Biosorption of lead by a soil isolate Aspergillus neoalliaceus

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Research Article

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**Abstract**

This study focused on Pb (II) elimination from aqueous solutions using fungal soil isolate which is identified as *Aspergillus neoalliaceus*. The sorption of lead with fungal mass studies was performed as a function of pH, biomass dose, contact time, and initial dye concentration. The solution pH value strongly affected the sorption of lead fungal mass. To examine the effect of hydrogen ions on biosorption in solutions containing lead, solutions with different pH values were used and pH 5 was found to be the most suitable pH value for lead removal. Lead biosorption was remained very low below pH 4 because of the competing effect of hydrogen ions in solution. It has been observed that the removal of lead ions based on biosorption with *Aspergillus neoalliaceus* is better explained by Langmuir isotherm and pseudo-second-order kinetic models compared to other used models.

**1. Introduction**

Along with the increase in industrialization, many environmental problems have emerged. Heavy metals play a major role in this environmental pollution burden. Heavy metal contamination of the environment including soil and water, which have a crucial role in the continuity of organism life, frequently emerges from human, agricultural and industrial activities. One of the most threatening pollutants as heavy metal is lead due to its accumulation in the environment [1].

Pb (II) becomes harmful to the central nervous system over the concentration of 0.05 mg/L. Besides, lead pollution can cause anemia, encephalopathy, hepatitis, and kidney diseases in humans [2].

The developments towards technological demands and industrial activities are the causes of Pb (II) releasing to the environment. Battery manufacturing, printing, metal-based processes are the main origins that play important role in the contamination of lead [3].

Many studies have been focused on the removal of heavy metal ions using various techniques. It has been shown that heavy metal removal is performed with various physicochemical techniques, examples of these techniques are membrane filtration, chemical precipitation, evaporation, and adsorption. [4]. However, these methods have several drawbacks associated with high reagent requirements, toxic waste generation, and unpredictable metal removal. [5]. In this context, research focused on biosorbent such as new environmentally friendly, economical, and effective metal adsorbents have been carried out by the application of microbial biomass [6].

Biosorption could be defined as the process of removing heavy metals using biological systems [7]. This removal platform has been reported as an easy and environmentally-friendly technique providing low-cost and effective removal capability [8]. Bio-active materials of bacteria, yeast, algae, fungi have been extensively used for the eliminating of metals from aqueous solutions [9]. Fungi have gained much more attention compared to bacterial strains and have strong potential due to their high removal potential against a large number of heavy metal ions [10, 11]. The functional residues on the cell wall of fungi such
as -COOH, -SH, -OH, -NH₂, -PO₄H₂ enable the interaction between the surface of the cell and target heavy metal [10, 12].

This study, it was aimed to perform Pb (II) biosorption using soil isolate fungal biomass as adsorbent.

2. Materials And Methods

2.1. Chemicals

Malt extract broth, sabroud dextrose agar, Lead nitrate (CAS No: 10099-74-8),

2.2. Isolation and identification of the fungus

Five grams of soil sample was suspended in 250 ml Erlenmeyer Flasks with malt extract broth. Afterward, the suspended soil sample in the growth medium was incubated at 25°C for 5 days. At the end of the incubation period, 0.1 ml was taken from the suspension and inoculated on sterile potato dextrose agar media. Fungal species were identified by ITS regions/18S rRNA sequencing. Genomic DNA was isolated with the EurX GeneMATRIX isolation kit (Poland). Spectrophotometric measurement was performed to check the purity of the DNA obtained after extraction (Thermo Scientific Nanodrop 2000). Samples were Sanger Sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and ABI 3730XL instrument (Applied Biosystems, Foster City, CA).

2.3. Characterization and zeta potential

The surface morphology of I heat-inactivated Aspergillus neoalliaceus was monitored with GAIA3 + Oxford XMax 150 EDS scanning electron microscope. Fungal samples characterized spectroscopically by 8000 Series (Shimadzu, Japan) FTIR spectrophotometer (Thermo Scientific).

Fourier transform infrared spectrums of lead-loaded and unloaded fungal masses were recorded between 4000 and 400 cm⁻¹ wavenumber regions. The net surface charge of heat-inactivated fungal biomass was analyzed by measuring zeta potentials at known pH values within 3.0 to 7.0.

2.4. Production of heat-inactivated biomass

Identified fungal species inoculated 1000 ml flasks with 500 ml malt extract broth and incubated for 5 days at 25°C (agitation rate:150 rpm). Pellets of Aspergillus neoalliaceus were separated from growth media via filter paper and collected. The fungal mass washed with distilled water was autoclaved for 15 minutes at 120°C to obtain heat-inactivated biomass. Heat-inactivated biomass was left to dry at 50°C for 2 days and ground to obtain a powder. powdered biomass was sieved through a 0.15 mm screen.

2.5. Pb²⁺ biosorption studies

Unless other was stated, all experiments were carried out in a 15 ml centrifuge tube containing 10 ml Pb²⁺ solution with 10 mg fungal biomass at 25 °C and stirred 150 rpm with a rotary shaker (Mikrotest, Turkey). Lead solutions were adjusted with 0.1 N NaOH and 0.1 N HCl solutions. The pH of the Pb (II) and 10 mg
biomass containing medium was adjusted from 2 to 7 to investigate the effect of medium pH on biosorption. To examine the effects of the amount of biomass, 5 mg to 50 mg dry heat-inactivated fungal biomass was added into Pb (II) solutions. The change of biosorption efficiency with a lead concentration in the medium was also studied by changing the metal concentration from 5 to 50 mg/L. To determine the effect of contact time on fungal sorption of Pb (II), samples were taken within the predetermined time intervals. Biomass-free solutions were prepared as a control for each run.

2.6. Pb (II) analysis

To determine lead biosorption capacity, Pb (II) solutions with biomass were filtered via syringe filters (0.45 μm). Initial and final metal concentrations in the solutions were determined by the Perkin Elmer Analyst 800 atomic absorption spectrometer in the flame module. The averages of three measurements were used in the calculations.

The Pb (II) sorption efficiency (Qe) and removal rate (%) of the fungal mass at equilibrium were calculated using the equations given below

\[
Q_e = \frac{[(C_i - C_e) V]}{m}. \text{Eq. (1)}
\]

\[
\text{Removal Rate (%) } = \frac{[(C_i - C_e) / C_i]}{\times 100}. \text{Eq. (2)}
\]

where \( Q_e \) is the amount of Pb\(^{2+} \) adsorbed by heat-inactivated dried fungal biomass at equilibrium (mg/g), \( C_i \) is the initial concentration of lead ions (mg/L), \( C_e \) is the equilibrium concentration of lead ions (mg/L), \( m \) is the amount of biomass in the lead solution (g), and \( V(L) \) is the volume of adsorption medium (L).

2.7. Isotherm studies

In isotherm studies, apart from the initial lead concentration, other variables such as pH, temperature, biomass dose, and contact time were kept constant. Ten milligrams of biomasses are put into each 10 ml Pb\(^{2+} \) solution which has different lead concentrations were used in studies. Following the biosorption period, lead concentrations of solutions were measured.

Since Langmuir and Freundlich's equations are the more common models used to describe the adsorption mechanism, these two isotherm models were used.

The Langmuir and Freundlich isotherm model is expressed by the equations given below [13]:

\[
\text{Langmuir: } C_e/Q_e = (Q_e/Q_{max}) + (1/Q_{max} \times K_L) \text{ Eq. (3)}
\]

\[
\text{Freundlich: } \ln Q_e = 1/n \ln C_e + \ln K_F \text{ Eq. (4)}
\]

2.8. Kinetics

Kinetic models pseudo-first order and pseudo-second-order kinetic models were tested for the biosorption of Pb\(^{2+} \) on biomass. Lagergren's equation, used for pseudo-first-order model [14]:

\[ \ln (q_e - q_t) = \ln(qe) - K_1 t \quad \text{Eq. (5)} \]

The equation for pseudo-second-order kinetic model [15]:

\[ \frac{t}{q_t} = \frac{1}{(K_2 q_e^2)} + \frac{t}{q_e} \quad \text{Eq. (6)} \]

3. Results And Discussion

3.1. Identification of fungus

The fungal species, isolated from soil was identified as *Aspergillus neoalliaceus* (GenBank Number: MH279421.1), based on the ITS regions/18S rRNA sequence. *Aspergillus* is a cosmopolitan genus of fungi that can live in a wide variety of environments [16].

3.2. FTIR analyses

FTIR spectrums of lead-loaded and unloaded fungal masses are given in figure 2. The broadband ranging from 3600 to 3000 may cause by the overlap of -OH and -NH stretching vibrations. The absorbance peaks from 3000 to 2800 cm\(^{-1}\) are belonged to -CH and -CH\(_2\) stretching vibrations. The peaks from 2363 to 2335 may represent –C=C– symmetry [17]. Absorbance peaks at 1742 (a) and 1743 (b) cm\(^{-1}\) may be attributed to C=O stretching vibrations. The adsorption band at 1643 cm\(^{-1}\)(a) that was shifted to 1632 cm\(^{-1}\)(b) after Pb\(^{2+}\) biosorption may be attributed to the C=O groups of primary amides playing a role in biosorption processes [13]. Amino and carboxyl groups play a major role in binding processes [18]. The band was observed at 411 cm\(^{-1}\) wavelengths, in lead-loaded biomass (b) may belong to the Pb-O stretching [19].

3.3. Effect of pH on biosorption

The pH of aqueous solutions is perhaps the most important factor for adsorption and biosorption studies. The pH value of the medium influences the ionization of functional groups (amino, phosphate, and carboxyl groups) on the cell wall [20]. In addition, the chemistry of the aqueous solution is closely related to the concentration of hydrogen ions in the environment. At high pH values, the solubility of heavy metals decreases significantly [10]. As depicted in Figure 3, little or no metal uptake was observed below pH 4.0. The maximum uptake capacity of Pb\(^{2+}\) biosorption was attained at pH 5.0 (19.49 mg/g). Low Pb\(^{2+}\) biosorption capacities were obtained at pH values less than 4.0 due to the protonation of functional groups on the cell wall [21]. Since the hydrogen ion concentration is high at low pH values, these ions compete with metal ions for active metal-binding regions. [20]. Hence adsorption capacity decreases with increasing H\(^{+}\) ions. The decrease in metal uptake at pH 6-7 can be explained by the formation of hydroxylated complexes of lead ions that compete with metal ions for active sites. [22]. Lead removal capacity of *Oceanobacillus profundus* increased with increasing pH up to pH 5 and then decreased [9]. The high yield of Pb\(^{2+}\) biosorption capacity with Moringa oleifera was observed in the range of pH 4-6 values [17]. Another study reported that the maximum lead removal was observed at pH 5
[23]. The different study reported that maximum Pb\(^{2+}\) biosorption with *Penicillium cryosogenum* was observed at pH 6 [24].

The heat-inactivated *Aspergillus neoalliaceus* biomass gains a negative charge at pH 5 (Fig. 1). The sudden increase in the metal uptake capacity of the biosorbent above pH 3 shows the importance of the surface charge. When fungus-based sorbent negatively charged at pH 5, biosorption of lead was reached maximum value. Although the biosorbent is negatively charged above pH 5, a decrease in its biosorption capacity was observed. A previous study attributed the decrease in lead adsorption over pH 5 to the hydroxylated lead compounds formed [22].

### 3.4. Effect of biomass dose

The dosage effect of *A. neoalliaceus* mass on the removal rate and \( Q_e \) were studied by varying the biomass concentrations ranging from 5 to 50 mg by keeping pH and the volume of the medium solution constant. The maximum biosorption efficiency was obtained with 1000 mg/L *A. neoalliaceus* biomass dosage. The biosorption capacity of Pb\(^{2+}\) ions was decreased with increasing biomass dosage in the solution. Contrary to biosorption capacity, the removal of heavy metal increases with increasing biomass dose (Fig. 4). The biosorption performance of divalent cations of Nickel, Copper, and Zinc decreased with increasing biomass amount in the solution. Also, the removal percentage of metals increased with increasing fungal biomass [25]. The increase in the removal percentage of heavy metal with increasing biomass can be attributed to an increase in the number of metal-binding active regions. In this study, maximum Pb\(^{2+}\) removal (93%) was achieved with a 1000 mg/L biomass dosage.

### 3.5. Effect of contact time and Kinetics

The altering lead concentration versus time is given in Figure 5, rapid biosorption was seen within the first 10 minutes. Nearly 85% of the lead ions in the medium were adsorbed the first 30 minutes and equilibrium was reached within 60 minutes. The rapid adsorption of lead was observed in the early stage because of an abundance of the active binding site on biosorbent, with lead ions binding with active sites, biosorption efficiency was decreased in the later stage [22]. Another study showed that 95% of lead ions are adsorbed within the first 60 minutes [23].

The validity of each model was evaluated by R Squared value \((R^2)\). The kinetics of Pb\(^{2+}\) biosorption onto heat-treated *Aspergillus neoalliaceus* biomass fits better to the pseudo-second-order kinetic model at pH 5 (Fig. 6). The values of pseudo-first-order and pseudo-second-order model parameters were given in Table 1.

**Table 1.** The first and second order kinetic model parameters
The theoretical Qe was found as 22.37 mg/g from the slope of the linear line. This value was determined as 22.47 mg/g experimentally. The theoretical Qe calculated for the pseudo-first-order was found to be 13.34, apparently quite far from the experimental Qe value. A different study reported that biosorption of lead with Pleurotus ostreatus biomass obeyed the pseudo-second-order kinetic model [26]. In another study, it has been reported that the pseudo-second-order kinetic model better explains the lead sorption phenomenon with *Penicillium simplicissimum* [20].

### 3.6. Effect of initial lead concentration

The lead uptake efficiency of fungal mass increased with increasing heavy metal concentration in the solution and achieved saturation at 40 mg/L Pb$^{2+}$ concentration (Fig. 7). The higher metal concentrations provide an impetus for processes involving biosorbent and aqueous phase transfers [27]. In this study, the highest metal uptake was observed at 40 mg/L initial metal concentration (29.95 mg/g).

### 3.7. Isotherms

The Freundlich and Langmuir isotherms models are two models that are widely used to explain biosorption mechanisms. The Freundlich equation is the oldest equation used to explain the adsorption phenomenon on heterogeneous surfaces [28]. The Freundlich adsorption isotherm explains the heterogeneous and multi-layered adsorption on the adsorbate surface [29]. The Langmuir adsorption isotherm explained the assumption of a fixed number of binding sites, no interaction between adsorbate molecules, all binding sites have the same energy level and monolayered adsorption [28].

According to experimental data, biosorption of Pb$^{2+}$ better fit Langmuir isotherm ($R^2$: 0.995) than Freundlich isotherm ($R^2$:0.898) at pH 5. Constants of isotherms were given in Table 2.

| Langmuir constants | Freundlich constants |
|--------------------|----------------------|
| $Q_{\text{max}}$ (mg/g) | $K_L$ (L/mg) | $R^2$ | $K_F$ (L/g) | n | $R^2$ |
| 32.05 | 0.69 | 0.995 | 10.86 | 1.93 | 0.898 |

In a study with activated carbon obtained from plant material, it was reported that lead adsorption followed the Langmuir isotherm [30]. Similar results were obtained with *Rhodococcus opacus* (bacteria)
and *Lepiota hystrix* (fungus) [23, 31]. On the other hand, a recent study reported that biosorption of lead with *Simplicillium chinense* was obeyed Langmuir isotherm model [32].

4. Conclusion

In this study, a fungal species which was obtained from soil and identified as *Aspergillus neoalliaceus* was used for the removal of lead ions, which are important to remove due to their harmful effects. Laboratory scale studies have shown that the parameter that most affects the removal efficiency of Pb (II) with fungal mass is the pH value of the solution, and the highest biosorption capacity is reached at pH 5. Studies conducted against time have shown that approximately 85% of lead uptake has been seen in 30 minutes. The fungal sorption of Pb (II) ions obeyed the Langmuir isotherm. Lead biosorption onto heat-treated *A. neoalliaceus* mass fits better to pseudo-second-order model, experimental and calculated Qe values were found to be very close to each other.

To sum up, it is thought that this biosorbent obtained from *Aspergillus neoalliceus* can be used in the removal of lead.

Declarations

Conflict of Interest: The authors declare that they have no conflict of interest

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Figures
Figure 1

Scanning electron micrograph of the heat-inactivated *A. neoalliaceus* biomass powder (2.5 kx).

Figure 2

FTIR spectra of pristine *Aspergillus neoallianceus* biomass (a) and Pb2+ loaded *Aspergillus neoallianceus* biomass (b)
Figure 3

The effect of pH on the Pb (II) biosorption of lead onto heat-inactivated fungal biomass (Initial concentration of lead: 20.0 mg/L, biomass amount: 10mg, volume: 10 mL, 25 °C. All data were reported as mean ± SD, n=3)

Figure 4
Effect of fungal sorbent dose on removal and biosorption of lead (Initial concentration of lead: 20.0 mg/L, volume: 10 mL, 25 °C, pH:5. All data were reported as mean ± SD, n=3)

Figure 5

Effect of contact time on biosorption of lead with heat-inactivated *A. neoalliaceus* biomass (Initial concentration of lead: 20.0 mg/L, volume: 10 mL, 25 °C, pH:5)

Figure 6

The graphs of pseudo-first-order (a) and pseudo-second-order kinetic model (b)
**Figure 7**

Effect of metal concentration on biosorption (biomass dose: 10 mg, volume: 10 mL, 25 °C, pH: 5, All data were reported as mean ± SD, n=3)