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Pb^{2+} Biosorption by Pretreated Fungal Biomass

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Abstract: The effect of pretreatment on the Pb^{2+} biosorption capacity of fungal biomasses, *Aspergillus versicolor*, *Metarrhizium anisopliae* var. *anisopliae*, and *Penicillium verrucosum*, was investigated. For this purpose, the biomasses were subjected to physical treatments such as heat and autoclaving, and chemical treatments such as sodium hydroxide, formaldehyde, gluteraldehyde, acetic acid, hydrogen peroxide, commercial laundry detergent, orthophosphoric acid and dimethyl sulfoxide.

Dimethyl sulfoxide, hydrogen peroxide and gluteraldehyde increased biosorption of Pb^{2+} in comparison with the *A. versicolor* live biomass. *M. anisopliae* var. *anisopliae* biomass pretreated with hydrogen peroxide, gluteraldehyde, commercial laundry detergent, dimethyl sulfoxide and formaldehyde significantly improved biosorption of Pb^{2+} in comparison with live biomass. Pretreatment with all methods of *P. verrucosum* increased biosorption of Pb^{2+} in comparison with live biomass. The maximum biosorption capacity of *A. versicolor* biomass subjected to dimethyl sulfoxide was 30.6 mg g^{-1}.

Key Words: *Aspergillus*, *Metarrhizium*, *Penicillium*, pretreatment, fungal biomass, lead.

Ön İşlem Görmüş Fungal Biyokütle ile Pb^{2+} Biyosorpsiyonu

Özet: Bu çalışmada *Aspergillus versicolor*, *Metarrhizium anisopliae* var. *anisopliae*, *Penicillium verrucosum*, fungal biyokütlelerinin Pb^{2+} biyosorpsiyon kapasitesi üzerine ön işlemlerin etkisi araştırıldı. Bu amaçla, biyokütleler ısı ve otoklav gibi fiziksel işlemlere, ve sodyum hidroksit, formaldehit, gluteraldehit, asetik asit, hidrojen peroksit, ticari çamaşır deterjanı, orto fosforik asit ve dimetil sülfoksit gibi kimyasal işlemlere maruz bırakıldı.

A. versicolor canlı biyokütlesine kıyasla dimetil sülfoksit, hidrojen peroksit ve gluteraldehitle işlem görmüş biyokütlelerin Pb^{2+} biyosorpsiyonunda artma olduğu bulundu. Hidrojen peroksit, gluteraldehit, ticari çamaşır deterjanı, dimetil sülfoksit ve formaldehit ile ön işlem görmüş *M. anisopliae* var. *anisopliae* biyokütleleri canlı biyokütleye oranla önemli derecede Pb^{2+} biyosorpsiyonunu artırdı. *P. verrucosum*'a uygulanan bütün ön işlemler canlı biyokütleye kıyasla Pb^{2+} biyosorpsiyon kapasitesini artırdı. En fazla biyosorpsiyon kapasitesi dimetil sülfoksitle ön işlem görmüş *A. versicolor* ile 30,6 mg g^{-1} olarak bulundu.

Anahtar Sözcükler: *Aspergillus*, *Metarrhizium*, *Penicillium*, ön işlem, fungal biyokütle, lead.

Introduction

Fungi, in common with other microbial groups, can accumulate metals from their external environment by means of physico-chemical and biological mechanisms. Biosorption is a process that utilizes inexpensive dead biomass to sequester toxic heavy metals and is particularly useful for the removal of contaminants from industrial effluents. Biosorbents are prepared from the naturally abundant and/or waste biomass of algae, moss, fungi or bacteria that have been killed while the biomass is pretreated by washing with acids and/or bases before final drying and granulation [1-3].

Nonviable microbial biomass frequently displays a higher affinity for metal ions compared with viable biomass, probably due to the absence of competing protons produced during metabolism. To avoid the problems of metal toxicity for microbial growth, or inhibition of metal accumulation by nutrient or excreted metabolites, the decoupling of the growth of the biomass from its function as a metal-sorbing material is seen as one of the major advantages of biosorption [4,5].

The use of dried, nonliving or chemically pretreated microorganisms seems to be a preferred alternative to the use of living cells in industrial applications for the removal of heavy metal ions from wastewater. The use of

dead cells offers the following advantages over live cells: the metal removal system is not subject to toxicity limitations, there is no requirement for growth media and nutrients, the biosorbed metal ions can be easily desorbed and biomass can be reused, and dead biomass-based treatment systems can be subjected to traditional adsorption models in use. As a result, the use of dead fungal biomass has been preferred in numerous studies on biosorption of toxic metal ions from aqueous solutions [6,7].

Living cells can be pretreated using physical or chemical means in order to increase metal biosorption capacity. Physical pretreatment methods have included heat treatment, autoclaving, freeze drying, and boiling. Chemical pretreatment methods, such as introducing fungal cells to acids, alkali and organic chemicals, showed enhancement of metal biosorption by different fungal biomasses [7-12].

The aim of this study was to investigate the effect of physical and chemical pretreatment of different fungal biomasses on biosorption of Pb^{2+}.

Materials and Methods

Microorganisms, medium and culture conditions

Aspergillus versicolor, *Metarrhizium anisopliae* var. *anisopliae*, *Penicillium verrucosum* were isolated from soil and identified in a previous study [13]. These fungal cultures were routinely maintained on potato dextrose agar (Merck). To produce the biomass for biosorption experiments, the seed cultures were prepared by loop inoculation and incubating the fungus in 100 ml of liquid medium (M1) at 25 °C on an orbital shaker at 130 rpm. The M1 medium contained (in g l^{-1}) sucrose, 20; bacto peptone, 5; neopeptone, 5; KH_2PO_4, 1; $NaNO_3$, 1 and $MgSO_4.7H_2O$, 0.5 [1]. After 24 h of incubation, 10 ml of the seed culture was transferred into 100 ml of M1 medium. The cultures were grown at 25 °C on an orbital shaker at 130 rpm for 1 week. All culture work was conducted aseptically. The fungal biomass was then harvested by filtration, washed with generous amounts of deionized water, resuspended and washed again.

Pretreatment of biomass

The live biomass so obtained will be referred to as type A in this paper. Thirty grams of wet biomass A was then pretreated in 9 different ways as described below:

- dried at 60 °C for 12 h in an incubator (type B),
- autoclaved for 15 min at 121 °C, 15 psi (type C),
- boiled for 15 min in 500 ml of 0.5 N sodium hydroxide solution (type D),
- boiled for 15 min in 500 ml of 15% (vol/vol) formaldehyde solution (type E),
- boiled for 15 min in 200 ml of 10% (vol/vol) acetic acid solution (type F),
- boiled for 15 min in 500 ml of 2% (vol/vol) gluteraldehyde solution (type G),
- boiled for 15 min in 300 ml of 10% (vol/vol) hydrogen peroxide solution (type H),
- boiled for 15 min in 500 ml of water in which 2.5 g of commercial laundry detergent was dissolved (type I),
- boiled for 15 min in 200 ml of 50% (vol/vol) dimethyl sulfoxide solution (type J),
- boiled for 15 min in 200 ml of 10% (vol/vol) σ-phosphoric acid solution (type K).

After each pretreatment with chemicals the biomasses were washed with generous amounts of deionized water and then dried at 60 °C for 12 h. The sodium hydroxide-pretreated biomass was washed with deionized water until the pH of the solution was in a near neutral range (pH 6.8-7.2).

Biosorption studies

Biosorption experiments were carried out using Pb^{2+} containing solutions added in the form of $Pb(NO_3)_2$ prepared in distilled water. The inlet-Pb^{2+} concentration in the solution was about 100 mg l^{-1}. Biosorption experiments were carried out adding dry biomass (0.15 g) in response to wet biomass (1 g). Known amounts of biomass were introduced to Pb^{2+} solution at pH 5.5. The reaction mixture was agitated at 130 rpm on a rotary shaker. After 180 min of contact time the biomass was separated by filtering the reaction mixture and the filtrate was analyzed for metal concentration.

Measurement of metals

Lead ion concentration in the solution was measured with a Perkin Elmer 3110 atomic absorption spectrometer. Before measurement, the solutions containing Pb^{2+} were appropriately diluted with deionized water to ensure that the Pb^{2+} concentration in the sample

was linearly dependent on the absorbance detected. Biosorption experiments were conducted in triplicate and average values were used in the analysis. The amount of Pb^{2+} (mg) biosorbed per gram of dried biomass was calculated using the following equation:

$$Q = \left(\frac{C_0 - C}{m}\right) . V$$

where Q = mg of metal ion biosorbed per gram of biomass; C_0 = inlet-metal ion concentration, mg l^{-1}; C = final metal ion concentration, mg l^{-1}; m = dry weight of biomass in the reaction mixture, g and V = volume of the reaction mixture, l.

Results and Discussion

The findings related to Pb^{2+} biosorption by live and pretreated fungal biomasses are presented in the Figure for each fungus. The live biomass of *A. versicolor* had the highest biosorbent activity for lead ions among the species studied. This was followed by *M. anisopliae* var. *anisopliae* and *P. verrucosum* live biomass. These species belong to different genera, and so their chemical characteristics are different from each other. Analyses of cell walls of certain fungi imperfecti reveal additional differences in composition. The isolated cell wall of *A. niger*, for instance, consists chiefly of neutral carbohydrate (73% to 83%) and hexosamine (9% to 13%), with smaller amounts of lipid (2% to 7%) and protein (0.15% to 2.5%). Purified cell wall preparations of the imperfect fungus *P. chrysogenum* consisted of at least 2 layers and contained the largest proportion of glucose, followed by glucosamine, galactose, and mannose in molar ratios of 9:4:5:3:1. Approximately 2% of the wall is protein [14]. Differences between the chemical characteristics of *A. versicolor* and *P. verrucosum* cells, belonging to the genera *Aspergillus* and *Penicillium*, respectively, may be one of the reasons why the metal biosorbent performances of these fungi are different.

The sequestering of metallic species by fungal biomass, which constitutes the basis of its biosorbent behavior, has mainly been traced to the cell wall. The cell wall is not necessarily the only site where the sequestered metals are located. They may also be found within the cell, associated with various organelles, or may crystallize in the cytoplasm [14]. The drying and then grinding of

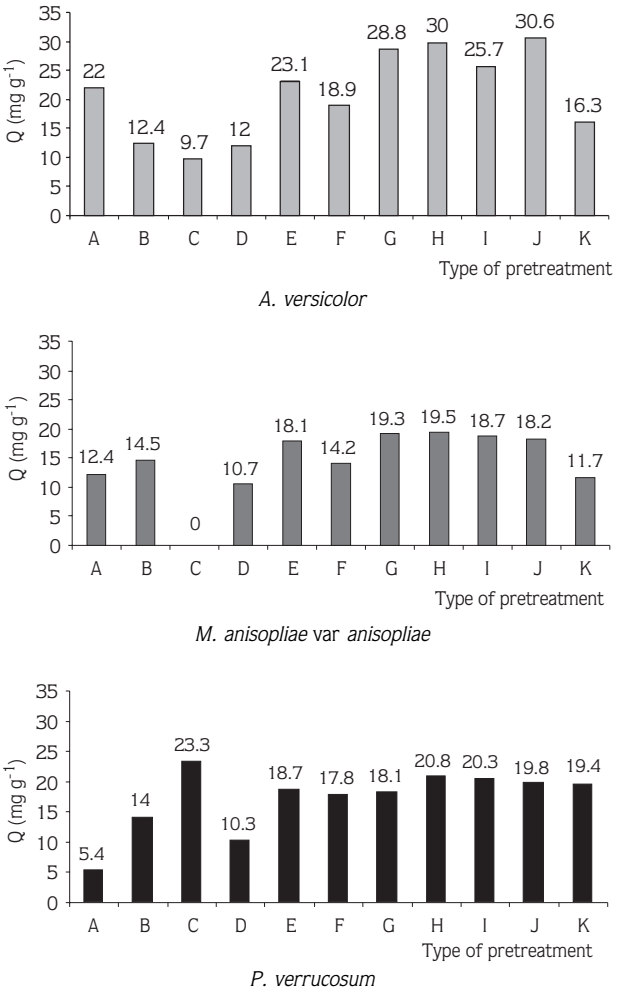

Figure. Pb^{2+} biosorption by live and pretreated biomasses. A: live, B: heat, C: autoclaved, D: NaOH, E: formaldehyde, F: acetic acid, G: gluteraldehyde, H: H_2O_2, I: detergent, J: DMSO, K: σ-phosphoric acid.

fungal biomass reveal sites where metal ions could be sequestered, and so increase the probability of encountering metal ions and such sites.

The findings showed that most of the pretreated fungal biomasses (types B-K) had high biosorption capacity in comparison with live cells (type A) for lead ions (Figure). An increase in biosorption of lead ions as a result of pretreatment could be due to an exposure of active metal binding sites embedded in the cell wall or chemical modifications of the cell wall components. Huang and Huang stated that the increase in metal biosorption after pretreating the biomass could be due to the removal of surface impurities and to the exposure of available binding sites for metal biosorption [15].

Biosorption of Pb^{2+} by pretreated *A. versicolor* either increased or decreased depending on the pretreatment method in comparison with biosorption using live biomass. Pretreatment with formaldehyde, gluteraldehyde, hydrogen peroxide, commercial laundry detergent and dimethyl sulfoxide resulted in an improvement in Pb^{2+} biosorption. Acetic acid and σ-phosphoric acid pretreatment reduced biosorption of Pb^{2+} to a certain extent while autoclaving, heat, and sodium hydroxide pretreatment significantly reduced biosorption of Pb^{2+}. It was observed that the Q values obtained were quite high for all the pretreated *M. anisopliae* var. *anisopliae* biomass in comparison with live biomass, except for autoclave, sodium hydroxide, and σ-phosphoric acid. Autoclave pretreatment completely inhibited Pb^{2+} biosorption. Pretreatment of *P. verrucosum* biomass with autoclave, hydrogen peroxide, gluteraldehyde and DMSO increased biosorption of Pb^{2+} by approximately four times in comparison with live biomass while pretreatment with heat, sodium hydroxide, formaldehyde, acetic acid, gluteraldehyde and σ-phosphoric acid also increased biosorption of Pb^{2+}.

The present results demonstrate that formaldehyde, gluteraldehyde, hydrogen peroxide, detergent and DMSO pretreatment increased Pb^{2+} biosorption for each fungal biomass in comparison with live cells. Among the fungal biomasses, *A. versicolor* biomass subjected to DMSO was found to have the highest biosorption capacity (30.6 mg g^{-1}) for Pb^{2+} (Figure). Heat and autoclave pretreatment increased Pb^{2+} biosorption for *P. verrucosum* whereas *A. versicolor* biomass was negatively affected by these pretreatments. However, pretreatment of *M. anisopliae* var. *anisopliae* biomass using heat increased Pb^{2+} biosorption while autoclave pretreatment completely inhibited it.

The reduction of biosorption capacity in heat and autoclaved *A. versicolor* and autoclaved *M. anisopliae* var. *anisopliae* biomasses may be attributed to the loss of intracellular uptake. Yan and Viraraghavan reported that Mucor rouxii biomass pretreated with autoclave reduced the biosorption of heavy metals [16]. In the same way, Kapoor and Viraraghavan reported that *A. versicolor* pretreated with autoclave reduced the biosorption of cadmium, copper, and nickel [7]. However, some researchers reported that heat and autoclave pretreatment increased the biosorption capacity of microbial biomass. According to Galun et al., this increase was due to the exposure of latent binding sites after pretreatment [17].

Pretreatment with detergent and DMSO significantly improved biosorption of Pb^{2+} for each fungal biomasses in this study. However, pretreatment of live *P. verrucosum* biomass using NaOH increased biosorption of Pb^{2+}, while pretreatment of *A. versicolor* and *M. anisopliae* var. *anisopliae* biomass using NaOH reduced it. Biosorption of Pb^{2+} by biomass of *P. verrucosum* pretreated with sodium hydroxide was approximately 2 fold higher than that of live biomass. Similar results were reported by Ashkenazy et al., who showed that Pb^{2+} biosorption by *Saccharomyces uvarum* was more efficient after sodium hydroxide treatment [18]. The explanation they offered is that the increase in the metal uptake after the protein removal steps is brought about by the unmasking of some of the cellular groups, which cannot participate in the sorption process without treatment with alkali.

Some researchers reported that alkali pretreatment significantly enhanced biosorption capacity in comparison with live cells [6,16,18,19]. In contrast, Kapoor and Viraraghavan (1998) reported that in nickel biosorption by sodium hydroxide pretreatment there was an approximately 45% reduction in comparison with live cells [7]. As a result of sodium hydroxide treatment, the number of protein amino groups that can be engaged in metallic ion binding markedly decreased. Deproteination should, theoretically, reduce metal retention. Since the cell wall composition can be characteristic of the fungal species, we may say that this conflict is normal. For example, the cell wall of *Aspergillus* species contains 8.3% protein.

Most commercial detergents also contain alkali as one of their ingredients. According to Yan and Viraraghavan this could be the reason why the alkaline detergent pretreatment resulted in an enhancement of biosorption of metal ions [16]. So we may say that the effect of weak alkali DMSO on the biosorption capacity of live biomass could vary in a similar way to those of sodium hydroxide and detergent.

Kapoor and Viraraghavan reported that biosorption of lead, cadmium and copper ions by *A. niger* was more efficient after formaldehyde treatment [7]. Similarly, in this study, the formaldehyde pretreatment increased the Pb^{2+} biosorption of each fungal biomass (Figure).

However, Huang and Huang suggested that when biomass was pretreated with formaldehyde, methylation of the amino groups present in the cell wall significantly reduced biosorption capacity [15]. It should be noted that in their study living biomass was only mixed, not boiled during formaldehyde pretreatment. The difference in results may be due to a specific pretreatment.

Gluteraldehyde is a cross-linking reagent with multi-functional groups. According to Jianlong, gluteraldehyde pretreated *Saccharomyces cerevisiae* biomass retains almost all its original biosorption capacity [20]. In this study, gluteraldehyde pretreatment significantly increased Pb^{2+} biosorption of each fungal biomass. The differences in the results may be due to fungal cell wall structure. While the vast majority of fungi have a chitin-glucan cell wall, *S. cerevisiae* possesses a mannan-glucan cell wall, which contains only 1% chitin.

Acid treatment (Types F and K) demonstrated variable results in this study. Both acid treatment procedures increased the Pb^{2+} biosorption capacity of *P. verrucosum*. However, acetic acid and σ-phosphoric acid pretreatment significantly reduced biosorption of Pb^{2+} by *A. versicolor*. Pretreatment using acetic acid resulted in an improvement in Pb^{2+} biosorption, but σ-phosphoric acid pretreatment little reduced Pb^{2+} biosorption for *M. anisopliae* var. *anisopliae*. Some researchers reported that acid treatment significantly reduced the biosorption capacity of heavy metals [7,16]. Nevertheless, Huang and Huang reported that acid pretreatment strongly enhanced the adsorption capacity for *Aspergillus oryzae* mycelia [15]. According to Yan and Viraraghavan, the difference in results after a specific pretreatment may be attributed to the different strains of fungi and whether the biomass was live or dead when used in biosorption of metal ions [15]. Our findings support this opinion.

Conclusion

According to the results, it is obvious that *A. versicolor*, *M. anisopliae* var. *anisopliae*, and *P. verrucosum* biomasses subjected to physical and chemical methods can be used for the removal of lead ions from aqueous solutions. If the removal of Pb^{2+} ions from aqueous solutions is required, then it may be advantageous to use *A. versicolor* pretreated with dimethyl sulfoxide.

More information on biosorption is required to determine the best combination of metals, biomass types and other conditions. Moreover, further detailed studies should be conducted in order to clarify the causes of enhancement or decrease in adsorption capacity for microbial biomasses.

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