equations for the sorption of solute from a liquid solution [10,28]. It may be represented as follows:

$$dq_t/dt = k_1(q_{eq} - q_t) \quad (9)$$

where k_1 is the rate constant of pseudo-first-order biosorption (min^{-1}) and q_{eq} and q_t denote the amounts of biosorption at equilibrium and at time t (mg/g), respectively. After integration by applying boundary conditions, $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, gives

$$\log(q_{eq}/q_{eq} - q_t) = k_1 t/2.30 \quad (10)$$

A plot of $\log(q_{eq} - q_t)$ against t should give a straight line to onfirm the applicability of the kinetic model. In a true first order process $\log q_{eq}$ should be equal to the intercept of a plot of $\log(q_{eq} - q_t)$ against t.

Ritchie proposed a method for the kinetic adsorption of gases on solids [29,30]. If metal ion biosorption medium is considered to be a second-order reaction, Ritchie equation is

$$1/q_t = (1/k_2 q_{eq} t) + (1/q_{eq}) \quad (11)$$

where k_2 (g/mg/min) is the rate constant of the second-order adsorption.

Table 4
The first-order and second-order kinetics constants for biosorption of UO_2^{2+} on the fungal preparations

Biomass form	Experimental	First-order kinetic			Second-order kinetic		
	q_{eqex} (mg/g)	k_1 ($\times 10^3$ min^{-1})	q_{eq} (mg/g)	R^2	k_2 ($\times 10^1$ g/mg/min)	q_{eq} (mg/g)	R^2
Untreated	268	5.53	1.27	0.902	2.95	284	0.994
Heat-treated	342	4.88	0.98	0.921	3.50	358	0.995
Alkali-treated	378	4.03	0.85	0.954	4.41	391	0.992

A plot of $1/q_t$ versus $1/t$ (Eq. (11)) should give a linear relationship for the applicability of the second-order kinetic. The rate constant (k_2) and adsorption at equilibrium (q_{eq}) can be obtained from the intercept and slope, respectively, and there is no need to know any parameter beforehand.

The comparison of experimental biosorption capacities and the theoretical values estimated from the first- and second-order rate equations and are presented in Table 4. The theoretical q_{eq} values estimated from the first-order kinetic model gave significantly different values compared to experimental values. In the case of second-order kinetic model, comparing the equilibrium capacities, q_{eq}, of the second-order kinetic model with the experimental equilibrium capacities, q_{eqex}, the calculated maximum capacity from the second-order equation is the most accurate, therefore, the second-order kinetic model best described the experimental data.

3.7. Desorption and reuse

The use of a biosorbent in the wastewaters treatment depends not only on the biosorptive capacity, but also on how well the biomass can be regenerated and used again. For repeated use of a biosorbent, adsorbed metal ions should be easily desorbed under suitable conditions. Desorption of the adsorbed UO_2^{2+} ions from the untreated, heat- and alkali-treated fungal biomass preparations were studied in a batch system. The UO_2^{2+} ions adsorbed onto fungal biomass preparations were eluted with 10 mM Na_2CO_3. Maximum amounts (93%) of uranium biosorbed by the biomass could be recovered by addition of 10 mM sodium carbonate. The effectiveness of Na_2CO_3 over mineral acids for desorption of biosorbed uranium ions has been reported previously for various microbial biomass [8].

In order to show the reusability of the biosorbents adsorption–desorption cycle of UO_2^{2+} ions was repeated six times by using the same preparations. In the first cycle, more than 90% biosorbed uranium ions recovered from fungal biomass preparations using 10 mM Na_2CO_3. In the sixth reuse cycle, the fungal biomass preparations reduced their initial adsorption capacity up to 81%. The regeneration of biosorbent shows that the untreated, heat- and alkali-treated *L. sajor-caju* biomass could be used repeatedly for adsorption/desorption of UO_2^{2+} ions.

4. Conclusion

In this study, untreated, heat- and alkali-treated *L. sajor-caju* biomass preparations were used to recover uranium ions from aqueous solutions. Some results were given as follows: the adsorption of uranium ions was quite sensitive to pH of the medium and the maximum biosorption was obtained at acidic pH between 4.5 and 5.5. Temperature has not a favourable effect on biosorption capacity of fungal biomass in the range of 5–35 °C. The higher UO_2^{2+} ions biosorption capacity was obtained by alkali-treated fungal biomass preparation may be due to the changes in biosorptive characteristics of the fungal cell wall constitute as a result of alkali treatment. The biosorption capacities of the untreated, heat- and alkali-treated fungal biomass for uranium ions were 268, 342 and 378 mg/g dry fungal biomass, respectively. The biosorption capacity order of the fungal biomass preparations was observed as follows: alkali-treated > heat-treated > untreated fungal biomass preparations. The biosorption isotherms for the untreated, heat- and alkali-treated fungal biomass were described well by the Freundlich equations. The biosorption of UO_2^{2+} ions on the biosorbents seems to be followed the second-order kinetic equations. These results suggest that untreated and treated *L. sajor-caju* biomasses have potential applications in the recover of metal ions from solutions. Furthermore, as *L. sajor-caju* biomass is an edible biomass, it can be employed for drinking water purification.

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