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Modification of surface properties of *Lentinus sajor-caju* mycelia by physical and chemical methods: evaluation of their Cr^{6+} removal efficiencies from aqueous medium

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Abstract

The hexavalent chromium biosorption onto untreated and heat-, acid- and alkali-treated *Lentinus sajor-caju* mycelia were studied from aqueous solutions. The particles sizes of the fungal mycelia ranged from 100 to 200 μm. The effect of pH, temperature, biosorbent dose, initial concentration of chromium ions, contact time parameters were investigated in a batch system. Biosorption equilibrium was established in about 4 h. The surface charge density of the fungal preparations varied with pH, and the maximum absorption of chromium ions on the fungal preparations were obtained at pH 2.0. The biosorption of chromium ions by the tested fungal preparations increased as the initial concentration of chromium ions increased in the medium. The maximum biosorption capacities of the untreated and heat, HCl- and NaOH-treated fungal biomass were 0.363, 0.613, 0.478 and 0.513 mmol Cr^{6+} per gram of dry biomass, respectively. The correlation regression coefficients and the Langmuir constant values show that the biosorption process can be well defined by Langmuir equation. The chromium adsorption data were analysed using the first- and the second-order kinetic models. The first-order equation is the most appropriate equation to predict the biosorption capacities of all the fungal preparations. In addition, the polarity and surface energy of the untreated and all the modified biomass film preparations were determined by contact angle measurement. All the tested fungal biomass preparations could be regenerated using 0.1 M NaOH solution.

Keywords: White-rot fungus; *Lentinus sajor-caju*; Biosorption; Cr^{6+}; Chemical and physical treatment; Contact angle; Surface energy

1. Introduction

Heavy metals are toxic because they are present as ions in an aqueous system and can be readily absorbed into the human body. Even a very small amount can cause severe physiological or neurological damage. One of the most dangerous metal ions for human life is Cr^{6+} which is found in industrial wastewater because of the extensive use of chromate and dichromate in electroplating, leather tanning, metal finishing, nuclear power plant, textile industries and chromate preparation [1,2]. Chromium, Cr^{6+}, is a powerful carcinogenic agent that modifies the DNA transcription process causing important chromosomic aberrations. The Cr^{6+} may also cause epigastric pain, nausea, vomiting, severe diarrhea, and hemorrhage [3]. Portable waters containing more than 0.05 mg/l of chromium are considered to be toxic [4,5]. Thus, the removal of metal ions from wastewaters has become an important and widely studied area where a number of technologies have been developed over the years. The most important of these methods include reverse osmosis, ion exchange adsorption, chemical precipitation and electro depositions [6]. These methods are highly expensive. Therefore, the use of microbial biomass for removal of toxic heavy metal ions from wastewaters has emerged as an alternative to the

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existing methods as a result of the search low cost, innovative methods [7,8].

"Biosorption" refers to different modes of non-active metal uptake by microbial biomass, where metal sequestration by cells can take place through adsorption, ion exchange, coordination, complexation, etc. The major advantages of biosorption technology are: its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials [9]. Fungal cell walls contain large quantity of polysaccharides and proteins. These biopolymers offer many functional groups, which can bind metal ions such as carboxyl, hydroxyl, sulphate, phosphate and amino groups [10]. The use of untreated and treated microbial biomass for metal ions removal has gained importance. Non-living microbial cells can accumulate heavy metal ions to the same or to a greater extent compared to growing or resting counterpart [9]. The use of non-living microbial cells in industrial application may offer some advantages over living cells, such as lower sensitivity to toxic metal ions concentration and adverse operating conditions [8,9,11]. The performance of a biosorbent depends on its surface properties [12–14]. Among these, the chemical structure, the hydrophobic and polar characters of the microbial cells are the most important [15]. The latter can be determined by contact angle 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. The purpose of this research was to study the enhancement of the adsorptive capacity of the *L. sajor-caju* biomass for the removal of Cr^{6+} ions from aqueous solution. For this purpose, *L. sajor-caju* biomass was modified via heat, acid and alkali treatment. The effects of contact time, solid/liquid ratio, initial concentration, and pH on the biosorption of Cr^{6+} ions have been investigated. The changes on the surface properties of *L. sajor-caju* mycelia 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 [16]. The biosorption of Cr^{6+} ions from aqueous solutions on the untreated and treated fungal biomass under different kinetic and equilibrium conditions is investigated in detail. Finally, elution-reuse of untreated and treated fungal preparations was evaluated. The information gained from these studies will indicate whether the untreated and/or treated biomass of *L. sajor-caju* have the potential to be used for the removal and recovery of Cr^{6+} ions from wastewater.

2. Materials and methods

2.1. Microorganism and media

Pure cultures of *Lentinus 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 [9]. Inoculates were obtained from 7 days old agar slant culture. The fungal mat (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 fungus

Lentinus 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 acid and alkali treatment of fungal biomass 0.1 M HCl and/or NaOH were used, respectively. The fungal biomass was treated with acid or alkali at ambient temperature for 6.0 h while continuous stirring at 200 rpm, and hereafter they called acid- and alkali-treated fungal biomass, respectively. All treated fungal 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. Surface area measurement

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.

2.4. FT-IR spectroscopy

FT-IR spectra of powdered untreated and heat-, acid- and alkali-treated mycelia were obtained by using an FT-IR spectrophotometer (Mattson 1000 FT-IR, England). The dry fungal mycelia (about 0.1 g) mixed with KBr (0.1 g) and pressed into a tablet form. The FT-IR spectrum was then recorded.

2.5. 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 and heat-, acid- and alkali-treated and Cr^{6+} covered mycelia were calculated using the contact angle data of the probe test liquids.

The results was analysed according to van Oss, Good and Chaudury's acid–base method [16]. The relevant equations are summarized below.

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

$$\gamma^{TOT} = \gamma^{LW} + \gamma^{AB} \quad (1)$$

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

Substituting the appropriate expressions, Eq. (2) is obtained,

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

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. (2) and the method equation was solved using CAM 200 software package.

2.6. Biosorption studies

The biosorption of Cr^{6+} ions, on the untreated and heat-, acid- and alkali-treated mycelia from aqueous solution was investigated in batch system. The solutions containing Cr^{6+} ions was prepared from the analytical grade potassium dichromate. A stock solution (1000 mg/l) of Cr^{6+} was obtained by dissolving dried potassium dichromate in Milli-Q water. The range of concentration of Cr^{6+} ions prepared from stock solution. To determine the effect of initial concentration of Cr^{6+} ions on the biosorption rate and capacity on the fungal preparations, the initial concentration of Cr^{6+} ions was varied between 20 and 600 mg/l in the medium.

The effects of the medium pH and temperature on the biosorption capacity of the fungal preparations were investigated in the pH range 1.0–8.0 (which was adjusted with H_2SO_4 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 40 °C). Cr^{6+} ions concentration in each solution (100 mg/l) was prepared in saline solution (7.5 ml) and 25.0 mg fungal preparations was transferred into the medium and agitated magnetically at 200 rpm. The effect of solid/liquid ratio experiments was carried out varying the amount of biosorbent in the adsorption medium at pH 2.0 and the Cr^{6+} concentration was 100 mg/l. At the end of the biosorption, the mycelia were separated from the medium by centrifugation. The concentration of remaining Cr^{6+} ions in the biosorption medium was determined spectrophotometrically at 540 nm by using a double beam UV–vis spectrophotometer (Shimadzu, Tokyo, Japan, Model 1601) after complexation with 1,5-diphenyl carbazide [17]. Before determination of the total quantity of chromium Cr^{6+} in the adsorption medium, Cr^{3+} and Cr^{2+} were converted to Cr^{6+} using $KMnO_4$ [18,19].

The amount of adsorbed Cr^{6+} ions per unit mycelia (mmol metal ions/g dry fungal biomass) was obtained by using the following expression:

$$q = \frac{(C_0 - C)V}{M} \quad (3)$$

where q is the amount of Cr^{6+} adsorbed onto the unit amount of the biomass (mmol/g); C_0 and C are the concentrations of the Cr^{6+} ions in the initial solution (mg/l) and after biosorption, respectively; V the volume of the aqueous phase (l) and M the amount of the biomass (g). Each experiment was repeated three times and results given are the average values.

The percentage removal of chromium was calculated as follows:

$$\%\ \text{removal of } Cr^{+6} = \frac{C_0 - C}{C_0} \times 100 \quad (4)$$

2.7. Reusability test

In order to determine the reusability of biosorbents consecutive biosorption–desorption cycles were repeated five times by using the same fungal preparations. Desorption of Cr^{6+} ions was performed by 0.1 M NaOH solution. Fungal biomass preparations loaded with Cr^{6+} ions was placed in desorption medium and stirred at 200 rpm for 4 h at 25 °C. The final Cr^{6+} concentration in the aqueous phase was determined by using a spectrophotometer as described above. After each cycle of adsorption–desorption, biosorbents was washed with saline solution and reconditioned for adsorption in the succeeding cycle. Desorption ratio was calculated from the amount of metal ions adsorbed on the biomass preparations and the final Cr^{6+} ion concentration in the adsorption medium. Desorption ratio was calculated from the following equation:

$$\text{Desorption ratio} = \frac{\text{amount of } Cr^{+6} \text{ desorbed}}{\text{amount of } Cr^{+6} \text{ adsorbed}} \times 100 \quad (5)$$

3. Results and discussion

3.1. Characterisation of biosorbent systems

Fungal biomass cell walls contain chitin, chitosan, proteins, lipids, polyuronides and melanin have been shown to sequester metal ions [15,17]. The mechanism of Cr^{6+} biosorption by untreated and heat-, acid- and alkali-treated *L. sajor-caju* biomass was elucidated on the basis of biomass treatment, BET, FT-IR and contact angle studies. It should be noted that Cr^{6+} behaves as an oxy-anion (CrO_4^{2-} or $Cr_2O_7^{2-}$) in aqueous medium, according to aqueous solution chemistry of chromium. Therefore, it may not bind to negatively charged functional groups on the biomass surface such as carboxylate, phosphate and sulphate, because of the respective charge repulsion. The amino groups of the major cell wall components (i.e., hexosamines and proteins) of the fungal biomass are protonated at low pH (i.e., pH 1.0–2.0)

and the negatively charged chromate ions become electrostatically attracted to the positively charged amine groups of the fungal cell wall [20]. Heat treatment can produce additional binding sites via denaturation of proteins in cell wall structures. Acid treatment can cause degradation of protein and hexoseamine in the cell wall structures and can increase number of binding sites (i.e., $–NH_2$), which is more easily protonated at adsorption pH 2. Alkali treatment can cause hydrolysis of protein and also deacetylation of chitin. All those changes control adsorption capacity of modified mycelia increased.

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 and heat-, acid- 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

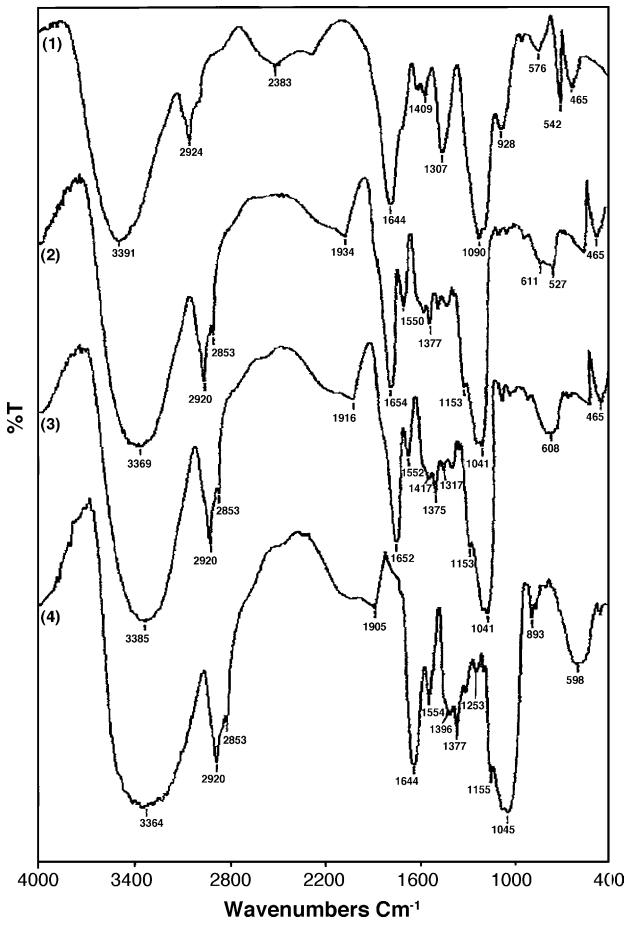

Fig. 1. FT-IR spectra: (1) untreated; (2) heat-treated; (3) acid-treated; (4) alkali-treated fungal biomass.

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 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 and heat-, acid- and alkali-treated fungal biomass preparations were measured by BET method and were found to be 0.545, 0.875, 1.23 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 Cr^{6+} ions due to the increase in the surface area of the modified fungal mycelia.

The variation of the wetting force is extremely sensitive to the surface characteristics since it reflects the effect of functional groups in a surface layer thick (less 10 Å) and in direct contact with the liquid phase [21]. The contact angle values for water, glycerol and diiodomethane on the untreated and treated-fungal preparations are presented in Table 1. The untreated and heat-, acid- and alkali-treated fungal preparations gave quite different contact angle values depending on the surface properties. The highest contact angles were obtained with water. The variation of the contact angle values after heat, acid and alkali treatment and Cr^{6+} coverage show that the hydrophilicity of the fungal preparations is 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 Cr^{6+} adsorption 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

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

Biomass form	Water θ (°) (γ_{erg} = 71.3)	Glycerol θ (°) (γ_{erg} = 64.0)	DIM θ (°) (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
HCl-treated	72.2 ± 1.3	81.1 ± 0.8	33.8 ± 0.7
NaOH-treated	81.8 ± 0.2	79.3.1 ± 1.9	31.0 ± 0.3
Cr^{6+}-sorbed	77.3 ± 0.3	76.9 ± 0.7	41.4 ± 0.6

γ_{erg}: surface tension of test liquid.

changes cause contact angles (Table 1) and later surface energy changes too (Table 2). The determined overall surface free energy (γ^{Total}) 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^{Total}) 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. As expected, the highest γ^{AB} value was observed for the acid-treated fungal biomass could be due the degradation of acid labile cell wall components into oligomers (i.e. chitin and chitosan). Therefore, the polarity of the acid-treated fungal mycelium increased up to 22.6% (Table 2). Heat, acid and/or base treatments of fungal biomass resulted in increase in the surface polarity and in the polar component of the surface free energy. As expected, the charge–charge interaction increased between the treated biomass and Cr^{6+} ion species 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

Chromium biosorption rate was obtained by following the decrease of the concentration of Cr^{6+} ions within the adsorption medium with time (Fig. 2). As can be seen from the figure, the Cr^{6+} removal rate was high at the beginning of biosorption and equilibrium was completely established at about 4 h. Cr^{6+} ions biosorption increased linearly with time during the first 2 h and remained nearly constant after 4 h. After this equilibrium period, the amount of adsorbed Cr^{6+} ions did not significantly change with time. This trend in binding of Cr^{6+} ions suggests that the binding may be through interactions with functional groups located on the surface of the biosorbents. Note that there are several parameters, which determine the biosorption rate such as stirring rate of the aqueous phase, structural properties both of the support and the biosorbent. Therefore, it is too difficult to compare the biosorption time reported in the literature for other biosorbents. For example, the biosorption equilibrium time of Cr^{6+} on the dead and immobilized biomass of *Rhizopus arrhizus* was 2 h [22].

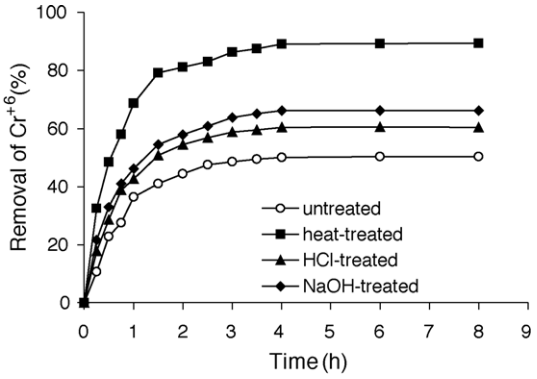

Fig. 2. Biosorption rates of Cr^{6+} on the untreated and heat-, acid- and alkali-treated fungal biomass preparations: biosorption conditions: initial concentration of Cr^{6+}: 100 mg/l; pH: 2.0; temperature: 25 °C.

3.3. Influence of biosorbent dose

One of the parameters that strongly affect the biosorption capacity is the concentration of the biosorbent in the liquid phase. The influence of the biosorbent dose on the biosorp-

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 forms	γ^{LW} (mN/m^2)	γ^+ (mN/m^2)	γ^- (mN/m^2)	γ^{AB} (mN/m^2)	γ^{Total} (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
HCl-treated	43.08	1.37	4.59	12.59	55.67	22.6
NaOH-treated	44.63	0.98	3.32	7.10	51.73	13.7
Cr^{6+}-sorbed	38.88	0.49	3.33	3.23	42.11	7.7

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

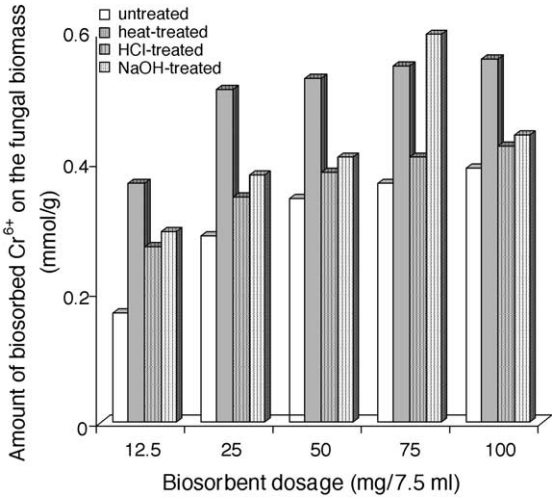

Fig. 3. Effect of biosorbent dosage on the removal of chromium by the untreated and heat-, acid- and alkali-treated fungal biomass preparations: biosorption conditions: initial concentration of Cr^{6+}: 100 mg/l; pH: 2.0; temperature: 25 °C.

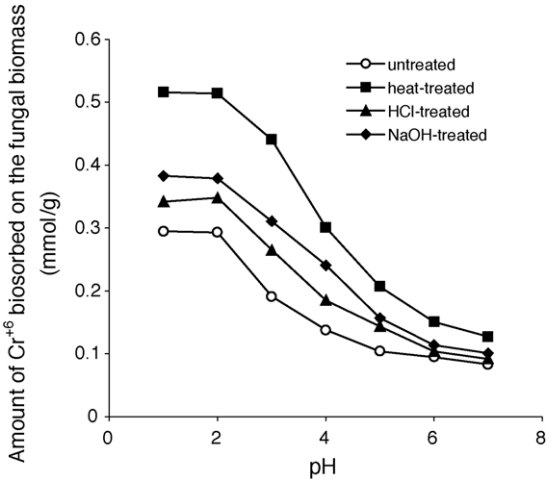

Fig. 4. Effect of pH on the biosorption capacities of the untreated and heat-, acid- and alkali-treated fungal biomass preparations for Cr^{6+}: biosorption conditions: initial concentration of Cr^{6+}: 100 mg/l; amount of mycelia 25 mg/7.5 ml reaction medium; temperature: 25 °C.

tion capacity of the untreated and treated fungal biomass was studied for an initial concentration of Cr^{6+} of 100 mg/l and the biosorbent dose was varied between 12.5 and 100 mg in 7.5 ml biosorption medium (Fig. 3). For all the fungal preparations, the increase in biosorbent dose from 12.5 to 25.0 mg/7.5 ml resulted in a rapid increase in removal of Cr^{6+} ions. Further, increment the biomass dose in the adsorption medium did not provide sufficient improvement in the biosorption efficiency of Cr^{6+} ions. Therefore, the proper fungal biomass dose was selected as 25.0 mg/7.5 ml for the rest of the experimental studies. The experimental data also show that the treated fungal preparations have higher level of performance for removal of Cr^{6+} from aqueous medium than those of untreated form. Several researchers reported that the increase in the percentage removal with increase in the adsorbent dosage is due to the increase in the number of adsorption sites [23].

3.4. Influence of pH and temperature

Fig. 4 shows that biosorption of Cr^{6+} ions by untreated and the treated fungal biomass preparations decreased when the medium pH increased. As seen from the figure, the maximum biosorption of Cr^{6+} ions on the untreated and heat-, acid- and alkali-treated biomass were observed at pH 1.0 and 2.0. It should be noted that the distribution of Cr^{6+} ions species is dependent on both the total concentration of Cr^{6+} and pH of the aqueous solution. The oxy-anions of chromium are know to exist in the following equilibrium [24]:

$$H_2CrO_4 \rightleftharpoons HCrO_4^- + H^+, \quad k_1 = 1.21 \tag{6}$$

$$CrO_7^{2-} + H_2O \rightleftharpoons 2HCrO_4^-, \quad k_2 = 35.5 \tag{7}$$

$$HCrO_4^- \rightleftharpoons CrO_4^{2-} + H^+, \quad k_3 = 3 \times 10^{-7} \tag{8}$$

$$HCrO_7^- \rightleftharpoons CrO_7^{-2} + H^+, \quad k_4 = 0.85 \tag{9}$$

Since the distribution of anionic species of Cr^{6+} is pH dependent, this could be the main variable for removal of Cr^{6+} ions by fungal biomass. The fungal cell wall is mainly composed of polysaccharides (i.e. chitin and chitosan), some of which may have associated proteins, with other components including lipids and melanins [15]. These bio-macromolecules on the fungal cell wall components have several functional groups (such as, amino, carboxyl, thiol, sulfydryl and phosphate groups). The metal biosorption depends on the protonation or unprotonation of these functional groups on the cell wall. The ionic forms of the metal in solution and the electrical charge of the fungal biomass depend on the solution pH. At acidic pH (i.e., 1.0–2.0), protonation of amino groups of the fungal cell wall components enhanced the biosorption capacities of the biosorbents to Cr^{6+} ions. The increased binding of Cr^{6+} ions at low pH can be explained due to the electrostatic binding to positively charged groups such as amines of chitosan in the fungal cell wall components [7,13,24–26]. The maximum Cr^{6+} biosorption was observed at pH 2.0 for various microbial biomasses such as *Rhizopus nigricans* [5], *Streptococcus equisimilis*, *Saccharomyces cerevisiae*, *Aspergilus niger* [27] and *Rhizopus arrhizus* [28]. The amount of adsorbed chromium species on the dry bases of the untreated and heat-, acid- and alkali-treated fungal biomass preparations at pH 2.0 were found to be 0.219, 0.514, 0.348 and 0.379 mmol/g biosorbent, respectively.

The temperature of the adsorption medium could be important for energy dependent mechanisms in metal biosorption by microbial cells. Mostly adsorption is an exothermic process, whereas some examples of endothermic adsorption have also been reported [26]. The biosorption of Cr^{6+} by the biosorbents appears to be temperature dependent over the temperature range tested (Fig. 5). From 5 to 40 °C, the

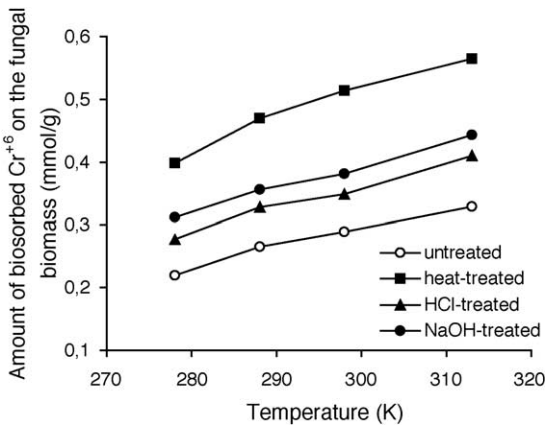

Fig. 5. Effect of temperature on the biosorption capacities of the untreated and heat-, acid- and alkali-treated fungal preparations for Cr^{6+}: biosorption conditions: initial concentration of Cr^{6+}: 100 mg/l; amount of mycelia 25 mg/7.5 ml reaction medium; pH: 2.0.

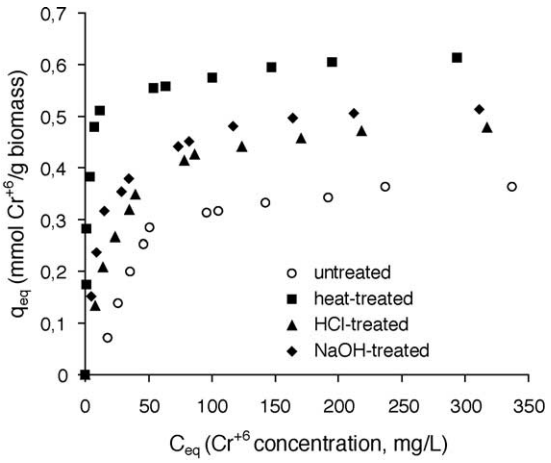

Fig. 6. Biosorption capacities of untreated and heat-, acid- and alkali-treated fungal biomass preparations for Cr^{6+}: biosorption conditions: amount of fungal biomass: 25 mg/7.5 ml reaction medium; pH: 2.0; temperature: 25 °C; initial concentration of Cr^{6+}: 20–600 mg/l.

biosorption capacities of all the tested fungal biomass preparations for Cr^{6+} ions increased between 1.4- and 1.5-fold. The increase in Cr^{6+} biosorption with increasing temperature is due to either higher affinity of sites for Cr^{6+} or an increase in binding sites on relevant biosorbent surfaces as a result of reorientation of cell wall components of the fungal mycelium. Increased biosorption at higher temperature also suggests for the possibility of formation of some coordinate type of bond between chromium atom of dichromate ion and electron rich donor atoms of fungal biomass. Similar observations have also reported by the other researchers [24,27,29–31].

3.5. Effect of initial Cr^{6+} concentration on biosorption

The Cr^{6+} ions biosorption capacities of the untreated and treated fungal biomass preparations were presented as a function of the equilibrium concentration of Cr^{6+} ions within the aqueous medium (Fig. 6). Biosorption capacities of the all tested biosorbents increased with increasing initial concentration of chromium 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 [32]. As seen from Fig. 6, the amount of biosorbed Cr^{6+} ions on the untreated and heat-, acid- and alkali-treated fungal biomass were 0.363, 0.613, 0.478 and 0.513 mmol/g biosorbents, respectively. The higher Cr^{6+} ions biosorption capacity was obtained with the heat-treated biomass may be explained by the increase in the availability of the binding sites by fixing the soluble protein in the cell wall after denaturation with heat. It should be noted that the biosorption capacity of the heat-treated biomass to Cr^{6+} ions was about 1.7 times higher than that of the untreated form. The fungal biomass was treated by acid solution to evaluate the potential for altering the biomass surface for adsorption enhancement. The acid-treated biomass enhanced 1.3-fold Cr^{6+} biosorption capacity compared to the untreated form. The acid treatment of the fungal biomass results in not only a physical cleaning or washing-out, but also some chemical transformation, such as denaturation of the protein molecules. The acid treatment may also degrade to some extent polysaccharide compounds of the fungal cell wall, and therefore produce additional available binding sites (amino group), which is more easily protonated at adsorption pH 2. 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 [17]. As expected, the biosorption capacity of the alkali-treated fungal biomass was also favourably affected and increased approximately 1.4-fold with respect to untreated form. The biosorption capacity order of the fungal biomass preparations was observed as follows: heat-treated > base-treated > acid-treated > untreated form. The biosorption capacity (q_{eqex}) defines the equilibrium capacity of fungal biomass for Cr^{6+} ions under given experimental conditions. The magnitude of the q_{eqex} was found to span to a range of values (0.363–0.613 mmol/g biomass) comparable to other types of biomass earlier reported (Table 3).

3.6. Adsorption isotherms

The degree of biosorption of a metal ion on a biosorbent has been found to be a function of the equilibrium metal-ion concentration in solution at constant pH and temperature conditions. The adsorption isotherm models used to characterize the interaction of Cr^{6+} with the fungal preparations. The single-solute adsorption isotherm models of Langmuir and Freundlich, which are widely used to analyse data for water and wastewater treatment applications have been shown to describe the biosorption equilibrium. The Langmuir model

Table 3
Comparison between the Cr^{6+} ions maximum biosorption capacity by *Lentinus sajor-caju* biomass and other microbial biomass found in the literature

Biomass	Biosorption conditions			q_{eqex} (mmol/g)	Reference
	pH	T (°C)	C_i (mg/l)		
Chlorella vulgaris	2.0	25	25–250	0.461	[10]
Spirogyra sp.	2.0	18	1–25	0.282	[13]
Rhizopus nigrican	2.0	30	50–400	0.308	[14]
Rhizopus arrhizus	2.0	25	50–300	0.455	[22]
Basillus circulans	2.7	28	50–200	0.663	[33]
Basillus megaterium	2.7	28	50–200	0.615	[33]
Zoogloea ramigera	2.0	25	125–150	0.162	[34]
Dunaliella sp. 1	2.0	25	50–250	0.725	[35]
Dunaliella sp. 2	2.0	25	50–250	0.532	[35]
Lentinus sajor-caju (untreated)	2.0	25	20–600	0.363	This work
Lentinus sajor-caju (heat-treated)	2.0	25	20–600	0.613	This work

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 [36,37]. The mathematical description of this model is

$$q = \frac{q_m C}{K_d + C} \tag{10}$$

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 [26,37]. This empirical equation takes the form:

$$q = K_F(C)^{1/n} \tag{11}$$

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 adsorption constants calculated from the corresponding isotherms with the correlation coefficients are presented in Table 4. The correlation coefficients show that the adsorption process could be described by the Langmuir equation. The Langmuir constant (q_m) values were fit the experimental data.

On the other hand, the magnitudes of K_F and n (Freundlich constants) show easy separation of Cr^{6+} 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 [38,39]. As seen from Table 4 for all experimentally tested untreated and treated fungal biomass, n values were found high enough for separation.

3.7. Biosorption kinetics modeling

The kinetics of Cr^{6+} biosorption on the fungal biomass preparations was determined with three different kinetic models i.e. the first-, second-order equations and intra-particle diffusion models. The first-order rate equation of Lagergren is one of the most widely used equations for the sorption of solute from a liquid solution [40]. It may be represented as follows:

$$\frac{dq_t}{dt} = k_1(q_{eq} - q_t) \tag{12}$$

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 (mmol/g), respectively. After integration by applying boundary conditions, $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, gives:

$$\log\left(\frac{q_{eq}}{q_{eq} - q_t}\right) = \frac{k_1 t}{2.30} \tag{13}$$

A plot of log $(q_{eq} - q_t)$ against t should give a straight line to confirm 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 [41]. If metal ion biosorption medium is considered to be a second-order reaction, Ritchie equation is:

$$\frac{1}{q_t} = \frac{1}{k_2 q_{eq} t} + \frac{1}{q_{eq}} \tag{14}$$

where k_2 (g $mmol^{-1}$ min^{-1}) is the rate constant of the second-order adsorption.

Table 4
Isotherm models constants and correlation coefficients for biosorption of Cr^{6+} ions from aqueous solutions

Biomass form	Experimental q_{eqex} (mmol/g)	Langmuir constant			Freundlich constant		
		q_m (mmol/g)	K_d ($\times 10^4$ M)	R^2	K_F	n	R^2
Untreated	0.363	0.425	8.61	0.989	0.046	2.20	0.921
Heat-treated	0.613	0.614	0.67	0.998	0.157	5.93	0.915
Acid-treated	0.478	0.511	3.86	0.999	0.107	3.08	0.939
Alkali-treated	0.513	0.535	2.51	0.999	0.129	3.77	0.942

Table 5
The first-order and second-order kinetics constants for biosorption of Cr^{6+} on the fungal preparations

Biomass form	Experimental q_{eqex} (mmol/g)	First-order kinetic			Second-order kinetic		
		k_1 ($\times 10^2$ min^{-1})	q_{eq} (mmol/g)	R^2	k_2 ($\times 10^2$ g/mmol/min)	q_{eq} (mmol/g)	R^2
Untreated	0.363	1.93	0.323	0.966	3.39	0.400	0.994
Heat-treated	0.613	1.85	0.571	0.994	2.39	0.714	0.993
Acid-treated	0.478	1.98	0.470	0.998	1.57	0.662	0.996
Alkali-treated	0.513	1.91	0.510	0.993	1.70	0.684	0.997

A plot of $1/q_t$ versus $1/t$ (Eq. (10)) 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 intra-particle diffusion model proposed by Weber and Morris [42], the initial rate of intra-particular diffusion is calculated by linearalisation of the curve $q = f(t^{0.5})$:

$$q = K_i t^{0.5} \tag{15}$$

where q is the amount of adsorbed metal ion on the mycelia at time t (mmol/g), t the time (s) and K_i the diffusion coefficient in the solid (mmol/g s$^{1/2}$). K_i has been determined by a plot $q = f(t^{0.5})$ taking account only of the initial period.

The comparison of experimental biosorption capacities and the theoretical values estimated from the first- and second-order rate equations and are presented in Table 5. The theoretical q_{eq} values estimated from the second-order kinetic model gave significantly different values compared to experimental values. In the case of first-order kinetic model, comparing the equilibrium capacities, q_{eq}, of the first-order kinetic model with the experimental equilibrium capacities, q_{eqexp}, the calculated maximum capacity from the first-order equation is the most accurate, therefore, the first-order kinetic model best described the experimental data.

The first-order rate constant of the biosorption reaction (k_1) is expressed as a function of temperature by the following Arrhenius type relationship:

$$k_1 = A_0 \exp\left(-\frac{E_a}{RT}\right) \tag{16}$$

where A_0 is the frequency factor, E_a the activation energy of biosorption, R the gas constant and T the temperature of aqueous medium. When $\ln k_1$ versus $1/T$ is plotted a straight line with slope $-E_a/R$ is obtained. The magnitude of activation energy may give idea about the type of adsorption (i.e., physical and chemical) [43]. In physical adsorption the equilibrium is usually rapidly attained. The activation energy for physical adsorption is usually no more than 4.2 kJ/mol, since the forces involved in physical adsorption are weak. On the other hand, chemical adsorption is specific and involves forces much stronger than in physical adsorption. Chemical adsorption means that the rate varies with temperature according to finite activation energy in the Arrhenius equation.

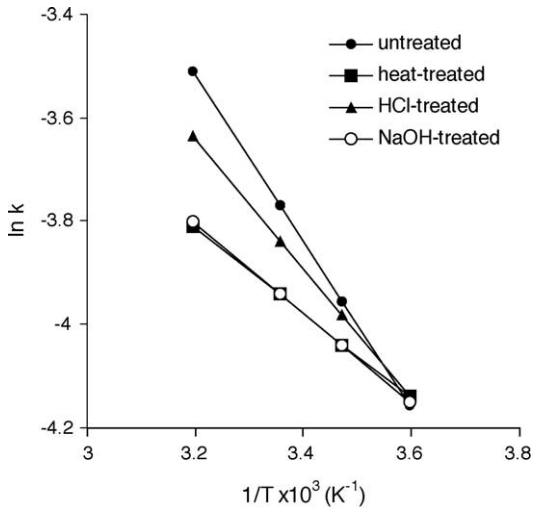

Fig. 7. Arrhenius ($\ln k_1$ vs. $1/T$) plot for untreated and heat-, acid- and alkali-treated fungal biomass preparations.

The E_a values were calculated from $\ln k_1$ versus $1/T$ linear plot for the untreated and heat-, acid- and alkali-treated fungal biomass with high correlation coefficients (R^2) of 0.975, 0.975, 0.967 and 0.996, respectively (Fig. 7). The activation energies (E_a) for the biosorption of Cr^{6+} ions onto the untreated and heat-, acid- and alkali-treated fungal biomass were found to be 13.34, 5.89, 10.36 and 7.22 kJ mol^{-1}, respectively. These values are of the same magnitude as the activation energy of chemical adsorption. These results indicated that Cr^{6+} biosorption on the untreated and treated fungal preparations are endothermic and involve chemical adsorption.

The K_i values are calculated for intra-particle model and tabulated in Table 6 for all the tested fungal biomass preparations. The low values of K_i suggest that the intra-particle diffusion is negligible, and the biosorption process take place on the surface of untreated and treated fungal biomass preparations.

Table 6
Values of K_i for the fungal preparations

Biosmass form	K_i (mmol/g s$^{1/2}$)
Untreated	0.020
Heat-treated	0.032
HCl-treated	0.023
NaOH-treated	0.024

Table 7
Cr^{6+} ions biosorption capacity of the untreated and heat-, acid- and alkali-treated fungal biomass preparations after repeated biosorption/desorption cycle

Cycle number	Untreated biomass		Heat-treated biomass		Acid-treated biomass		Alkali-treated biomass	
	Biosorption (mmol/g)	Desorption (%)	Biosorption (mmol/g)	Desorption (%)	Biosorption (mmol/g)	Desorption (%)	Biosorption (mmol/g)	Desorption (%)
1	0.289	93	0.514	96	0.349	98	0.381	93
2	0.287	91	0.508	92	0.341	94	0.374	95
3	0.285	94	0.499	94	0.326	92	0.365	97
4	0.275	92	0.501	95	0.326	96	0.358	92
5	0.282	92	0.496	92	0.322	93	0.361	96

Biosorption conditions: initial concentration of Cr^{6+} ions: 100 mg/l; amount of *Lentinus sajor-caju* biomass 25 mg/7.5 ml reaction medium; temperature: 25 °C; pH: 2.0.

3.8. 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 Cr^{6+} ions from the untreated and treated fungal biomass preparations were studied in a batch system. The Cr^{6+} ions adsorbed onto fungal biomass preparations were eluted with 0.1 M NaOH. In order to show the reusability of the biosorbents adsorption–desorption cycle of Cr^{6+} ions was repeated five times by using the same preparations (Table 7). The adsorption capacities for all the tested untreated and treated fungal preparations did not noticeably change (only a maximum 6% change was observed) during the repeated adsorption–desorption operations. The regeneration of biosorbent shows that the untreated and heat-, acid- and alkali-treated *L. sajor-caju* biomass could be used repeatedly without significantly loosing their biosorption capacities for Cr^{6+} ions.

4. Conclusion

As conventional methods for removing heavy metal ions from wastewaters are very expensive and not sufficient at low metal ions concentration, therefore, alternative methods should be considered. Food and industrial process can provide a cheap and constant supply of fungal biomass or the fungal biomass can be cultured using inexpensive growth media and unsophisticated culture techniques. The products of the above processes can be used successfully in the selective removal of the hexavalent form of chromium ions from solutions. Some pre-treatments were done to increase the biosorption capacity of the fungal biomass and among the treatment methods, heat treatment was found to be most efficient method on the others to increase the biosorption capacity for removal of Cr^{6+} ions from aqueous solution. As expected, heat, acid and/or alkali treatment of fungal biomass caused surface properties change. Such changes cause contact angle and later surface energy changes too. All those changes control the biosorption capacity of modified fungal biomass increased. The adsorption of chromium ions was quite sensitive to pH of the aqueous solution and the maximum biosorption was obtained at acidic pH 1.0 and 2.0. The higher Cr^{6+} ions biosorption capacity was obtained by heat-treated fungal biomass preparation may be due to the changes in biosorptive characteristics of the fungal cell wall constitute as a result of heat treatment. Temperature also has a favourable effect on biosorption in the range of 5–40 °C. The biosorption isotherms for the untreated and treated fungal biomass were described well by the Langmuir equations. The biosorption of Cr^{6+} ions on the biosorbents seems to be followed the pseudo-first-order biosorption kinetics. Since the Cr^{6+} ions binding capacity of *L. sajor-caju* was found to be most promising further study using real wastewater with presence of other toxic substance are proposed.

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