Sorption Performance of Live and Heat-Inactivated Loofa-Immobilized *Phanerochaete chrysosporium* in Mercury Removal from Aqueous Solution

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ABSTRACT: *The sorption behavior of loofa-immobilized Phanerochaete chrysosporium mycelia in two forms, Live (L) and Heat-Inactivated (HIA), was studied for the removal of Hg^{2+} ions from aqueous solution. Using the Langmuir isotherm, the two key parameters for the sorption performance, q_m and the coefficient b, were obtained; the q_m values for Hg^{2+} ions were 72.46 mg/g and 92.59 mg/g and the b coefficients were 0.073 L/mg and 0.114 L/mg for the L and HIA biosorbents, respectively. Using the Freundlich isotherm, the values of k_F were determined as 13.28 and 21.30, and the values of the coefficient n were 3.22 and 3.51 for the L and HIA biosorbents, respectively. Although the biosorption data were well fitted by both the Langmuir and Freundlich models, the Langmuir isotherm gave a better fit, with a higher correlation coefficient than the Freundlich model. Moreover, the essential characteristic of the Langmuir isotherm model, described as the separation factor, was indicative of the favorable adsorption of Hg^{2+} onto both of the test biosorbents ($0<R<1$). A pseudo-second-order rate equation, as suggested by Lagergren and modified by Ho and Mckay, was used for the kinetic analysis, and the resulting values of K_{2ads} were 1.16×10^{-3} g/mg·min and 1.08×10^{-3} g/mg·min for the L and HIA biosorbents, respectively. Regenerating the biosorbents was possible using hydrochloric acid to leach the sequestered mercury ions, providing an easy way to reduce the cost of the process. The prevailing criteria in the industrial selection and use of biosorbents were satisfactorily met by this system.*

KEY WORDS: *Phanerochaete chrysosporium, Loofa sponge, Immobilization, Mercury ions, Langmuir adsorption isotherm, Biosorption kinetics.*

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INTRODUCTION

The persistent problem of heavy metals in the environment, mainly due to large-scale industrial activities, poses a real threat to human health and natural ecosystems. Mercury is introduced to environment from a variety of sources and has the potential to be converted by abiotic processes and microorganisms into more toxic organomercury forms such as methylmercury chloride and ethylmercury chloride [1]. A variety of physicochemical methods for removing metal ions from industrial effluents, such as ion exchange, chemical precipitation, reverse osmosis and evaporative recovery, have been researched and generally found to be expensive and/or inefficient; for example, difficulties usually exist in the handling of solid waste generated during industrial waste treatment [2]. Economic considerations and practicability of a given technique are among the major factors in decision making by researchers or authorities in performing a selected waste-treatment process. The presence of a mercury-resistance operon in certain microorganisms, as with other operons in cellular systems, acts as a coordinated unit of gene expression, which by synthesizing appropriate proteins gives microbes the abilities required to survive and grow in the presence of the toxic metal (i.e., mercury) [3]. The mercury-resistance characteristic of these microbes is a well-studied mechanism of metal resistance; the process consists of transportation of Hg^{2+} into the cell via the MerT transporter protein and intracellular conversion to non-toxic volatile elemental mercury by mercuric reductase [4].

The current popularity of the use of white-rot fungi in biotechnology is mainly due their peroxidase-type enzyme activities, capable of catalyzing the degradation of the lignin heterobiopolymer of woody plants as well as a variety of organic pollutants with structures similar to lignin [5]. Manganese is an essential heavy metal required for the enzymatic activity of these fungal peroxidase (i.e., manganese peroxidase, MnP) although high concentrations of heavy metals are toxic, affecting morphological and physiological characteristics of the fungi [6]. Besides the action of peroxidases, the adsorption and accumulation of metals by fungi, especially those with a mycelial growth habit and possessing sufficient mechanical strength, provides unique opportunities for using these fungi as biosorbents which act selectively towards heavy metals [5,7]. Biosorption, a characteristic of live microbial cells or dead biomass, has been defined as a non-metabolically directed, passive process of metal-ion binding.

Immobilized fungal cells were found to be more stable during continuous operation in the bioreactor as compared to the free-growing form of the cells [8]. Moreover, live cells show greater sensitivity to heavy metal concentrations and adverse operating conditions such as pH, than do heat-inactivated biomass, and the applicability of these sorption characteristics is more relevant to industrial processes.

Loofa (*Luffa cylindrica*) grows well in the northern region of Iran and also in other tropical regions in Asian and African countries [9]. The loofa's low density (0.018 to 0.05 g/cm^3, high specific pore volume (26 to 34 cm^3/g) and porosity (85-95%) provide ideal structural characteristics as a natural cellular support for filamentous fungi compared with other cell supports [9, 10]. The structure and material of the support directly influence microbial immobilization and biochemical performance, for instance the presence of large void spaces in loofa and efficient mass-transfer characteristics of loofa pieces in a fixed-bed bioreactor explain the proper growth of the immobilized cells and emphasize the point that high cell densities increase the productivity of fermentation processes [11, 12].

The objective of the present work was to study the sorption performance of two mycelial forms of loofa-immobilized *Phanerochaete chrysosporium*, live (L) and heat inactivated (HIA), on mercury removal from aqueous solution. The capacity of these biosorbents for Hg^{2+} removal was determined using Langmuir and Freundlich adsorption isotherm models corresponding to the experimental equilibrium data. The biosorption kinetics were also analyzed using the pseudo-second-order rate equation described by Lagergren according to the modification suggested by *Ho & McKay* [13].

EXPERIMENTAL SECTION

Microorganism and inoculum preparation

Phanerochaete chrysosporium was obtained from the Iranian Research Organization for Science and Technology and maintenance of the fungal strain was by subculturing on a YMG 2% agar growth medium consisting of 2 g/L yeast extract, 10 g/L malt extract and

4 g/L glucose [14]. The medium was adjusted to pH = 6 and the fungus was grown for seven days at 27ºC. Then, two to three loops of the YMG agar medium were transferred to a 500-mL flask containing 150 mL YMG broth and incubated with agitation at 150 rpm at 27ºC for five days. The grown mycelia were then blended for 15 s and used as the inoculum in this study. The loofa sponges were cut into discs of approximately 2.5 cm diameter and 2-4 mm thickness, and the pieces were then soaked in boiling water for 30 min, washed thoroughly with tap water and allowed to stand in distilled water for 24 h, with the distilled water replaced 2-3 times. These pieces of loofa were then oven-dried at 70ºC [15]. Four pre-weighed pieces of the loofa were then placed in a 250 mL erlenmeyer flask containing 93 mL of growth medium prepared according to the formulation given by *Iqbal & Edyvean* [10] with slight modifications: 10 g/L glucose, 2.0 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.1 g/L NH_4Cl, 0.1 g/L $CaCl_2 \cdot H_2O$, 0.1 g/L yeast extract and 1 mg/L thiamine. The pH of the medium was adjusted to 4.5 and the flasks were autoclaved at 121°C for 20 min.

The blended biomass, prepared as described above (the inoculum), was added at a level of 7% v/v to the growth medium with four loofa pieces and agitated at 100 rpm at 35ºC for two days. Following growth of the fungus within the porous structure of the loofa discs, the compact block of the biomass was denoted the loofa-immobilized *P. chrysosporium* (live mycelia, L). The dry weight of the biomass was determined by the difference in the weight of the loofa discs before and after growth of the fungus. The heat inactivated loofa-immobilized fungus (HIA) was obtained by autoclaving the L form of mycelia at 121ºC for 20 min.

Biosorption studies

Qualification of the test biosorbents was studied by the uptake of mercury ions by each of the two biosorbent forms: known amounts of either of the two biosorbents were placed in 250-mL flasks containing 100 mL of Hg^{2+} solutions of varying initial concentrations (10-300 mg/L) and the equilibrium adsorption times were obtained when no further changes in the metal concentrations of the solutions were recorded. The initial Hg^{2+} concentration was set at 100 mg/L after the preliminary testing above. The influence of the initial pH was also examined and the subsequent tests were all performed at pH 6. Moreover, the results of the preliminary experiments indicated that the system equilibrated within three hours. The metal concentration at equilibrium was determined after taking a sufficient number of samples from the clear supernatant of the test solution. Inductively-Coupled Plasma Atomic-Emission Spectroscopy (ICP-AES, Perkin Elmer, Optima 2100 DV) was used for measuring the mercury concentration. The amount of biosorbed metal was determined from:

$$q = \frac{(c_0 - c)\,v}{m} \tag{1}$$

where q is the amount of the mercury ions biosorbed onto the unit amount of the biosorbent (mg/g);, c_0 and c are the mercury concentrations before and after biosorption (mg/L) and v and m are volume of the test solution (L) and amount of the biosorbent used (g), respectively[16].

The experimental biosorption data were fitted with the Langmuir and Freundlich sorption isotherm models, where the linearized form of each was used. The Langmuir equation is:

$$\frac{c_e}{q_e} = \frac{1}{bq_m} + \frac{c_e}{q_m} \tag{2}$$

where q_m is indicative of the maximum sorption capacity of the sorbate upon complete monolayer coverage of the sorbent surface (mg/g). The coefficient b (L/mg) is related to the affinity between sorbent and sorbate molecules. The terms c_e and q_e are the residual Hg^{2+} ions concentration and the amount of mercury ions adsorbed at equilibrium, respectively [17]. The Freundlich isotherm in linearized form is:

$$\ln q_e = \ln k_f + \frac{1}{n} \ln c_e \tag{3}$$

where k_F and n are the Freundlich constants, which are the indicators of the adsorption capacity and adsorption intensity, respectively [18, 19].

To study the metal adsorption kinetics, biosorption experiments using time-based analyses were performed, and the exposure time necessary for the tested sorbent to reach the equilibrium value was determined. The test solutions were prepared in 250-mL flasks containing 100 mL of the mercury solution at an initial metal concentration of 100 mg/L; a known amount of the biosorbent (102 mg of P. chrysosporium biomass in 284 mg of dry loofa)

was placed in the flask and it was agitated at 100 rpm at 35ºC. The initial pH was adjusted to pH 6 by adding 0.1 M HCl or NaOH as needed. Appropriate aliquots were then taken from the clear supernatant of the test solutions and the mercury concentration determined by ICP-AES at various time intervals over the two hours of the experiment (the time needed for the biosorption system to reach equilibrium). The mercury biosorption kinetics were analyzed using the pseudo-second-order Lagergren rate equation as modified by *Ho & McKay* [13]:

$$\frac{dq_t}{dt} = k_{2ads}\left(q_e - q_t\right)^2 \quad (4)$$

where q_e and q_t (mg/g) are the amounts of sorbate on the sorbent at equilibrium and at any time t, respectively, and k_{2ads} is the equilibrium rate constant of the pseudo-second-order sorption kinetics (g/mg min).

The presence of other ionic species (notably anionic species) is among the most influential environmental factors. The effect of the presence of Pb^{2+} on sorption performance of the test biosorbents in mercury removal was also studied.

Desorption tests and reusability of the biosorbents were examined by placing the Hg^{2+}-loaded fungal biomass (L and HIA) in the desorption medium (0.1 M HCl) and stirring the flasks at 100 rpm for 60 min at 25ºC. The desorption test was repeated three times, and after each cycle the biomass was washed thoroughly with deionized water. Desorption efficiency was determined as the ratio of the amount of Hg^{2+} desorbed to the amount previously adsorbed×100 [10].

Infrared spectra of the L and HIA fungal biomass before and after Hg^{2+} sorption were obtained with a Fourier-transform infrared (FT-IR) spectrometer [20]. Analyses of FT-IR spectra were done by the KBr method, with the samples prepared as KBr discs [16, 21]. Data are reported as the means of the results of two independent experiments performed separately for each of the test variables.

RESULTS AND DISCUSSION

Growth of P. chrysosporium immobilized on loofa

The growth pattern of P. chrysosporium immobilized within the open-structured loofa discs is shown in Fig. 1, and the amount of biomass was comparable with that reported by *Igbal & Edyvean* [10]. In the present work the stationary growth phase was reached within 40 h and

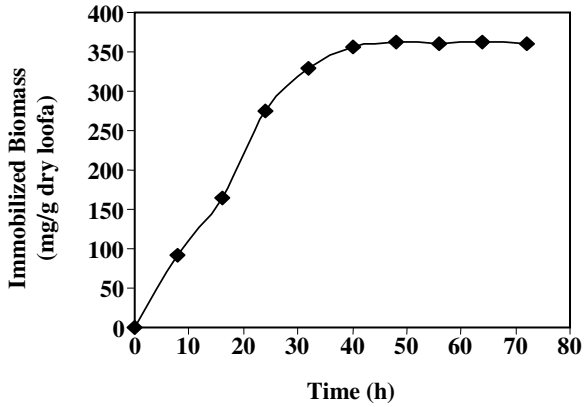


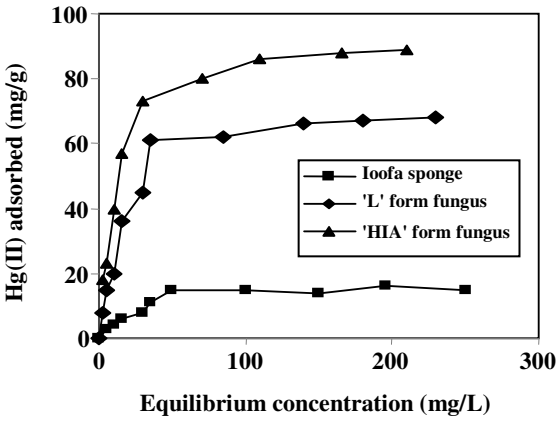
Fig. 1: Growth curve of the loofa- immobilized P. chrysosporium.

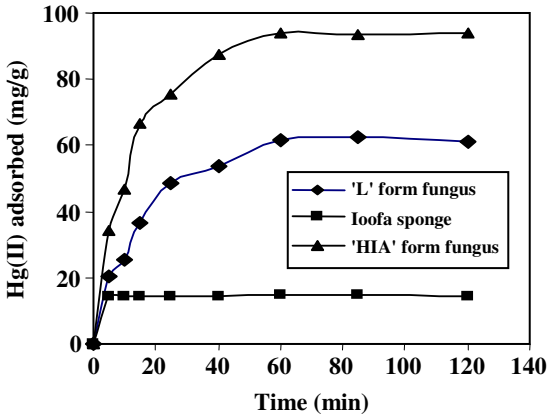
the loofa discs were completely covered with the mycelium in less than two days (350 mg/g of loofa sponge). This amount of the loofa-immobilized biomass was almost the same as the quantity reported by *Igbal & Edyvean* and the time events were considerably shorter than the times reported by *Igbal & Edyvean* [10]. Three-fold higher fungal biomass growth on loofa sponge than within calcium alginate beads [8] was reported by *Iqbal & Edyvean* [10]. Although the structure and material of the cell support directly affected fungal growth, and the suitability of loofa sponge in different biotechnological applications has been addressed [12], The inclusion of some chemical supplements in the culture media has also proven to be advantageous. For instance, the phenol- degrading ability of Ralstonia eutropha has been improved considerably by including yeast extract in the test culture media [21]. Yeast extract used in the present study was only difference between the medium in this work and that used by *Igbal & Edyvean* [10]. Moreover, studies on the removal of several heavy metals (Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}) by Mucor rouxii as a representative soil fungus have shown increased biomass production (by 13%) when yeast extract was included in the culture medium, and a low pH at equilibrium was found to be the reason for the low biosorption capacity of the culture medium which did not contain yeast extract [22].

Biosorption studies

Mercury ion concentrations in the range of 10-300 mg/L were used to test the biosorbent's properties for uptake of the test metal (Fig. 2(a)). The maximum Hg^{2+}

ions biosorption by the L and HIA biosorbents were 68.01 mg/g and 89.67 mg/g, respectively. The maximum mercury sorbed onto the bare loofa sponge was 15.3 mg/g, and P. chrysosporium immobilization on this lignocellulosic cell support markedly increased the test metal uptake: 4.5- and 5.6-fold increases for the L and HIA forms of the fungus, respectively. An initial Hg^{2+} concentration of 100 mg/L was used to study the effect of contact time on the biosorption (Fig. 2(b)). As seen in this figure, most of the mercury was biosorbed in less than 60 min, and there was almost no increase in the quantity of biosorbed mercury after 60 min. High biosorption rates were observed at the beginning of the biosorption experiment (biosorption rate is indicated here as the initial slope of the curve), and the sorption curves reached plateau values at ≥ 60 min, which was indicative of the adsorption equilibrium. No sorption occurred with loofa sponge only, and the HIA biosorbent showed higher mercury adsorption than the L form of the loofa-immobilized P. chrysosporium (adsorption of more than 90 mg Hg^{2+}/g HIA biosorbent in less than 60 min versus 50 mg mercury/g L biosorbent). Structural properties of the biosorbent including the cellular support and several other factors are known to affect the biosorption rate [19]. Measuring the time of adsorption plays a determining role in choosing a particular sorbent for a continuous-flow system. The plots shown in Fig. 2(a) were used to find the biosorption capacities of the test biosorbent and the quantity of test biosorbent as a function of the equilibrium concentration of Hg^{2+}, (see Fig. 2(b)). The effect of pH on the biosorption of Hg^{2+} is shown in Fig. 2(c), where the maximum biosorption of the test metal on the loofa-immobilized P. chrysosporium was observed at pH 6.0. The biosorption properties of several metal ions (cations) depend on the pH of the metal ion solution, as has been shown in other biosorption studies [6]. The role of pH in the sorption capacity of biosorbents is mainly due to its influence on the various chemical functional groups located on the microbial cell surfaces. These groups are mostly known to be negatively charged due to the ionization states of functional groups such carboxyl, sulfhydryl, etc. [4]. The contribution of these negatively charged groups to the sorption of metal cations may be interrupted when pH of the solution decreases, i.e., unionized carboxyl groups do not interact with metal cations. Fig. 2(c) shows effect of the pH of the test





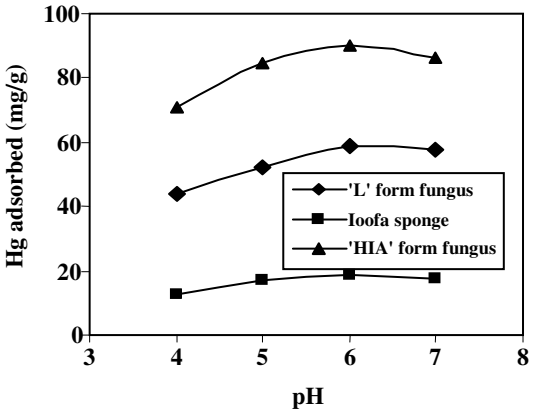


Fig. 2: Effect of initial concentration of Hg^{2+} on the biosorption of the test biosorbents (a). Equilibrium biosorption time of the test metal by the loofa-immobilized fungal cells (b). Effect of pH on the Hg^{2+} biosorption capacity (c). Adsorption conditions: initial concentration of Hg^{2+} ions: 100 mg/L, pH 6.0, temperature 35ºC.

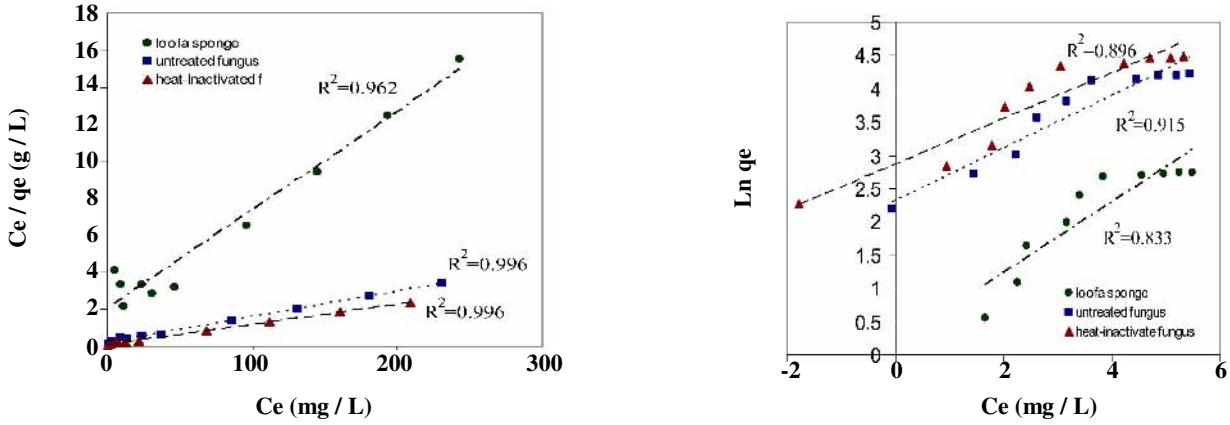


Fig. 3: Langmuir (a) and Freundlich (b) isotherms for the adsorption of Hg^{2+} by the test biosorbent (pH 6, 35ºC, 100 rpm agitation and 0.102 g of biomass).

solution on the biosorption of Hg^{2+} by the test biosorbents. The maximum capacity for mercury biosorption was at pH ~6.0 for the HIA biosorbent. The amount of sorbed Hg^{2+} on the test biosorbents increased as the pH of the metal solution was increased from pH 4 to pH 6.0. The adsorption of hydrogen ions from the metal solution by the fungal hyphal biomass (biosorbent) or the neutralization of H^+ by released hydroxyl ions from the fungal biomass can increase the final pH of the solution [22].

Adsorption isotherms have been used to characterize the interaction of metal ions (adsorbed species) with an adsorbent. This relationship between the concentration of aqueous Hg^{2+} and the quantity adsorbed on the test biosorbents at equilibrium between the liquid and solid phases was described in the present work using two common adsorption-isotherm models. Namely, Langmuir and Freundlich. In Fig. 3(a)-(b), the adsorption data are plotted applying the Langmuir and Freundlich isotherms.

Table 1 compares the adsorption constants obtained here and the relevant values reported by other researchers. The maximum possible sorbate uptake by the test sorbents, or q_m values, were 72.46 and 92.59 mg/g for the L and HIA biosorbents, respectively. The initial slope of this linearized plot gives the parameter $q_m b$ and the higher the initial slope, the greater the affinity of the adsorbent for the adsorbate [23]. The coefficient b is related to the affinity between the adsorbate and adsorbent and the value of this constant is indicative of the reciprocal of the metal-ion concentration at which 50% of the saturation of the adsorbent is achieved [16]. The low values of b are indicative of the high affinity of this biosorbent for mercury, a desirable property [17]. In the present work the affinity of the L-form biosorbent for Hg^{2+} was the higher of the two.

One meaningful characteristic of the Langmuir isotherm model has been defined in terms of *separation factor*, a dimensionless constant ($R = 1 / 1+bc_0$) in which the Langmuir coefficient *b* and the initial concentration of the adsorbent describe the shape of the isotherm, i.e., when R is between zero and one, the adsorption is categorized as favorable [24]. By this metric, the biosorbents tested in the present study both exhibited a favorable adsorption for Hg^{2+} (Table 1).

The Freundlich constants were also determined from plotting the experimental data as shown in Fig. 3(b) (see also Table 1). The k_F constant shows the adsorption capacity of the adsorbent for a particular adsorbate while *n* indicates the affinity of the adsorbate towards the adsorbent, i.e., an adsorption intensity (1/n) value of <1 reflects a favorable adsorption.

FT-IR analysis

The interference of radiation between two beams to yield an interferogram is the principle on which FT-IR spectroscopy is based [20]. The interferogram signal is produced as a function of the changes in path length between the two beams. The mathematical method of Fourier transformation provides an interconversion between distance and frequency of the beams [20]. FT-IR spectra of the test biosorbent 'L' before and after Hg^{2+} adsorption are presented in Fig. 4, while Table 2

Table 1: Comparison of the Langmuir and Freundlich constants for Hg^{2+} biosorption onto test biosorbents. The values of the separation factor for different initial concentrations of Hg^{2+} are also presented.

Biosorbent	Langmuir			Freundlich		References
	q_m (mg/g)	b (L/mg)		k_F	n	
L	72.46	0.093		13.82	3.22	Present study
HIA	92.59	0.114		21.30	3.51	Present study
WFB_P*	269.0	0.07		31.6	2.29	[23]
WFB_T**	161.0	0.5		59.0	3.85	[23]
Alginate-immobilized microalgal system	116.0	0.036		15.27	2.91	[19]
Lentinus edodes						
Live	358.1	0.077		36.5	2.08	[16]
Heat-inactivated	419.1	0.145		61.7	2.29	

** Waste biomass (Penicillium oxalicum);*

*** waste biomass Tolypocladium sp.*

	Initial Hg^{2+} concentration (mg/L)									
Biosorbent	10	20	30	50	70	100	150	200	250	300
L	0.578	0.406	0.314	0.215	0.164	0.12	0.084	0.064	0.052	0.044
HIA	0.467	0.305	0.226	0.419	0.111	0.081	0.055	0.042	0.034	0.028

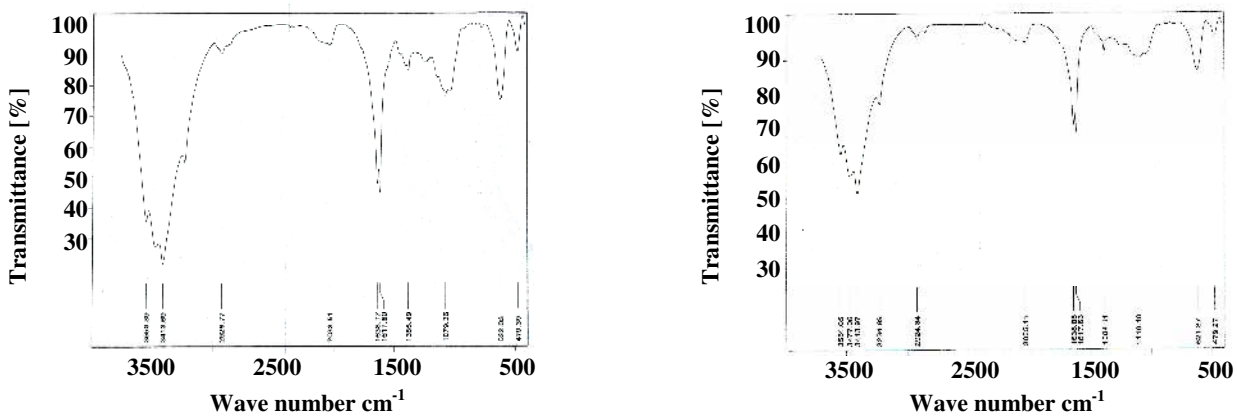


Fig. 4: FT-IR spectra of the L form of the fungus before (a) and after (b) Hg^{2+} biosorption.

summarizes the characteristic absorbance bands of the chemical groups usually found on the fungal cell surface. The intensities of the absorption bands at 3,555 and 3,413 cm^{-1} for the L form decreased markedly after mercury biosorption (Fig. 4). These frequencies correspond to vibrations of different chemical bonds in the molecule absorbing radiation at different infrared wavelengths, i.e., bond stretching or bond bending (see Table 2). The major structural components of the cell surfaces of fungi are carbohydrate polymers, mainly chitin (poly-N-acetylglucosamine), proteins, lipids and polyphosphates [25]. According to FT-IR characteristic group frequencies given in Table 2, the presence of all these chemical groups on the fungal cell surfaces were

Table 2: Identifying characteristics of various chemical groups in FT-IR spectral analysis*.

Wavenumber (cm^{-1}) 4000 3000 2000 1500 1000	Group vibration	Description	Mainly seen in
	-OH stretch	Hydroxyl	liquid phase
	=C-H stretch	unsaturated bond	lipids
	-C-H stretch	Saturated bond	lipids
	C=O stretch	Ester	lipids, amino acid
	C=O stretch	carboxylic acid	lipids, amino acid
	C=O stretch	amide I	proteins
	C=C stretch	Trans	lipids
	C=C stretch	cis-bond	lipids
	N-H bending	amide II	proteins
	C-H scissoring	Aliphatic-CH_2	lipid
	C-O stretch	Carboxylates	amino acids, lipids
	N-H bending	amide III	proteins
	P=O stretch	phosphate ester	lipids, nucleic acids
	C-O stretch	Ether	carbohydrates

confirmed, and at two wavenumbers noticeable decreases in absorbance intensity were seen after Hg^{2+} biosorption: 3,413 and 1,617 cm^{-1} (Fig. 4). The signals corresponding to several chemical groups and characteristics of their absorbance intensities are presented in Table 2.

Kinetic analysis

The mercury concentration profile versus the incubation time, shown in Fig. 2(b), was used for the adsorption kinetic analysis according to equation (4). By integrating and rearranging terms in Eq. (4), the following linearized from was obtained [13]:

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{q_e^2 k_{2ads}} \qquad (5)$$

The k_{2ads} and q_e values were computed from a plot of t/q_t versus time, confirming the applicability of the pseudo-second-order rate equation to the experimental data obtained in the present study (Fig. 5). The values for the correlation coefficients were 0.994 and 0.997 for the L and HIA biosorbents, respectively. The q_e values were fairly close to the experimental values: 69.4 and 103.09 mg/g for L and HIA, respectively. The values of k_{2ads} were 1.16×10^{-3} and 1.08×10^{-3} g/mg min for L and HIA, respectively.

Effect of Pb^{2+} ions

The complexity of microbial cell structures, particularly cell surfaces, plays a key role in metal biosorption. The presence of various anionic functional groups on the cell surface gives all microorganisms a negative charge [2]. Fig. 6 shows how the presence of Pb^{2+} ions in the solution affected the biosorbent capacity for Hg^{2+} removal: Pb^{2+} ions at a 50-mg/L level decreased the Hg^{2+} biosorption by 15% and 10% for L and HIA, respectively.

The performance of *P. chrysosporium* in three cellular forms (live, dead 'heat-treated' and resting cells) on Pb^{2+} removal from aqueous solution has been evaluated previously [26]. The general idea regarding heat treatment is that the cell-surface integrity is compromised by heating, and passive movement of the metal into the cellular interior through the now-leaky walls becomes possible [27]. However, the adsorptive capacity of the heat-treated fungal cells for Pb^{2+} was lower compared that of resting cells [26]. Metabolism-dependent biosorption of heavy metal may be involved in the resting form of the fungal cells, and metal transport across the cell membrane may cause intracellular accumulation followed by precipitation. Extracellular accumulation of heavy metal may be also possible, where certain metabolites e.g., phosphates, oxalates, carbonates and

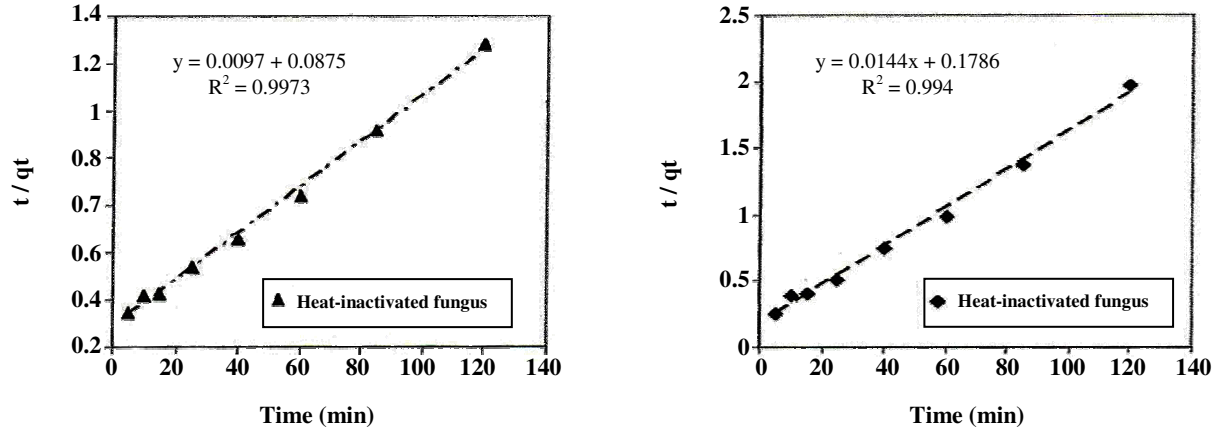


Fig. 5: Linearized form of the pseudo-second-order kinetic model for Hg^{2+} biosorption by the test biosorbents (initial concentration of mercury ions, 100 mg/L; temperature, 35°C; pH 6; agitation, 100 rpm; and amount of the biosorbent, 0.102 g).

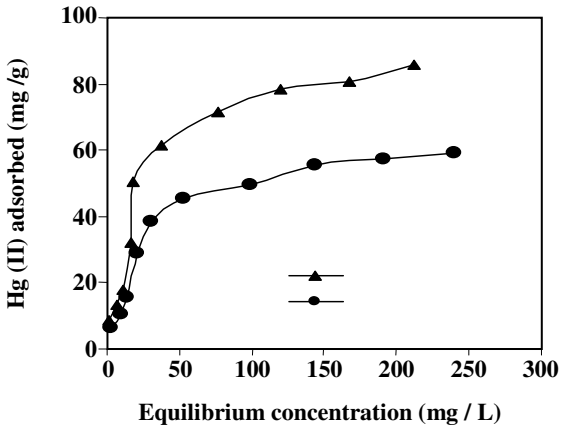


Fig. 6: Effect of presence of Pb^{2+} (50 mg/L) in the test solution on the biosorption of Hg^{2+} by the test biosorbent (initial concentration of Hg^{2+}, 100 mg/L; pH 6; 35°C).

other organic acids which normally synthesized during exponential growth may be involved in metal binding [5, 26].

Desorption, regeneration and reusability tests

The reusability of the test biosorbent was examined by quantifying the desorption of the adsorbed Hg^{2+} from the mercury-loaded biosorbents. Hydrochloric acid (100 mM) was used to elute the metal, and more than 90% of the Hg^{2+} adsorbed onto the biosorbents was released back into solution (Hg^{2+} ions concentration: 100 mg/L). The formation of anionic complexes such as those between Cl^- and Hg^{2+} may be involved in the desorption mechanism [19]. In further tests, the performance of Hg^{2+}-loaded biosorbents was examined by applying consecutive adsorption-desorption cycles; three cycles were completed and the biosorbents performed well. Concerning the practical applications of this desorption process, any potential damage to the biosorption sites could be prevented by using the lowest possible concentration of HCl as the eluting agent, and 100 mM HCl has been considered to be the usual level in these desorption processes [10, 19].

CONCLUSIONS

Considerable attention has been directed towards the use of natural materials combined with microorganisms for the removal of soluble heavy metals and organic compounds from industrial wastewater. The loofa sponge, a lignocellulosic material naturally dried in the field, has a unique porous structure, which was used in the present study for the supported growth and immobilization *of P. chrysosporium*. Batch experiments were conducted to evaluate the performance of the live and the heat-inactivated mycelia on the loofa as a biosorbent for Hg^{2+} removal from aqueous solution. Equilibrium data were fitted to two isotherm models, and the Langmuir isotherm gave the best fit for the data. The time dependency of the mercury removal was analyzed using a pseudo-second-order rate equation, as expressed by Ho and McKay, and the high correlation coefficient revealed a good fit of the data. The results of the present study, including FT-IR analysis, showed that the major mechanism mediating metal adsorption appeared to be passive in this case, while cell complexity remains a barrier for elucidating the exact mechanism of mercury biosorption by the test fungus. However, these basic experiments show the potential of biomass for use as a natural and inexpensive source of lignocellulosic material

for toxic heavy-metal removal from industrial wastewater. The considerable interest generated in recent years in this area is expected to continue, and the emergence of an innovative approach such as this would be valuable.

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