Biosorption characteristics of *Aspergillus niger* spores for removal of Pb (II) ions from an aqueous solution

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Abstract—Different from the conventional biosorbent, our preliminary results show that the fungal spores have the biosorption capacity to remove the heavy metals ions. In this study, the biosorption of Pb(II) ions onto inactive *A. niger* spores were investigated in a batch biosorption system. The effect of initial pH, contact time, initial Pb(II) ions concentration and biosorbent dosage was systematically investigated. The biosorption equilibrium was attained within 120 min. The maximum biosorption capacities of Pb(II) ions was found to be 45.95mg/g. The optimum of Pb(II) ions uptake was at pH5.0, 2.0g/L biosorbent dosage at 30°C. The significant changes of the spores' surface with or without-loading Pb(II) ions were showed by Scanning Electron Microscope. The results indicated that *A. niger* spores are a suitable biosorbent for the removal of Pb(II) ions from aqueous solution.

Keywords—fungi; *Aspergillus niger*; spores; lead; biosorption.

I. INTRODUCTION

The contamination of aqueous environment by heavy metals from industrial effluents is a worldwide environmental problem due to their toxic effects and accumulation through the food chain [1]. Consequently, it is important to remove the heavy metal ions by the adsorbents before the environment is polluted. Conventional treatment material mainly include various porous material [2], nanomaterials [3] and composite materials [4] for removal of heavy metals from aqueous solution which are not economical and generate huge quantity of toxic chemical sludge [5]. Compared with conventional methods, biosorption is considered as an alternative process that offers potential advantages such as low cost, minimization of excess sludge production, and high efficiency in detoxifying dilute effluents [6,7].

Lead is widely used in the production of batteries, being a alkyl additives for gasoline. Lead, cadmium and mercury are acute toxicity which form the “big three” of metals with the greatest potential hazard [5,8]. At present, quite a number of researches focused the effect of biosorption of heavy metals on *Aspergillus niger* [9]. *A. niger* hyphae are being proposed suitable sources of the biosorbent due to its advantages, such as simply and inexpensive media, easy to cultivation. However, my co-worker had only a report which is associated with the biosorption of chromium from an aqueous solution by *A. niger* spores. It has not yet been investigated that biosorption of *A. niger* spores for removal of Pb(II) ions from an aqueous solution. In the present study, *A. niger* spores uptake the Pb(II) ions was investigated as a function of initial pH, contact time, initial Pb(II) ions concentration and biosorbent dosage. The significant changes of the spores' surface with or without-loading Pb(II) ions were showed by Scanning Electron Microscope.

II. MATERIALS AND METHODS

A. Growth and preparation of biosorbent

A strain of *A. niger* was grown in Czapek's soild medium at 30 °C in a incubator for a week. The composition of the medium was (g/L): sucrose 30, NaNO₃ 3, KH₂PO₄ 1, MgSO₄ • 7H₂O 0.5, KCl 0.5, FeSO₄ 0.01, agar 20. The pH of the medium was adjusted to 6.0 using 1M hydrochloric acid. When the cultured cell was over, the spores of *A. niger* were thoroughly knocked down and used for biosorption studies.

B. Preparation of metal solutions

The test solutions containing single Pb(II) ions was prepared from the analytical grade chemicals. Pb(II) solutions used in this study was prepared from 1 g/L stock solution obtained by dissolving Pb(NO₃)₂ in deionized water. Batch studies were carried out in 250 mL Erlenmeyer flasks to elucidate the optimum operating conditions which enhanced Pb(II) biosorption.

C. Analysis method

The concentration of unadsorbed Pb(II) in the biosorption medium was determined at 283 nm by atomic absorption spectrometer.

D. Batch biosorption experiments

In this experiments, biosorption of Pb(II) from an aqueous solution containing a single metal ion was investigated. Optimun conditions (pH, contact times, initial metal ion concentration and biomass dosage

leves, respectively) for biosorption of Pb(II) were determined.

Over the course of this study, the biomass was added in the conical flask containing 50 mL Pb(II) aqueous solution. Then the conical flask was shaken for the different contact times in an electrically thermostatic reciprocating shaker at 150 rpm. Finally, the biomass was centrifugated and the supernatant was analyzed for the concentration of metal ions by using atomic absorption spectrometer (Persee TAS-990). The effect of pH on the biosorption of Pb(II) on spores of *A. niger* was investigated from 2.0 to 6.0. By using stock solution containing 1000 mg/L metal, 50mL solutions containing 100 mg/L Pb(II) was prepared. The initial pH of each metal solution was adjusted to the required pH value by using 1 M HNO₃. Then 0.1g of dried spores was added to the metal solution and the mixture was shaken at 150rpm and 30 °C for 3h, which was sufficient for biosorption equilibrium. Similarly, metal solutions with varing concentrations (ranging from 20 to 300 mg/L) were used to assess the effect of initial metal ion concentration. In the later experiments, the pH of the Pb(II) solutions was adjusted to 6.0. The contact time was varied for the determination of the optimal biosorption time. For this purpose, the flasks from an experimental set were removed at intervals of 30 min (up to 300 min). The effects of the biomass doses on the heavy metal ions adsorption were also investigated. A certain amounts of biomass doses (from 50 to 250 mg) were put into 50 mL heavy metal solutions. Their contents were centrifuged at 3600g for 5 min and the supernatant was analyzed for residual metal ion concentration. The amount of adsorbed metal ions per gram of biomass was obtained by using the general equation:

$$q = [(C_i - C_e) * V] / M \quad (1)$$

where q is the amount of metal ions biosorbed on the biomass (mg/g); C_i is the initial metal ion concentration in solution (mg/L); C_e is the equilibrium metal ion concentration in solution (mg/L); V is the volume of the medium (L); M is the amount of the biomass used in the reaction mixture (g).

E. Characterization of the Biosorbent

Surface morphology was studied with an electron microscope. The scanning electron micrographs (SEMs) of composite biosorbent were obtained with an Environmental Scanning Electron Microscope.

III. RESULTS AND DISCUSSION

A. Effect of initial pH on biosorption capacity

In this research, the effects of initial pH on biosorption capacity of *A. niger* spores was investigated

and showed in Fig 1. There is an increase in the adsorbed amount of Pb(II) ions with increasing pH from 2.0-5.0. The maximum biosorption was observed at pH 5.0. Several researchers have also investigated the effect of initial pH on the biosorption of Pb(II) ions by using different biomass and similar results were reported. For instance, Tamer Akar and Sibel Tunali found that the Pb(II) biosorption capacity of *Aspergillus flavus* increased with increasing pH of the solution and reached a constant value at pH 5.5 [10].

The pH of the biosorption medium affects the solubility of metal ions (i.e. hydrolysis, complexation by organic and/or inorganic ligands, redox reactions) and the ionization state of the functional groups (i.e. carboxylate, phosphate and amino groups) [11,12]. The observed from Fig 1. increase could be attributed to the strong relation of biosorption to the number of surface negative charges, which depends on the dissociation of functional groups. Relatively low biosorption capacity at pH 2.0 can be attributed to protonation of active sites, resulting in competition between H^+ and Pb^{2+} for occupancy of the binding sites [13]. At the pH values of greater than 6.0 the Pb(II) ions became precipitate as $Pb(OH)_2$ due to increasing concentration of OH^- ions in the solution. For this reason, the experiments were not conducted with higher pH values.

Fig 1 . Effect of pH on the Pb(II) biosorption on spores of *A. niger*

B. Biosorption time of Pb(II)

It has been determined that rapid biosorption of Pb(II) ions by biomass was observed in first 30 min , the Pb(II) biosorption capacity of biomass did not significantly change up to 120 min and then the biosorption of metal ions was very slow. (Fig 2.). After this equilibrium period the amount of adsorbed metal ions did not change significantly with an increase in contact time, the contact time was chosen at 120 min in this work. The rapid biosorption is a significant

parameter for large-scale application in industrial processes.

Figure 2 . Effect of contact time on the Pb(II) biosorption on spores of *A. niger*

C. Effect of initial Pb(II) ion concentration

The experiments were carried out using various concentrations of Pb(II) solution under the determined optimum pH values and contact time. The effect of initial Pb(II) ion concentration was investigated in the range of 20-300 mg/L. The results were presented in Fig. 3. The Pb(II) biosorption capacity of the biomass firstly increased with increasing of the initial concentration of Pb(II) ions concentration, becoming saturated at 200 mg/L for Pb(II) ions. Then the value did not significantly change with the initial Pb(II) ion concentration.

Figure 3 . Effect of initial Pb(II) ions concentration onto biosorption of the spores of *A. niger*.

D. Effect of biosorbent dosage

It is a important parameter that the dosage of biosorbent determines the capacity of biosorbent at a given initial concentration of Pb(II) ions. To determine the effect of the biosorbent dosage on the biosorption capacity of the spores of *A. niger* the amount of biosorbent added into metal solution were varied from

0.5 to 5.0 g/L. The maximum removal of the most Pb(II) ions was observed at an adsorbent dosage of 2.0 g/L in Fig. 4.

Figure 4 . Effect of biosorption dosage on the biosorption efficiencies for Pb(II).

E. Scanning electron microscope analysis

Fig. 5 shows the SEM images of the spores of *A. niger* before and after Pb(II) adsorption. The spores before adsorption are characterized as ellipse shape with smooth surface (Figure 5a). However, the spores changes after biosorption, such as the distortion with roughness surface (Figure 5b).

Figure 5a. Autoclaved spores of *A. niger*

Figure 5b. Spores of *A. niger* loaded with Pb(II)

Figure 5 .Scanning electron microscopes (SEMs) of spores

IV. CONCLUSIONS

The present investigation suggested that the *A. niger* spores without the aerial mycelium possess great potentiality as a low-cost, a high-efficient biosorbent and to be applied for the removal of heavy metals from aqueous solutions. The initial pH, contact time, initial metal ion concentration and biosorption dosage significantly influence the biosorption process while the maximum biosorption capacities of the spores for Pb(II) was found to be 45.95mg/g. The optimum for the removal of Pb(II) ions was at pH5.0, 2.0g/L biosorbent dosage and 120min.

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REFERENCES

- [1] Y. Ding, D. B. Jing, H. L. Gong, L. B. Zhou, X. S. Yang, "Biosorption of aquatic cadmium (II) by unmodified rice straw", *Bioresour. Technol.* vol. 114, Dec. 2012, pp.20-25, doi: 10.1016/j.biortech.2012.01.110
- [2] C. A. Bell, S. V. Smith, M. R. Whittaker, A. K. Whittaker, L. R. Gahan, M. J. Monteiro, "Surface-functionalized polymer nanoparticles for selective sequestering of heavy metals", *Adv. Mater.* vol. 18, Dec. 2006, pp. 582–586, doi: 10.1002/adma.200501712
- [3] X. Z. Lin, T. Y. Ma, Z. Y. Yuan, "Titania–silica–phosphonate triconstituent hybrid mesoporous materials as adsorbents in gas and liquid phases", *Chem. Eng.* vol. 166, Dec. 2011, pp. 1144–1151, doi: 10.1016/j.ccej.2010.11.086
- [4] Katherine, G. Dunphy, R. T. Margaret, F. B. Jillian, "Environmental risks of nanotechnology: national nanotechnology", *initiative funding.* vol. 40, Dec. 2000–2004, pp. 1401–1407, doi: 10.1021/es0515708
- [5] S. S. Ahluwalia, D. Goyal, "Microbial and plant derived biomass for removal of heavy metals from wastewater", *Bioresource Technology.* vol. 98, Dec. 2007, pp. 2243–2257, doi: 10.1016/j.biortech.2005.12.006

- [6] D. Kratochvil, B. Volesky, "Advances in the biosorption of heavy metals", *Trends Biotechnol.* vol. 16, Dec. 1998, pp. 291–300, doi: 10.1016/S0167-7799(98)01218-9
- [7] S. K. Sen, S. Raut, T. K. Dora, D. P. K. Mohapatra, "Contribution of hot spring bacterial consortium in cadmium and lead bioremediation through quadratic programming model", *Hazard. Mater.* vol. 265, Dec. 2014, pp. 47-60, doi:10.1016/j.jhazmat.2013.11.036
- [8] Volesky, "Removal and Recovery of Heavy Metals by Biosorption. Biosorption of Heavy Metals", CRC Press, Boca Raton, FL, Dec. 1990, pp. 3–43 doi:10.1021/bp00033a001
- [9] Park, Y. S. Yun, J. M. Park, "Use of dead fungal biomass for the detoxification of hexavalent chromium: screening and kinetics", *Process Biochemistry*, vol. 40, Dec. 2005, pp. 2559-2565. doi:10.1016/j.procbio.2004.12.002
- [10] Tamer, T. Sibel, "Biosorption characteristics of *Aspergillus flavus* biomass for removal of Pb(II) and Cu(II) ions from an aqueous solution", *Bioresource Technology*, vol. 97, Dec. 2006, pp. 1780–1787. doi: 10.1016/j.biortech.2005.09.009
- [11] F. Esposito, F. Pagnanelli, "pH-related equilibrium models for biosorption in single metal systems", *Chemistry. Engineering Science*, vol. 57, Dec. 2002, pp. 307-313. doi: 10.1016/S0009-2509(01)00399-2
- [12] M. Y. Arica, C. Arpa, A. Ergene, G. Bayramoglu, O. Genc, "Ca-alginate as a support for Pb(II) and Zn(II) biosorption with immobilized *Phanerochaete chrysosporium*", *Carbohydr. Polym.* vol. 52, Dec. 2003, pp. 167–174. doi: 10.1016/S0144-8617(02)00307-7
- [13] X. Ma, W. Cui, L. Yang, Y. Yang, H. Chen, K. Wang, "Efficient biosorption of lead(II) and cadmium(II) ions from aqueous solutions by functionalized cell with intracellular CaCO₃ mineral scaffolds", *Bioresource Technology*, vol. 185, Dec. 2015, pp. 70-78. doi:10.1016/j.biortech.2015.02.074