

Study on the Characterization of Lead (II) Biosorption by Fungus *Aspergillus parasiticus*

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Abstract

The lead (II) biosorption potential of *Aspergillus parasiticus* fungal biomass has been investigated in a batch system. The initial pH, biosorbent dosage, contact time, initial metal ion concentrations and temperature were studied to optimize the biosorption conditions. The maximum lead (II) biosorption capacity of the fungal biosorbent was found as $4.02 \times 10^{-4} \text{ mol g}^{-1}$ at pH 5.0 and 20°C. The biosorption equilibrium was reached in 70 min. Equilibrium biosorption data were followed by the Langmuir, Freundlich and Dubinin–Radushkevich (D–R) isotherm models. In regeneration experiments, no significant loss of sorption performance was observed during four biosorption-desorption cycles. The interactions between lead (II) ions and biosorbent were also examined by FTIR and EDAX analysis. The results revealed that biosorption process could be described by ion exchange as dominant mechanism as well as complexation for this biosorbent. The ion exchange mechanism was confirmed by *E* value obtained from D-R isotherm model as well.

Index Entries: *Aspergillus parasiticus*; biosorption; equilibrium; isotherm; lead (II); mechanism.

Introduction

The disposal of heavy metals by contaminated effluents into receiving water bodies causes a major concern because of their non-biodegradability and toxic natures as well as tendency for accumulation throughout food chain (1). Lead is one of the common contaminants used in the several industrial process especially production of batteries, varnishes, paints and

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antiknock compounds in gasoline (2). The presence of lead in the water systems is of great importance due to its toxicity even at low concentrations and potential carcinogenicity. The hazardous effects of lead are on central and peripheral nervous systems, hematopoietic, renal, gastrointestinal, cardiovascular and reproductive systems. The other damaging effects of lead are anemia, tenderness, loss of cognitive abilities, nausea (3), and suppression of the mental capacity of children (4). Hence, there is significant interest regarding lead removal from contaminated water systems.

The traditional treatment techniques for the elimination of toxic metals from contaminated effluents usually include chemical precipitation, ion exchange, electrolysis, filtration, evaporation, reverse osmosis and membrane separation. However these techniques have certain constraints such as high cost, high reagent and energy requirement, incomplete removal, generation of toxic sludge and other secondary problems (5,6). For these reasons interest has been recently focused on the use of biosorption technology to sequester metal ions from contaminated effluents which based on the metal-microbe interactions (7). The distinct advantages of biosorption over traditional treatment techniques can be ordered as follows: low operating cost, minimization of the chemical and/or biological sludge volume, high efficiency in detoxifying very dilute effluents, no nutrient requirements, regeneration of sorbent material, and possibility of metal recovery (8,9). Also, biosorption can be used *in situ* and with proper design may not need any industrial process operations and can be integrated with many systems in the most eco-friendly manner (5). A wide variety of microbial origin materials such as algae, fungi, yeast and bacteria can be employed as sorbent material in biosorption technology (6). The surface properties of the biosorbent materials play an important role in this process. Because several interactions include ion exchange, complexation, chelation and adsorption occur between metal ions and potential metal binding sites within the structure of the biosorbent material (10,11). The same metal ions can be sorbed with different mechanisms by different microbial cells (12). The use of several fungal species, e.g., *Mucor rouxii* (13), *Aspergillus flavus* (14), *Botrytis cinerea* (15), and *Neurospora crassa* (16) has been investigated for their lead removal potentials. As far as we are aware, there is no report available in the literature about the heavy metal removal properties of *Aspergillus parasiticus* biosorbent.

The objective of this study was to examine the lead sorption ability of *A. parasiticus* cells from aqueous solutions in the batch mode. Reusability potential and temperature effect on the biosorption capacity of biosorbent in aqueous systems were investigated in addition to the effects of design parameters such as pH, biosorbent dosage and contact time. Freundlich, Langmuir and Dubinin-Radushkevich (D-R) isotherms were used for the biosorption isotherm modelling. Furthermore the lead-biosorbent interactions were investigated with FTIR, SEM and EDAX analysis to elucidate the biosorption mechanism.

Materials and Methods

Preparation of the Biosorbent Material

The pure culture of *A. paraciticus* (NRRL 502) was obtained from the collection of Biology Department of Eskişehir Osmangazi University. The cultures were routinely maintained by subculturing on malt extract agar slants. The composition of the liquid medium used for cell cultivation was described in our previous study (14). The final pH of the medium was adjusted to 5.5 with 1 N HNO₃ before autoclaving at 121°C for at least 20 min. One cubic centimeter spore suspension of *A. paraciticus* was inoculated into above liquid media in 250-cm³ Erlenmeyer flasks and incubated at 25°C for 7 d on a rotary shaker operating at 150 rpm. After the fungal growth, the mycelial pellets were separated from the culture medium by filtration through Whatmann No. 1 filter paper and the harvested mycelia were washed several times thoroughly with deionized water. The filtration process was followed by drying in an oven at 60°C overnight. The resulting biomass was ground and sieved to obtain particle fraction less than 150 µm.

Preparation of Lead (II) Solutions

A stock solution of 1 g dm⁻³ lead (II) was prepared by dissolving an accurate quantity of anhydrous Pb(NO₃)₂ of analytical grade in deionized water. Other concentrations ranging from 50 and 300 mg dm⁻³ were prepared by diluting of the stock solution. The initial pH values of the experimental solutions were adjusted to a desired value in the range of 1.0–5.5 using 0.1 M HCl and 0.1 M NaOH. Fresh dilutions were used for each biosorption study. All the chemicals used were of analytical grade and supplied by Merck (Germany).

Batch Biosorption Studies

The biosorption of lead (II) ions onto dried biomass of *A. paraciticus* was investigated in the batch mode at 20°C. Batch experiments were carried out using a series of beakers (50 cm³) on a magnetic stirrer operating at 200 rpm. The effects of initial pH, biosorbent dosage, contact time and temperature were studied to elucidate the optimum operating conditions. The effect of initial pH on the biosorption capacity of *A. paraciticus* was determined by equilibrating the biosorption mixture containing 0.1 g of dried biomass and 100 mg dm⁻³ of lead (II) solutions at different pH values of 1.0, 2.0, 3.0, 4.0, 5.0, and 5.5. The effect of biosorbent dosage was studied by using different dose of biomass (0.4–3.0 g dm⁻³). The optimum pH and biosorbent concentration were determined as 5.0 and 1.6 g dm⁻³, respectively and used throughout all the biosorption experiments. The period of contact time was varied up to 90 min determined by using the procedure described above. For the assessment of the effect of initial metal ion concentration on the biosorption at optimum conditions, the lead (II) solutions at

the concentrations ranging from 50 to 300 mg dm⁻³ were prepared and used. The effect of the temperature on the uptake of lead (II) ions was also investigated using dried biomass. When the biosorption process completed, the experimental mixtures were centrifuged at 4500 rpm for 2 min and the residual lead (II) ion concentrations in the supernatant liquids were analyzed.

Regeneration and Reusability of Biomass

The recovery and reusability of biosorbent material is an important parameter related to the application potential of biosorption technology (6). Different mineral acids revealed metal elution efficiency close to 100%. In this work consecutive batch biosorption and desorption experiments were performed using the same biosorbent in order to test for its ability to be reutilized after regeneration. For this purpose, 0.08 g of biosorbent was contacted with 50 cm³ lead (II) solution (100 mg dm⁻³) at pH 5.0. Following the biosorption process, lead (II)-loaded biomass separated by centrifugation and suspended into eluent solution (50 cm³ of 0.1 M HCl). Each biosorption and desorption cycle was allowed 70 min of contact time in solutions containing biosorbent-lead (II) ions or biosorbent-desorbent agent for achieving sorption or desorption equilibrium. The concentration of the lead (II) ion released into eluent solution was analyzed. The eluted biosorbent was thoroughly washed with deionized water and placed into metal solution for the next biosorption cycle. Desorption efficiency was calculated by using the following equation.

$$\text{Desorption efficiency} = \frac{\text{Amount of lead(II) desorbed}}{\text{Amount of lead(II) biosorbed}} \times 100 \quad (1)$$

Metal Analysis

The experimental solutions were analyzed for lead (II) concentrations using Hitachi 180-70 model flame atomic absorption spectrophotometer (FAAS) with a deuterium background correction. All the instrumental conditions were optimized for maximum sensitivity as indicated below:

Flame:	air-acetylene
Hallow cathode lamp:	lead
Lamp current:	7.5 mA
Working wavelength:	283.3 nm
Spectral slit width:	1.3 nm

The instrument response for lead was checked using a known lead standard solutions in every 10 readings. The amount of sorbed lead (II) ions (q_e) per g biomass was calculated using the general mass-balance equation as follows:

$$q_e = [(C_i - C_e)] \cdot V/M \quad (2)$$

where, C_i and C_e are the initial and equilibrium concentrations of lead (II) ion in solution (mg dm⁻³), V = volume of the medium (dm³), and M = weight of the biomass used in the reaction mixture (g).

Statistical Analysis

All the biosorption experiments were carried out in triplicate to check reproducibility of the results and the data presented are the mean values from these independent experiments. Standard deviation and error bars are indicated wherever necessary. All statistical analysis was done using SPSS 9.05 for Windows, with which it is possible to evaluate whether the effect and the interaction among the investigated factors are significant with respect to the experimental error.

FTIR, SEM, EDAX and ζ -Potential Analysis of Biosorbent Material

To determine the functional groups that might be involved in lead (II) sorption the chemical characteristics of dried *A. parasiticus* biomass before and after lead(II) sorption were analyzed and interpreted by FTIR spectroscopy. The spectra were obtained with a Bruker Tensor 27 FTIR apparatus with the samples prepared as KBr discs. The spectra were recorded in the wavenumber region of 400–4000 cm^{-1} .

The surface morphology analysis of the biosorbent was made by using scanning electron microscopy. The scanning electron microscope (Cam Scan Oxford Link SEM) equipped with energy-dispersive X-ray microanalyzer (EDAX) was used to take microscopic surface pictures and EDAX spectra of biosorbent. For these analyses biomass samples were mounted on a stainless steel stab with a double-stick tape followed by coating with a thin layer of gold and palladium under vacuum to increase the electron conduction and to improve the image quality. Gold and palladium were used to coating material for the biosorbent surface because they can form very thin films without suppressing fine surface features (17). The surface charge of the biomass was measured using the Zeta potential analyzer (Malvern Zetasizer nano ZS).

Results and Discussion

Effect of Initial pH on Lead (II) Biosorption

It is well documented that solution pH is an important variable governing the uptake of heavy metals by biosorption process as it not only affects metal species in solution, but also influences the surface properties of biosorbents in terms of dissociation of binding sites and surface charge (18–21). The effect of the solution pH on the biosorption process can be varied from the type of biomass to the type of metal ion being studied (22). Figure 1 represents the biosorption results of lead (II) for 100 mg dm^{-3} solution at different initial pH values by dried biomass of *A. parasiticus*. As can be seen from the figure, at pH 1.0 the biosorption was almost negligible. However the lead (II) removal capacity of the biosorbent increased from 9.19 ± 0.41 to $37.29 \pm 1.18 \text{ mg g}^{-1}$ with an increase in solution pH from 2.0 to 5.0 and reached a plateau at pH 5.0. Figure 2 shows the surface charge of the biomass at different pH conditions. As can be seen from the figure the zeta

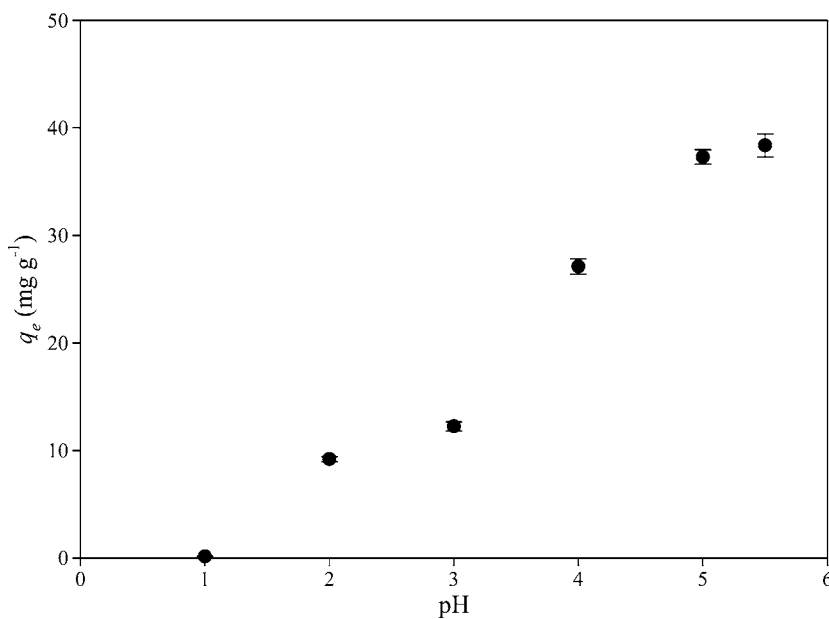


Fig. 1. The effect of pH on the biosorption of lead (II) by *Aspergillus parasiticus* at 20°C.

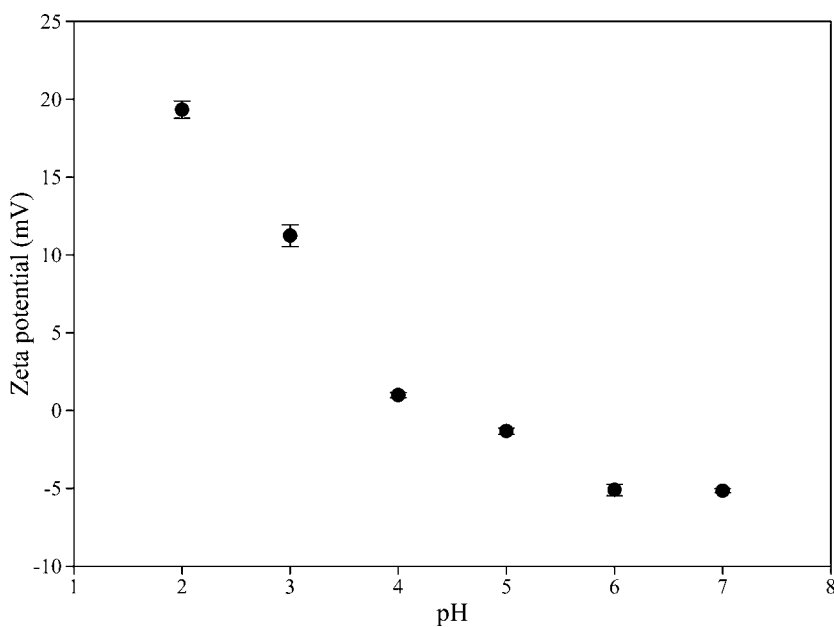


Fig. 2. The surface charge of *Aspergillus parasiticus* biomass at different pH values.

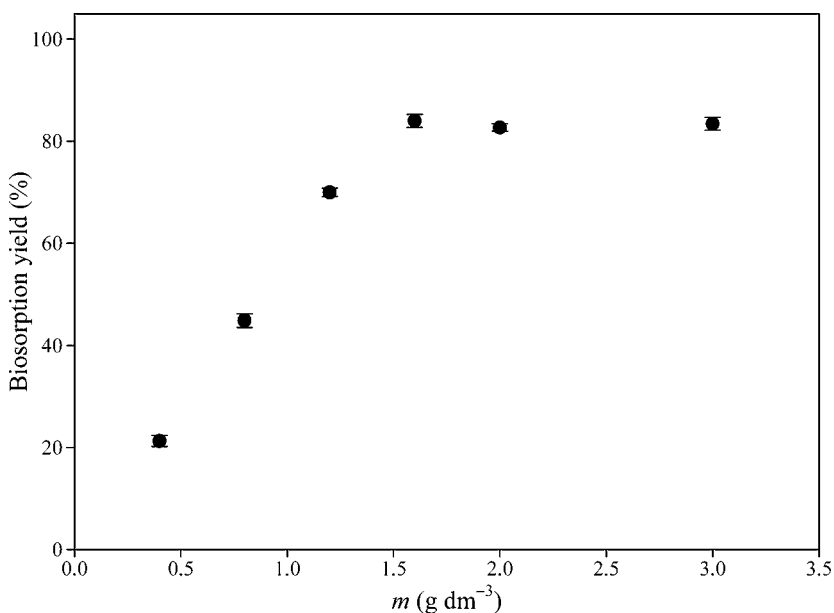


Fig. 3. The effect of biosorbent dosage on the biosorption of lead (II) by *Aspergillus parasiticus* at 20°C.

potential values of the biomass were measured as positive in the pH range of 2.0–4.0 and the overall surface of the biomass was negatively charged at the pH values 5.0, 6.0, and 7.0. The relatively low biosorption values at strong acidic conditions can be attributed to proton-metal ion competition for occupancy of the binding sites on the biosorbent surface. In the other words at highly acidic conditions biosorbent surface becomes saturated with protons thus reducing the interaction between biosorbent and metal ions. In contrary as the pH increases, more negatively charged surface become available due to deprotonization thus facilitating the approach of the metal ions to the binding sites and metal uptake increases (20,23,24). No pH values greater than 5.5 were studied as a result of a precipitation of lead (II) ions in the form of hydroxide due to high concentration of OH⁻ ions in the solution.

Effect of Biosorbent Dosage on Lead (II) Biosorption

Lead (II) removal data of *A. parasiticus* for different values of biosorbent dosage are represented in Fig. 3. When the biosorbent dosage increased from 0.4 to 1.6 g dm⁻³ the percentage of lead (II) biosorption significantly increased from 21.26 ± 1.83 % to 83.98 ± 2.22 % ($p < 0.05$). This was attributed to availability of binding sites increased (5,25). Further increase in the biosorbent dosage did not changed the lead (II) biosorption yield. The constant trend observed with the biosorbent dosage between 3.0 and 1.6 g dm⁻³ ($p > 0.05$) can be explained by the binding of almost all ions to the

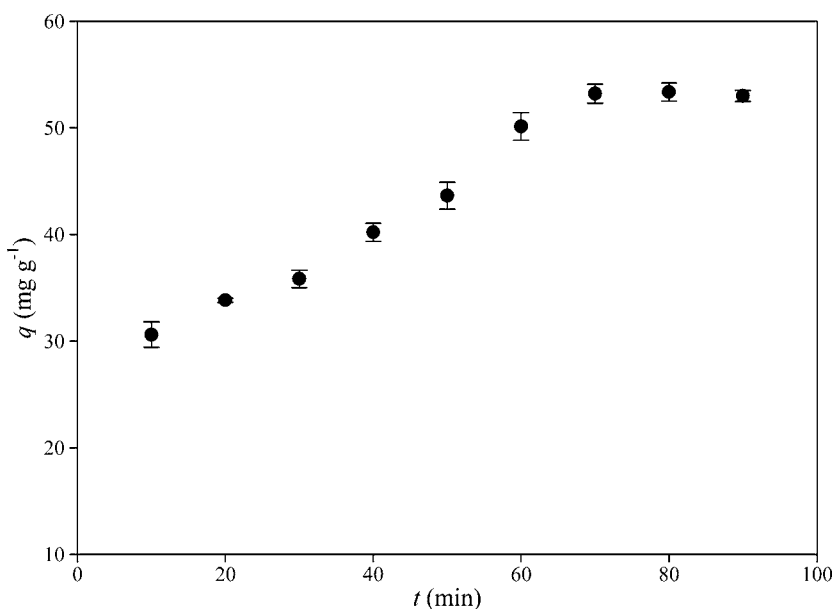


Fig. 4. The effect of contact time on the biosorption of lead (II) by *Aspergillus parasiticus* at 20°C.

biosorbent and the establishment of equilibrium between the ions bound to the biosorbent and those remaining unsorted in the solution (25). However, the biosorption capacity of *A. parasiticus* showed a reduced trend as biosorbent concentrations increased. This may be due to several causes, including partial cell aggregation causing a decrease of active metal binding sites (26), availability of solute, electrostatic interactions, and interface between binding sites (27,28).

Effect of Contact Time on Lead (II) Biosorption

The lead (II) removal capacity of dried *A. parasiticus* biomass as a function of time is presented in Fig. 4. It can be seen that fast rate of biosorption was observed within the first 10 min, and that this was followed by a slower phase until the attained of equilibrium. Equilibrium time was found to be 70 min and after the equilibrium period no further increase in the amount of biosorbed lead (II) ($p > 0.05$). The similar fast biosorption trends have been also reported by the other researchers (23,28,29). The initial rapid phase is probably due to the abundant of availability of active metal binding sites on the biosorbent surface and with the gradual occupancy of those sites. The sorption becomes less efficient in the slower stage (30,31) as a result of competition for decreasing availability of active binding sites intensities by the metal ions remaining in solution. The rate of metal-sorption is of great significance for developing a microbial origin biosorbent-based water-treatment technology (23) and practical application of process (18).

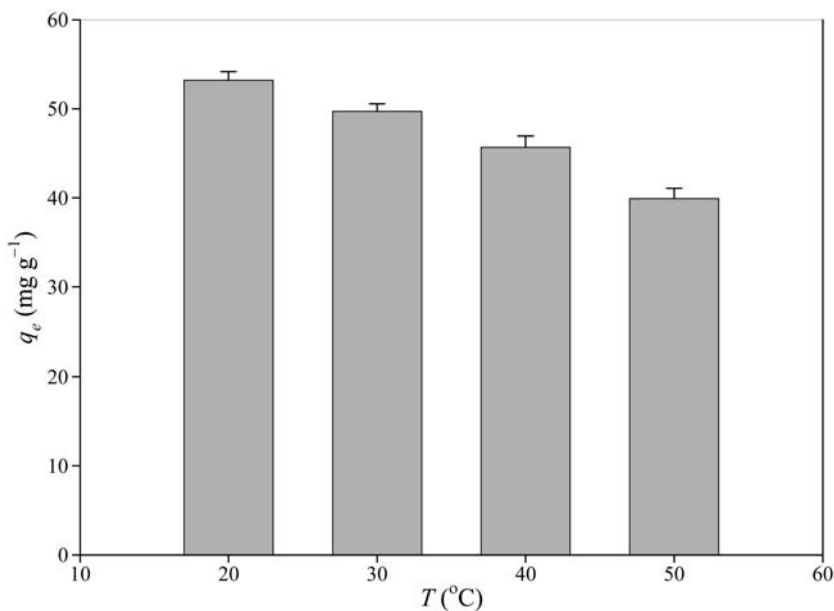


Fig. 5. The effect of temperature on the biosorption of lead (II) by *Aspergillus parasiticus*.

Effect of Temperature on the Biosorption of Lead (II)

The variation of the biosorption capacity with temperature is presented in Fig. 5. As seen from this figure, the lead (II) biosorption by *A. parasiticus* appears to be temperature dependent in the temperature range studied. When the temperature was increased from 20 to 50 the lead (II) loading capacity of biosorbent decreased from 53.21 ± 1.67 to 39.91 ± 2.02 mg g⁻¹ ($p < 0.05$). The exothermic behavior of biosorption may be attributed to weakening of sorptive forces between the active binding sites of the biosorbent and sorbate species and also between the adjacent molecules of the sorbed phase (32–34). Similar observations were also reported by the other researchers (32,35).

Regeneration and Reusability

Reusability of biosorbents after regeneration is very important factor in water-treatment process. Because regeneration can influence the cost of the whole process and the possibility of the metal recovering from liquid system as well, which is very important for practical application of the process (6,36). The results obtained from the desorption experiments of lead (II) ions by 0.1 M HCl solution are shown in Fig. 6. It can be seen that the desorption of lead (II) from metal-loaded biosorbent was resulted with more than 96% recovery of lead (II) ions. The biosorption efficiency did not change significantly ($p > 0.05$) and only a maximum 10% decrease was

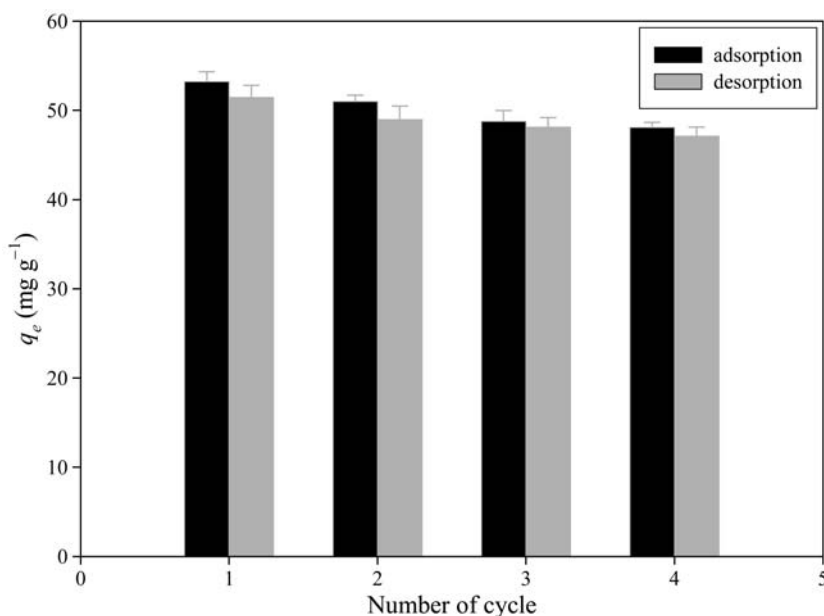


Fig. 6. The biosorption–desorption cycles for the biosorption of lead (II) by *Aspergillus parasiticus* at 20°C.

observed after four biosorption–desorption cycles. These results showed that the *A. parasiticus* biomass has a good potential for the removal of lead (II) ions repeatedly from aqueous solution without any detectable loss in the total biosorption capacity.

Biosorption Isotherms

Analysis of the equilibrium sorption data is important to determine the mechanistic parameters and to understand the mechanism of the sorption. In the present study, lead (II) biosorption data were analyzed for Langmuir, Freundlich, and D–R isotherm models and the general biosorption isotherm is shown in Fig. 7.

The Langmuir isotherm model is based on the assumption of a monolayer sorption which takes place at complete homogeneous surfaces. The linearized form of the Langmuir equation is represented by the following expression (37).

$$\frac{1}{q_e} = \frac{1}{q_{\max}} + \left(\frac{1}{q_{\max} K_L} \right) \frac{1}{C_e} \quad (3)$$

where q_e and q_{\max} are the equilibrium and monolayer lead (II) biosorption capacities of the biosorbent (mol g^{-1}), respectively, C_e is the equilibrium lead (II) concentration in the solution (mol dm^{-3}), and K_L is the Langmuir adsorption constant ($\text{dm}^3 \text{mol}^{-1}$) and is related to the free energy of adsorption.

The effect of isotherm shape has been discussed (38) with a view to predict whether a biosorption system is favorable or unfavorable. The

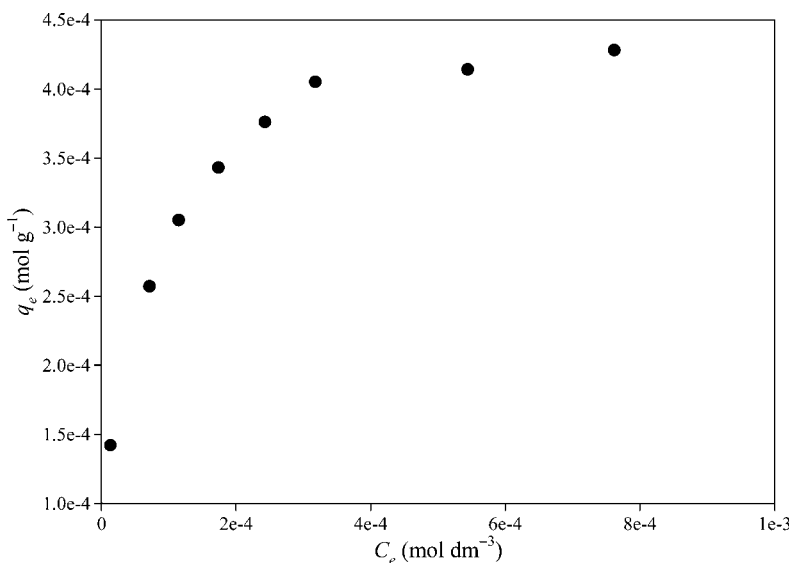


Fig. 7. The biosorption isotherm plot for the biosorption of lead (II) by *Aspergillus parasiticus* at 20°C.

essential feature of the Langmuir isotherm can be expressed by means of “ R_L ” a dimensionless constant referred to as separation factor or equilibrium parameter and R_L is calculated using the following equation:

$$R_L = \frac{1}{1 + K_L C_o} \quad (4)$$

where K_L is the Langmuir constant (dm³ mol⁻¹) and C_o the highest initial lead (II) concentration (mol dm⁻³).

The Freundlich isotherm is an empirical equation employed to describe heterogeneous systems. The linearized form of the Freundlich equation is as follows (39).

$$\ln q_e = \ln K_F + \ln C_e \quad (5)$$

where K_F (dm³ g⁻¹) and n are Freundlich isotherm constants, being indicative of the extent of the biosorption and the degree of nonlinearity between solution concentration and biosorption, respectively.

The D–R isotherm model is more general than the Langmuir isotherm. It was applied to distinguish the nature of biosorption as physical or chemical (40). The D–R isotherm equation (41) is:

$$\ln q_e = \ln q_m - \beta \epsilon^2 \quad (6)$$

where β is a constant related to the mean free energy of biosorption (mol² J⁻²), q_m is the theoretical saturation capacity, and ϵ is the Polanyi potential, which is equal to $RT \ln \left(1 + \frac{1}{C_e} \right)$, where R (J mol⁻¹ K⁻¹) is the gas constant, and T (K) is the absolute temperature.

Table 1
Biosorption Isotherm Model Constants for the Biosorption of Lead (II)
by *Aspergillus parasiticus* at 20°C

Langmuir Isotherm	q_{max} (mol g ⁻¹) 4.02×10^{-4}	K_L (dm ³ mol ⁻¹) 3.93×10^4	r_L^2 0.971	R_L 1.73×10^{-2}
Freundlich Isotherm	n 3.56	K_F (dm ³ g ⁻¹) 3.64×10^{-3}	r_F^2 0.950	
D-R Isotherm	q_{max} (mol g ⁻¹) 1.04×10^{-3}	β (mol ² kJ ⁻²) 4.93×10^{-5}	r_{D-R}^2 0.975	E (KJ mol ⁻¹) 14.34

The constant β gives an idea about the mean free energy E (kJ mol⁻¹) of biosorption can be calculated using the following relationship (42–44):

$$E = \frac{1}{(2\beta)^{1/2}} \quad (7)$$

E value gives information about biosorption mechanism as chemical ion-exchange or physical biosorption. The numerical value of the mean free energy of biosorption is 14.34 kJ mol⁻¹, which may correspond to a chemical ion exchange.

The Langmuir, Freundlich, and D–R parameters for the biosorption of lead (II) onto *A. parasiticus* are given in Table 1. It is indicated that the Langmuir, Freundlich, and D–R isotherm models are suitable for describing the lead (II) biosorption equilibrium by *A. parasiticus* in the studied concentration range with the regression coefficient (r^2) values more than 0.97. As the R_L values lie between 0 and 1, the adsorption process is favorable (38,45). The R_L value for this study was 1.73×10^{-2} , therefore, the biosorption of lead (II) was favorable.

The Freundlich constants K_F and n indicate the biosorption capacity of the biosorbent and a measure of the deviation from linearity of the biosorption, respectively. The values of K_F and n at equilibrium were 3.64×10^{-3} dm³ g⁻¹ and 3.56, respectively. The well description of the experimental results with all of the isotherm models investigated in this study implies that the biosorption of lead (II) ions onto *A. parasiticus* biomass is complex and involving more than one mechanism.

Mechanism of Lead (II) Biosorption

The SEM microscopic photos of *A. parasiticus* biomass at a magnification of $\times 300$, $\times 600$, and $\times 1000$ are given in Fig. 8. As shown in these pictures, many different pores and irregular particles were observed on the corrugated biomass surface, which is considered helpful for the binding of heavy

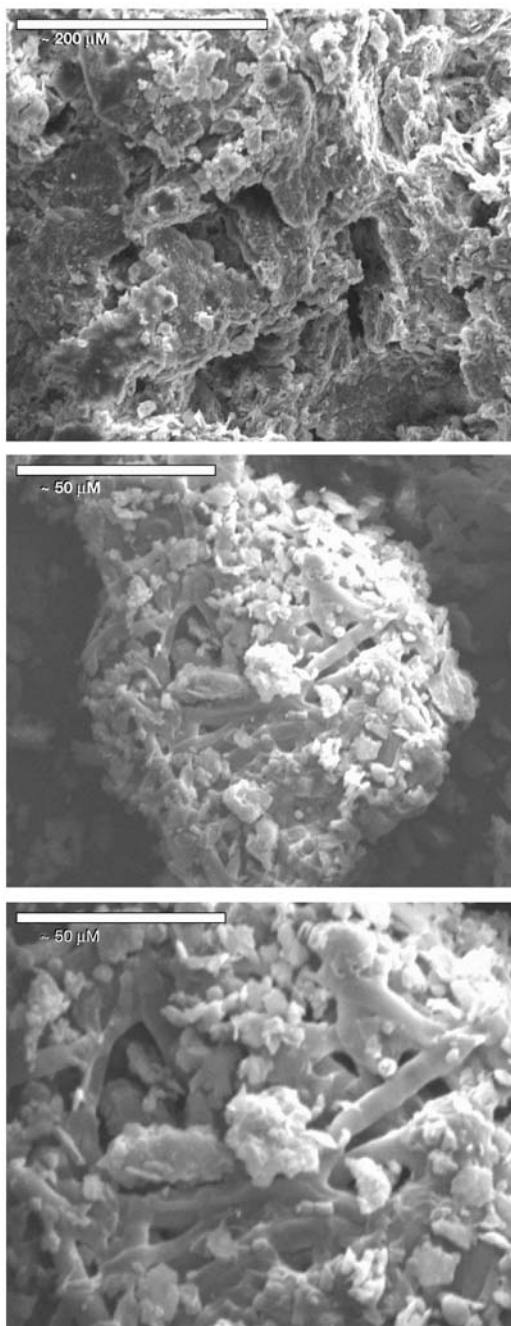


Fig. 8. Typical SEM micrographs of *Aspergillus parasiticus* biomass, magnification: $\times 300$ (A), $\times 600$ (B), and $\times 1000$ (C).

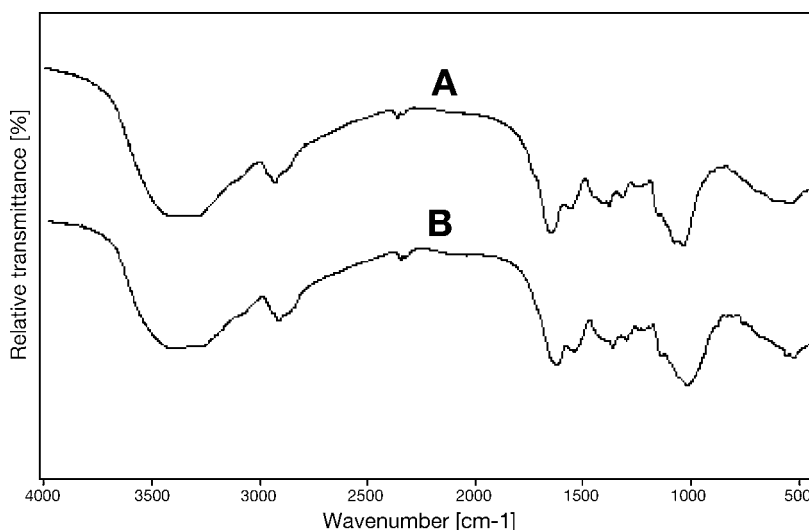


Fig. 9. FTIR spectra of *Aspergillus parasiticus* before (A) and after (B) lead (II) biosorption.

metals to biosorbent. The mechanism of the lead (II) biosorption by *A. parasiticus* biosorbent was elucidated on the basis of FTIR and EDAX analysis. As shown in Fig. 9, the FTIR spectrum of biosorbent displays a number of absorption peaks, reflecting the complex nature of examined biosorbent material. It has been known that many functional groups like sulfhydryl, hydroxyl, and phosphate provide favorable binding sites for metal cations (46). The broad stretching absorption peak at 3391 cm^{-1} representing -NH and bonded -OH groups. The band observed at 2927 cm^{-1} could be assigned to symmetric and asymmetric stretching vibrations of the -CH_3 and -CH_2 groups and their bending vibrations at 1315 cm^{-1} . The bands at 1644 and 1557 cm^{-1} correspond to carbonyl stretching vibrations of amide considered to be due to the combined effect of double bond stretching vibrations (47) and -NH deformation band. The 1033-cm^{-1} band is caused by C-N and C-O stretching (48) and the band at 534 cm^{-1} can be assigned to $\text{P}=\text{O}$ vibrations (49). FTIR spectrum of biosorbent after lead (II) biosorption indicated no significant changes in any of the characteristic absorbance bands present in unloaded biomass with the exception of peak shifts from 3391 cm^{-1} to 3400 cm^{-1} and from 534 cm^{-1} to 539 cm^{-1} and a small reduction in the intensity of the peak at 1644 cm^{-1} . These results implied that not only involvement of the related functional groups in biosorption of lead (II) but also the possibility that biosorption could be taken place through ion exchange mechanism. When comparing the typical EDAX spectra of the lead (II) loaded biomass (Fig 10A) with that of unloaded biomass (Fig 10B), it was observed that the intensity of the phosphorus signal at about 2.1 keV was considerably reduced after biosorption. This could be indicative of the complexation of lead (II) ions with phosphate groups on the biomass sur-

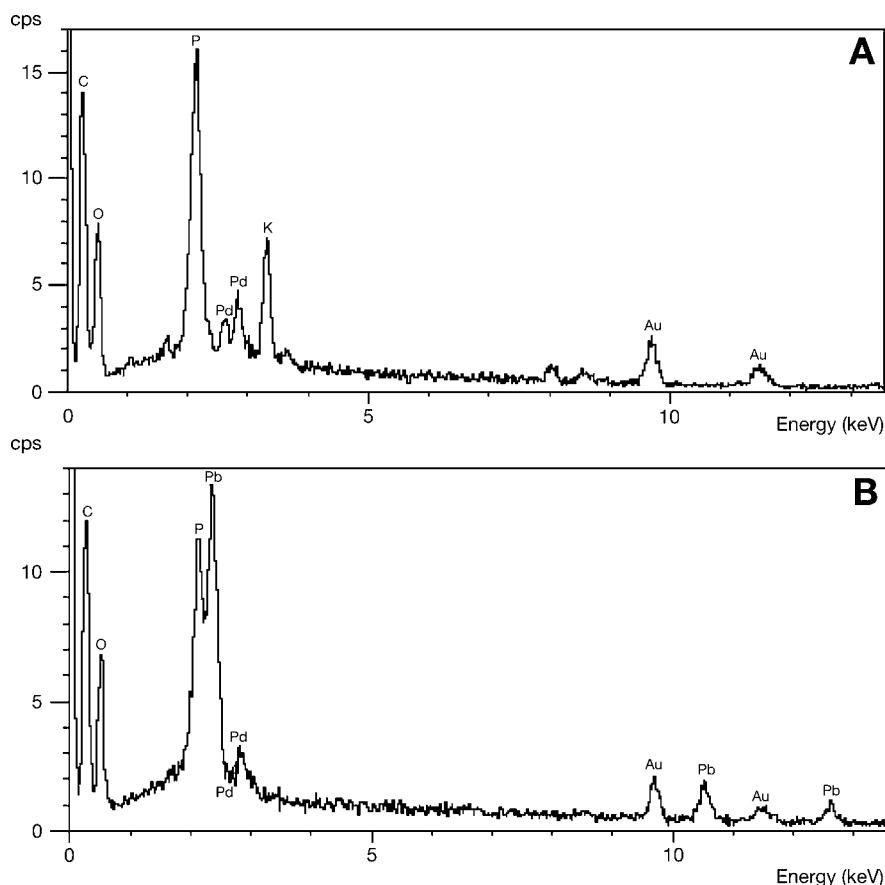


Fig. 10. Typical EDAX spectra of *Aspergillus parasiticus* before (A) and after (B) lead (II) biosorption.

face. Also, the appearance of lead (II) signal at about 2.2 keV and the disappearance of K^+ signal at about 3.4 keV were observed after lead (II) biosorption. These findings indicated that biosorption process also included ion-exchange mechanism for the removal of lead (II) ions by this strain which is confirmed by the E value obtained from D-R isotherm model.

Conclusions

The filamentous fungus, *A. parasiticus*, has a potential to be used as an alternative biosorbent material for the removal of lead (II) ions from aqueous solutions because of its easy cultivation, low cost, high biosorption capacity ($4.02 \times 10^{-4} \text{ mol g}^{-1}$), and reasonably rapid rate of biosorption.

The biosorption behavior of lead (II) ions onto *A. parasiticus* cells has been investigated with the variations in the parameters of pH, metal ion concentration, contact time and temperature. The experimental data were evaluated by Langmuir, Freundlich, and D-R isotherms and fit well to all

of the isotherm models. The biosorption process is also exothermic in nature. The regeneration and reusability of *A. parasiticus* would also enhance the economics of biosorption of lead (II) ions from wastewater. The interactions between metal ions and the functional groups on the cell wall surface of the biomass were confirmed by FTIR and EDAX analysis. Thus, the mechanism of lead (II) biosorption by *A. parasiticus* fungal biosorbent could be a combination of ion exchange and complexation with the functional groups present on the biosorbent surface.

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