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THE EFFECT OF CELL SURFACE TREATMENT ON LEAD(II) BIOADSORPTION BY *PHANEROCHAETE CHRYSOSPORIUM*

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ABSTRACT. In this study the biosorption of Pb(II) ion from aqueous solution on non-living mycelial pellets of *Phanerochaete chrysosporium* treated with caustic, heat and ethanol was studied using batch technique with respect to initial concentration and temperature. *Phanerochaete chrysosporium* was grown in a liquid medium containing mineral and vitamin materials with a complex composition. The biomass of *P. chrysosporium* treated with ethanol revealed that it was mechanical stable and had increased adsorption capacity for Pb(II) compared to caustic, heat and untreated cells. Increasing temperature upto 32 ºC the bioadsorption capacity decreased for ethanol treated fungal cells, while it had not effect on bioadsorption by heat and untreated fungal biomass. For caustic treated fungal biomass the Pb(II) removal increased with increasing temperature.

Keywords: *bioadsorption, Pb(II), Phanerochaete chrysosporium, fungal cell surface treatment, temperature*

INTRODUCTION

Heavy metal pollution is one of the most important environmental problems today.

Heavy metals are toxic and hazardous materials and their increasing amount have been documented **[1,2]**. Various industries produce and discharge wastes containing different heavy metals into the environment such as energy and fuel production, iron and lead metallurgy, mining, smelting of metalliferous, surface finishing industry, fertilizer and pesticide industry and application, electroplating, electrolysis, electro-osmosis, leatherworking, photography, electic

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appliance manufacturing, metal surface treatment, aerospace and atomic energy installation etc. Metal as a kind of resource is becoming shortage and also brings about serious environmental pollution, threatening human health and ecosystem. **[1, 2]**.

Different technological methods have been developed to remove heavy metals from environment. At present, many technologies, such as sulfuration method, electrolysis, membrane and ion-exchange process, can be used for the treatment of wastewater polluted by heavy metals. However, these methods are less effective and more expensive when heavy metal concentration in the wastewater is low, and some of them can easily cause the second pollution. In recent years, applying biotechnology in controlling and removing metal pollution has been paid much attention, and gradually becomes hot topic in the field of metal pollution control because of its potential application.

Biosorption can be defined as the removal of metal or metalloid species, compounds and particulates from solution by biological material **(3).** Large quantities of metals can be accumulated by a variety of processes dependent and independent on metabolism. Both living and dead biomass as well as cellular products such as polysaccharides can be used for metal removal **(3).**

Removal of heavy metals by biosorption has many advantages, such as fast adsorption speed, removing heavy metal ions selectively under low concentration, high adsorption efficiency, wide range of pH and temperature, less investment and running cost, in addition, some heavy metals can be recovered. Biosorption, as an efficient treatment means, is gaining increasing attention.

In the concept of biosorption, several chemical processes may be involved such as adsorption, ion exchange, and covalent bonding with the biosorptive sites of the microorganisms **[6]**. Biomass cell walls, consisting mainly of polysaccharides, proteins and lipids offer many functional groups which can bind ions such as carboxylate, hydroxyl, sulphate, phosphate and amino groups.

A large quantity of materials has been investigated as biosorbents for the removal of metals or organics extensively. The tested biosorbents can be basically classified into the following categories: bacteria, fungi, yeast, algae, industrial wastes, agricultural wastes and other polysaccharide materials (2). Different kind of fungi can be used as natural biosorbent such as *Saccharomyces cerevisiae* **[2,7 - 10]**, *Aspergillus niger* **[13-14]**, *Polyporus ostreiformis* **[15]**, *Phanerochaete chrysosporium* **[16-19]**, *Trametes versicolor* **[15]**.

Phanerochaete chrysosporium is a well-known white-rot fungus and it has a strong ability to degrade various xenobiotics and exist in bleaching effluents from pulp and paper mills **[20]**. It could also be used to remove heavy metals from wastewaters by adsorbing the metals on its mycelium.

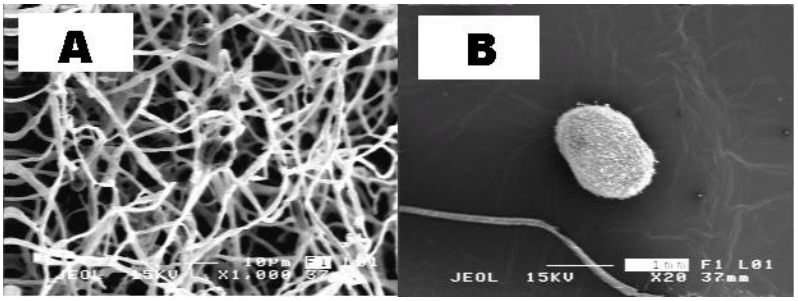

Figure 1. SEM image of *Phanerochaete chrysosporium* white-rot fungi.
A) The picture shows the mycelium of the fungi at a magnification of 1000 fold.
B) The picture shows the spherical-like fungal pellet at a magnification of 20 fold.

The treatment of native biomass improves its biosorption capacity, changes the possible binding groups **[21]**. Immobilization of these organisms improves mechanical strength, rigidity, size, porosity characteristics, and resistance to environmental restrains **[22, 23]**.

Kacar et al. (2002) have compared the biosorption capacity of alginate and immobilized live and heat inactivated *Phanerochaete chrysosporium* for Hg(II) and Cd(II) ions. The biosorption of Hg(II) and Cd(II) ions on the biosorbents depended on the experimental conditions, particularly on the pH of medium and the concentration of metals ion in the medium. Their experiment showed that the biosorption capacity decreased in order of non-living, living and immobilized cells **[15].**

Li et al (2004) studied the simultaneous biosorption of cadmium(II) and lead(II) ions by heat treated biomass of *Phanerochaete chrysosporium.* In the single-ion situation and under the optimum adsorption conditions (pH = 4.5), the maximum uptake was obtained as 15.2 mg g^{-1} at initial Cd(II) concentration of 50 mg l^{-1}, for Pb (II) ions it was 12.34 mg g^{-1}. The biomass concentration was 2 g l^{-1} in the suspension. In the binary metal solutions, both metal uptake and adsorption yield for one kind of ion decreased with increasing concentration of the other metal ion. Thus the interaction of Cd (II) and Pb (II) ions on their biosorption by *P. chrysosporium* was generally found to be antagonistic. The most logical reason for the antagonistic action was claimed to be the competition for adsorption sites of the cell surface. Pb (II) ions were found to be bound more effectively to *P. chrysosporium* than Cd (II) ions in binary metal solution **[23]**. Yetis et al. (2000) have studied the adsorption capacity of Pb(II) ion by living and non-living *P. chrysosporium* cells in aqueous solution. The resting cells of *P. chrysosporium* were able to uptake up to 80 mg Pb(II)/g dry cell at pH 5. However, live and dead cells exhibited lower capacities. The uptake capacity for Pb (II) ions of young resting fungal cells was higher than that of older fungal cells **[18].** Say, Denizli and Arica (2001) investigated the biosorption from artificial wastewaters of

Cu(II), Pb(II) and Cd(II) ions onto the dry fungal biomass of P. *Chrysosporium* fungal cells. The maximum adsorption of different heavy metal ions on the fungal biomass was obtained at pH 6.0 and the biosorption equilibrium was established after about 6 h. The experimental biosorption data for Cd(II), Pb(II) and Cu(II) ions were in good agreement with those calculated by the Langmuir model. The order of affinity for competitive conditions was as follows: Cu(II) > Pb(II) > Cd(II). This order was the same as in the non-competitive condition **[19]**.

Biosorption of heavy metals on the pretreated *P. chrysosporium* biomass has not yet been investigated completely. This paper is unique from the aspect that no one has compared the effect of different cell surface treatment of *Phanerochaete chrysosporium* on Pb(II) bioadsorption. The main objectives of the present study are the followings:

- using different pretreatment methods on *Phanerochaete chrysosporium* cells,
- comparison of the Pb(II) biosorption capacity by treated cells,
- study the effect of temperature on the bioadsoprtion process.

RESULTS AND DISCUSSION

The effect of ethanol, heat and NaOH treatment of non-living *Phanerochaete chrysosporium* mycelial pellets on Pb(II) biosorption was investigated in aqueous suspension. Figure 2a and 2b shows the effect of different treatments on Pb(II) biosoption capacity at 25 and 50 mg l^{-1} initial Pb(II) concentrations at 22^{o}C. The biomass concentration was 1 g l^{-1}. The adsorbed amounts of Pb(II) are shown in Figure 1a and the bioadsorption efficiencies (expressed in percent, %) of Pb(II) are shown in Figure 1b for different cell surface treatments in the case of both initial concentrations. The alteration of Pb(II) bioadsorption capacity on the effect of cell surface treatment was the same at both initial concentrations, although the bioadsorption capacities did not change significantly due to the morphology of mycelial fungal pellets. Non-living fungal cells were used as reference biosorbent. For untreated fungal cells at 25 mg l^{-1} Pb(II) concentration the bioadsorption capacity q_s is 16.34 mg g^{-1} (65.35 %), at 50 mg l^{-1} Pb(II) concentration q_s is 42.05 mg g^{-1} (84.11 %). The fungal cells treated with ethanol had the maximum bioadsorption capacity, at 25 mg l^{-1} initial concentration q_s is 16.91 mg g^{-1} (67.64 %), at 50 mg l^{-1} initial concentration q_s is 42.60 mg g^{-1} (85.20 %).

The caustic and heat treatment of cells slightly reduced the Pb(II) bioadsorption capacity in comparison with untreated cells. In the case of heat treated cells, at 25 mg l^{-1} Pb(II) concentration the bioadsorption capacity q_s is 15.68 mg g^{-1} (62.73 %), at 50 mg l^{-1} Pb(II) concentration q_s is 41.54 mg g^{-1} (83.07 %). In the case of caustic treated fungal cells, at 25 mg l^{-1} Pb(II) concentration the bioadsorption capacity q_s is 16.06 mg g^{-1} (64.22 %), at 50 mg l^{-1} Pb(II) concentration q_s is 41.31 mg g^{-1} (82.62 %).

The results show that increasing initial concentration of Pb(II) increased the adsorbed amount of Pb(II) and adsorption efficiency for Pb(II) by biomass. Göksungur et al. (2005) studied the biosorption of Pb(II) and Cd(II) ions from artificial aqueous solution using waste baker's yeast. The yeast cells were treated by ethanol, caustic and heat for increasing their biosorption capacity and the highest metal uptake values were obtained by ethanol treated yeast cells.**[9]**.

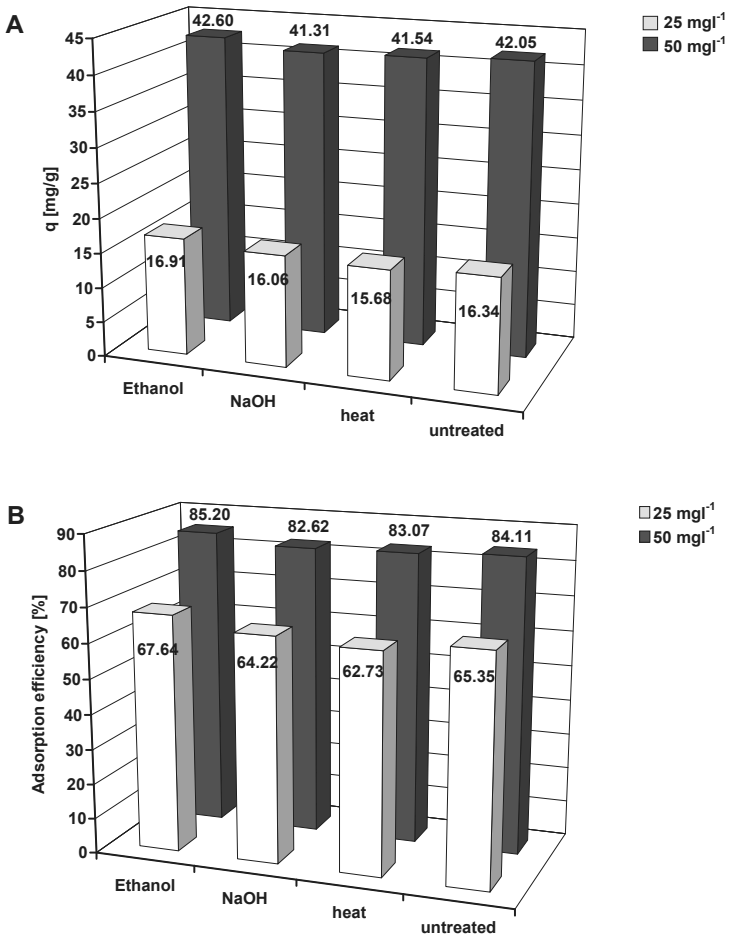

Figure 2: Effect of ethanol, heat, caustic treatment of fungal cells on Pb(II) bioadsorption process.
(a) The adsorbed amounts of Pb(II) and (b) the bioadsorption efficiencies are presented in the case of treated fungal cells.
Experimetal conditions: T = 22ºC, biomass concentration: 1 g l^{-1}, initial Pb(II) concentration: 25 and 50 mg l^{-1}, cell diameter: 0.5 – 1 mm, pH = 5.9.

The effect of temperature on Pb(II) bioadsorption by treated ***P. chrysosporium*** *biomass*

The effect of temperature on Pb(II) bioadsorption was also investigated at initial concentration of 50 mg l^{-1} at suspension concentration of 1 g l^{-1}. In Figure 3a the adsorbed amounts of Pb(II) and in Figure 3b the adsorption efficiencies for Pb(II) are presented in the case of treated fungal cells.

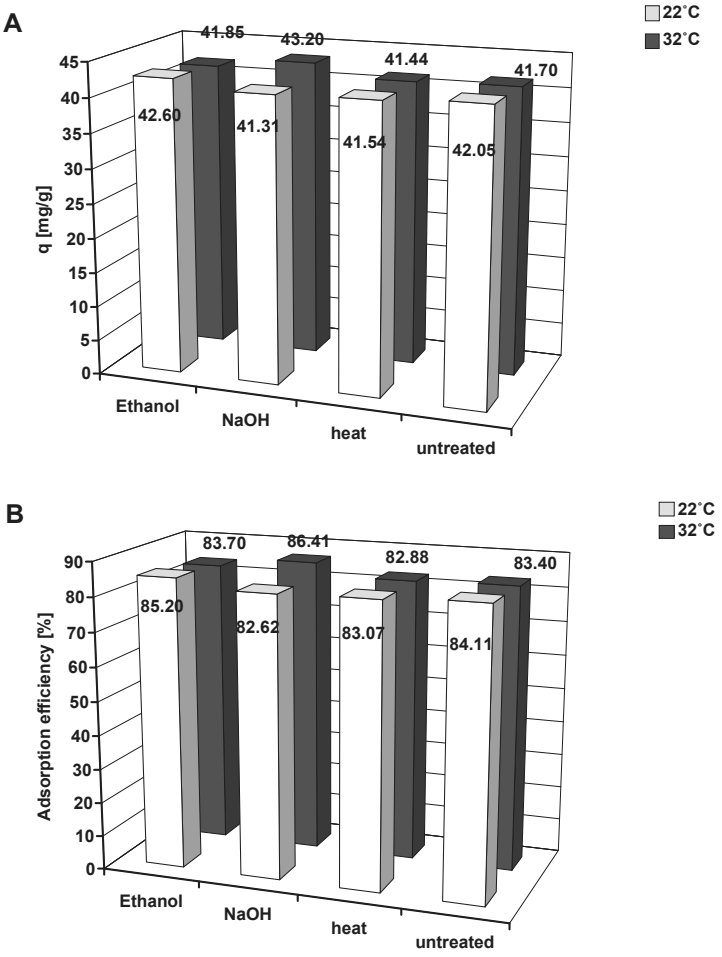

Figure 3. Effect of temperature on Pb(II) bioadsorption by ethanol, heat, caustic and untreated fungal cells. (a) The adsorbed amounts of Pb(II) and (b) the bioadsorption efficiencies are presented in the case of treated fungal cells. Experimetal conditions: T = 22.5 and 32 ºC, biomass concentration: 1 g l^{-1}, initial Pb(II) concentration: 25 and 50 mg l^{-1}, cell diameter: 0.5 – 1 mm, pH = 5.9.

The experimental results showed that increasing temperature slightly decreased the Pb(II) uptake by heat and ethanol treated fungal cells. This effect is well known and could be explained with the phenomena of ionic radius. The ionic radius of Pb (II) ion is 121 pm. A stronger physical affinity to Pb (II) ions was expected at the biosorption sites on the cells. This argument is consistent with the stronger temperature dependence of Pb (II) because physical adsorption forges a relatively loose and unstable combination that can be affected easily by thermal movement of the ions **[19]**.

For reference fungal cells at 22^{o}C the adsorption capacity q^s *i*s 42.05 mg g^{-1} (84.13 %), at 32^{o}C the adsorption capacity q^s *i*s 41.70 (83.40 %) mg g^{-1}. For heat treated fungal cells at 22^{o}C the adsorption capacity q^s *i*s 41.53 mg g^{-1} (83.07 %), at 32^{o}C the adsorption capacity q^s *i*s 41.44 mg g^{-1} (82.88 %). For ethanol treated fungal cells at 22^{o}C the adsorption capacity q^s *i*s 42.60 mg g^{-1} (85.2 %), at 32^{o}C the adsorption capacity q^s *i*s 41.85 (83.70 %) mg g^{-1}.

The heat treated biomass had the lowest adsorption capacity at 32^{o}C with 41.44 mg g^{-1}. Surprisingly the caustic treatment of fungal cells resulted a higher adsorption with 43.20 mg g^{-1} (86.41 %) at 32^{o}C in comparison with 41.31 mg g^{-1} (82.62 %) value at 22^{o}C The caustic treated biomass had the highest bioadsorption capacity for Pb(II) at 32^{o}C. This effect of caustic treatment on metal uptake was explained by the removal of protein groups of the cell wall that make non-adsorbable protein complexes with heavy metals **[25].**

CONCLUSION

In this study the effect of ethanol, heat and caustic cell surface treatment of *Phanerochaete chrysosporium* biomass on Pb(II) bioadsorption was studied at room temperature and elevated temperature (T = 32 ^{o}C). The ethanol treatment of fungal biomass resulted a higher adsorption capacity for Pb(II), while the caustic and heat treatment of cells slightly decreased the bioadsorption capacity at 22^{o}C. The experimental results showed that increasing temperature slightly decreased the Pb(II) uptake by heat and ethanol treated fungal cells, while the bioadsorption capacity of caustic treated fungal cells for Pb(II) increased in comparison with reference fungal cells. On the basis of results it can be revealed that the heat, ethanol and caustic cell surface treatment does not affect significantly the bioadsorption capacity of mycelial pellets of *Phanerochaete chrysosporium* for heavy metal due to the cell morphology. The treated and untreated *Phanerochaete chrysosporium* biomass can be used effectively as a biosorbent to remove Pb(II) from wastewaters.

EXPERIMENTAL SECTION

Chemicals

All chemicals were used in reagent grade. Stock Pb(II) solution were prepared by dissolving 0.799 g lead nitrate in 1 l of distilled water (500 mg l^{-1}). The Pb(II) concentration of the prepared solution varied between 25 and 50 mg l^{-1} for the biosorption experiments. The pH value was adjusted by using 0.1 M NaOH and HCl solutions.

Biomass cultivation

Phanerochaete chrysosporium was obtained from the Department of Environmental Microbiology, Faculty of Science, University of Pécs, Hungary was used in this study. It was cultivated as prevously described by Kirk et al **[24].** After 5 day incubation at 35^{o}C on a shaker (app. 180 rpm), the mycelial pellets were removed from the medium through filtration and inactivated in a pressure cooker at high temperature (120^{o}C) for 20 min.

Then, the mycelial pellets were washed several times with distilled water. These mycelial pellets were treated in the next step.

Preparation of treated biomass

Heat, caustic, ethanol treated *P. chrysosporium* fungal biomass were used in this study. For ethanol treatment 5 g of fungi was dissolved in 70 % (v/v) ethanol solution for 20 min. Then washed several times with distilled water 1 g of fungal cells was dissolved in 20 ml of 1 mol dm^{-3} NaOH solution for 15 min in the case of caustic treatment. For heat treatment 1g of fungi was put in 20 ml of distilled water and dried for 15 min at 120^{o}C in oven, so the heat treatment of biomass was duplicated.

Morphological study with Scanning Electron Microscope (SEM)

SEM studies were investigated in the Central Electron Microscope Laboratory, Faculty of Medicine, University of Pécs. Jeol JSM-6300 scanning electron microscope was used in this study.

Samples were only liophilized as a drying procedure before golden layer labelling. No further fixation procedure were done during the sample preparation protocol.

Study of bioadsorption

Biosorption experiments were carried out in batch mode to determine the adsorption capacity of the treated *Phanerochete chrysosporium* biomass. The biomass concentration was 1 g l^{-1}, 0.025 g dry mycelial pellets were mixed with 25 ml of solution containing a pre-determined concentration of

Pb(II) ion. Mycelial pellets and lead solution were placed in a test-tube. Tubes were agitated on a shaker at 150 rpm at constant temperature (22.5 and 32 ± 0.2°C). All experiments was performed at 22 and 32°C.

Samples were taken after 12 hours when the solution reached the equilibrium. Then they were centrifuged at 10 000 rpm for 10 min. The supernatant was used for analysis of the residual lead.

The adsorbed amount of lead(II) ion at equilibrium, q (mg/g), was obtained as follows:

$$q = \frac{(c_0 - c_e)V}{m} \quad (1)$$

where,

c_0 and c_e are the initial and equilibrium liquid phase concentrations (mg l^{-1})
V is the volume of the solution (l) and
m is the weight of the dry biomass used (g).

Analysis

Atom absorption spectrophotometer (AAS) was used to determine the equilibrium concentration of Pb(II) in the solution using standard calibration curve. Lead nitrate dissolved in nitric acid as standard solution (1000 mg/l) was from Scharlau Chemie (Germany). The Perkin Elmer AAS system was contained an Interlocked Gas Control System, and a wavelenght drive. The experiments were performed on an UV/VIS photo diode array detector at a wavelenght of 217 nm.

Standard calibration curve in the range of 1 -10 mg l^{-1} was made from lead stock solution (Scharlau, 1000 mg l^{-1}).

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