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EFFECT OF PRETREATMENT ON BIOSORPTION OF HEAVY METALS BY FUNGAL BIOMASS

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Abstract: One of the recent developments in biotechnology is the identification of a new type of adsorbents of biological origin which have high sequestering capacity for organic or inorganic pollutants. The effect of pretreatment on the heavy metal biosorption capacity of *Penicillium lanosa-coeruleum* biomass was investigated as a new biosorbent. The biomass was subjected to heat and chemical treatments including sodium hydroxide, formaldehyde, gluteraldehyde, acetic acid, hydrogen peroxide, detergent and dimethyl sulfoxide to study their effects on biosorption of copper, lead and nickel.
It was found that heat, sodium hydroxide and detergent pretreatments significantly improved biosorption of lead (27%) and copper (106%, 95%, 162%) whereas gluteraldehyde increased nickel biosorption (72%) in comparison with the living biomass. The fungal biomass of *P. lanosa-coeruleum* may be applied to develop an inexpensive, effective biosorbent for removing lead, copper and nickel from waste waters.

Key words: Biosorption; pretreatment; fungal biomass; heavy metals.

Ağır Metallerin Fungal Biyokütle İle Biyosorpsiyonu Üzerine Ön İşlemin Etkisi

Özet: Biyoteknolojide son gelişmelerden biri de organik ve inorganik kirleticileri yüksek oranda tutma kapasitesi olan biyolojik kökenli yeni adsorbentlerin belirlenmesi yönündedir. Yeni bir biyosorbent olarak *Penicillium lanosa-coeruleum* biyokütlesinin ağır metal biyosorpsiyon kapasitesi üzerine ön işlemlerin etkisi incelenmiştir. Bakır, kurşun ve nikel iyonlarının biyosorpsiyonu üzerine etkisini incelemek üzere, biyokütle ısı ve kimyasal işlemlere (sodium hidroksit, hidrojen peroksit, ticari çamaşır deterjanı ve dimetil sulfoksit) maruz bırakılmıştır.
Canlı biyokütle ile kıyaslandığında, biyokütlenin ısı, sodyum hidroksit ve deterjanla ön işlemi kurşun (%27) ve bakır (%106, %95, %162) biyosorpsiyonunu önemli oranda artırırken, glutaraldehitle ön işlemi nikel (%72) biyosorpsiyonunu artırmıştır. *P. lanosa-coeruleum* biyokütlesi, atık sulardan kurşun, bakır ve nikel giderimi için düşük maliyetli, etkili bir biyosorbent geliştirilmesinde kullanılabilir.

Anahtar kelimeler: Biyosorpsiyon; ön işlem; fungal biyokütle; ağır metaller.

Introduction

The waste waters discharged from chemical industries which may contain heavy metal ions have toxic effect on all living organisms. Because of this, disposal of them to the environment is a major threat to both human health and ecosystem (Azab and Peterson, 1989). So the development of new technologies is required to treat waste waters as an alternative to traditional physichochemical processes. Biosorption, the process of passive cation binding by dead or living biomass, represents a potantially cost- effective way of eliminating toxic heavy metals from industrial waste waters.

The uptake of heavy metals by biomass is usually classified into three categories: (1) cell surface binding, (2) intracellular accumulation and (3) extracellular accumulation. Being metabolism independent, the cell surface binding can occur in either living or inactivated microorganisms, whereas the intracellular and extracellular accumulation of metals are usually energy-driven processes, and thus can take place only in living cells (Gadd, 1990; Volesky, 1990a; Macaskie, 1990; Sağ and Kutsal, 2000). Non-viable microbial biomass frequently exhibits 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 toxicity of metals 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 (Fourest and Roux, 1992; Sağ and Kutsal, 2000).

The first major challenge for the biosorption field was to select the most promising types of biomass from an extremely large pool of readily available and inexpensive biomaterials. Although this task is not complete, a large number of biomass type have been tested for their metal binding capability under various conditions. Furthermore, dead fungal biomass has been subjected to physical and chemical treatments to enhance its performance (Kratochvil and Volesky, 1998).

Application of fungal biomass to remove heavy metal from industrial waste water and/or to recover economically valuable metals is attractive for industry. Food and industrial fermentation processes can provide a cheap and constant supply of fungal biomass or the biomass can be cultured using inexpensive growth media and unsophisticated fermentation techniques. Living cells can be pretreated using physical or chemical means with the objective of increasing the metal biosorption capacity. Physical pretreatment methods have included heat treatment, autoclaving, freeze-drying and boiling. Chemical pretreatment methods, such as contacting microbial cells with acids, alkali and organic chemicals, have showed enhancement or reduction in metal biosorption, depending on the fungal strains, and treatment procedures used (Gardea-Torresday et al, 1995; Kapoor and Viraraghavan, 1998; Yin et al., 1999).

Fungal cell walls and their components have a major role in biosorption and also take up suspended metal particulates and colloids (Sağ, 2001). Dead fungal cells sequester metals through chemical functional groups of the material comprising the cell and particularly the cell wall which constitutes a large percentage of the cellular dry weight. Fungal cell surfaces can be regarded as a mosaic of different functional groups where coordinations complexes with metals can be form. Among these group are carboxyle (-COOH), amide ($-NH_2$), thiol (-SH), phosphate (PO_4^{3-}), and hydroxide (-OH) (Volesky, 1990b).

The fungal cell wall composition can be characteristic of the fungal species (Volesky, 1990). So, the biosorption heavy metals using various fungal biomasses has been studied. Yan and Viraraghavan (2000) investigated the effect of pretreatment on the bioadsorption of heavy metals on *Mucor rouxii*. Kapoor and Viraraghavan (1998) studied the effect of pretreatment of *Aspergillus niger* biomass on biosorption of lead, cadmium, copper and nicel. Jianlong (2002) investigated the biosorption of copper (II) by chemically modified biomass of *Saccharomyces cerevisiae*. Fungi belonging to the genera *Rhizopus*, *Mucor*, *Aspergillus* and *Saccharomyces* have already been studied as potantial biomass for the removal of heavy metals from aqueous solutions (Gardea-Torresday et al, 1995; Huang and Huang, 1996; Kapoor and Viraraghavan, 1998; Kapoor et al, 1999; Yin et al, 1999; Yan and Viraraghavan, 2000). However, little was reported on the bioadsorption of heavy metals on fungi in *Penicillium* genera.

The aim of this study was to investigate the effect of pretreatment of *Penicillium lanosa-coeruleum* biomass on biosorption of copper, lead and nickel.

Material and methods

Microorganisms, medium and culture conditions

P. lanosa-coeruleum had been isolated from soil in previously our study and it was routinely maintained on potato dextrose agar (Tokur and İlhan, 1996, İlhan *et al.*, 2000). The composition of growth medium is given as follows (g L^{-1}) sucrose, 20; bacto peptone, 5; neopeptone, 5; KH_2PO_4, 1; $NaNO_3$, 1; $MgSO_4.7H_2O$, 0.5 (Yerushalmi, 1990). Seed culture was propagated in the liquid medium at 25°C, on a rotary shaker agitated at 130 rpm for 24 hours and then 10 ml of the seed culture was transferred to the same medium of 100 ml. The cultures were growth at the conditions mentioned above for five days. All culture work was conducted aseptically. The fungal biomass was then harvested by filtration, washed with generous amounts of deionized water, resuspended and washed then filtered again.

Pretreatment of biomass

The resting cells of *P. lanosa coeruleum* will be referred to as type A in this paper. Wet biomass A was then pretreated in nine different ways as described below:

- only dried at 60°C for overnight (type B)
- autoclaved for 15 min at 121 °C and 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 2% (vol/vol) gluteraldehyde solution (type F)
- boiled for 15 min in 500 ml of 10% (vol/vol) acetic acid 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 containing 2.5 g of commercial laundry detergent (type I)
- boiled for 15 min in 200 ml of 50% (vol/vol) dimethyl sulfoxide solution (type J).

All biomasses, including those exposed to pretreatment were washed with generous amounts of deionized water and ground after dried at 60°C for overnight. The biomass pretreated with NaOH was washed with deionized water until the pH of the solution was in near neutral range (pH 6.8-7.2) before drying.

Biosorption studies

Biosorption experiments were conducted using separate solutions containing Pb^{2+}, Cu^{2+} and Ni^{2+} added in the form of $Pb(NO_3)_2$, $CuSO_4$ and $Ni(NO_3)_2$, respectively. Biosorption conditions had been determined in previous our study (İlhan et al, 2003). The initial metal concentration of solution was prepared as 100 mg L^{-1} using distilled water and pH was adjusted 5.5. Biosorption experiments were carried out adding dry biomass (0.3 g) corresponding to wet biomass (2.162 g). These conditions were fixed in the batch biosorption studies for all metals. The reaction mixture was agitated at 130 rpm on a rotary shaker. After 180 min of contact time biomass was separated by filtering the reaction mixture and the filtrate was analyzed for metal concentration.

Measurement of metals

Total metal concentration in the solution was measured with a Perkin Elmer 3110 Atomic Absorption Spectrometer. Before measured by the Atomic Absorption Spectrometer, the heavy metal solutions were appropriately diluted with deionized water to ensure that the heavy metal 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 metal ion (mg) biosorbed per gram (dry weight) of biomass was calculated using the following equation:

$$Q = (\frac{C_0 - C}{m}) \cdot V$$

where, Q = amount of metal ion biosorbed per gram of biomass, mg g^{-1}; C_0 = initial metal ion concentration, mg L^{-1}; C = final metal ion concentration, mg L^{-1}; m = dry weight of biomass in the reaction mixture, g; V = volume of the reaction mixture, L (Yan and Viraraghavan, 2000).

Results and discussion

The effect of pretreatment of *P. lanosa-coeruleum* on biosorption of lead is shown in Figure 1. Biosorption of lead either increased or decreased depending on pretreatment method in comparison with biosorption using living biomass. Pretreatment of living biomass using heat (type B), sodium hydroxide (type D), commercial laundry detergent (type I) and dimethyl sulfoxide (type J) resulted in an improvement in lead biosorption in comparison with living biomass (from 19.0 to 20.1-24.2 mg g^{-1}). Formaldehyde (type E), glutaraldehyde (type F) and hydrogen peroxide (type H) pretreatment marginally decreased lead biosorption (from 19.0 to 15.13-16.73 mg g^{-1}) while acetic acid (type G) and autoclave (type C) pretreatment significantly reduced biosorption of lead in comparison with living biomass (from 19.0 to 6.63-9.36 mg g^{-1}).

Figure 2 shows the effect of pretreatment of *P. lanosa-coeruleum* on biosorption of copper. It was observed that Q values obtained for all pretreated biomasses were high in comparison with living biomass (from 5.95 to 6.23-15.66 mg g^{-1}). Pretreatment of biomass by using commercial laundry detergent (type I) increased biosorption of copper by three times in comparison with living biomass (15.66 mg g^{-1}).

Figure 3 shows the effect of pretreatment of *P. lanosa-coeruleum* on biosorption of nickel. Pretreatment of biomass using gluteraldehyde (type D) and commercial laundry detergent (type I) increased biosorption of nickel by approximately two times in comparison with living biomass (from 4.56 to 7.16-7.86 mg g^{-1}). Pretreatment with sodium hydroxide (type D), hydrogen peroxide (type H) and dimethyl sulfoxide (type J) of biomass also resulted in improvement of nickel biosorption in comparison with living biomass (from 4.56 to 5.5-5.93 mg g^{-1}). Heat (type B), autoclave (type C), formaldehyde (type E) and acetic acid (type G) pretreatments reduced biosorption of nickel (from 4.56 to 2.5-3.93 mg g^{-1}).

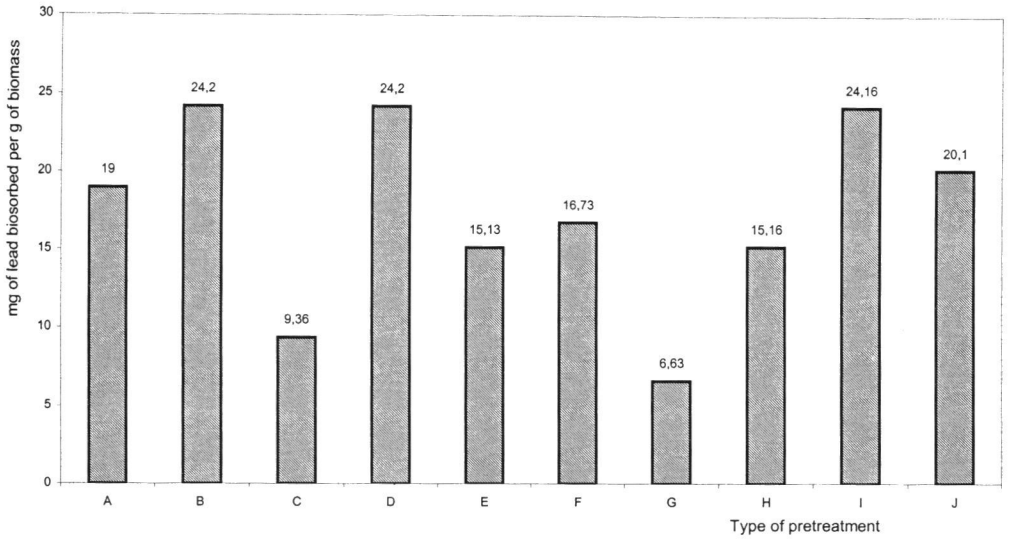

Fig 1. The effect of pretreatment on biosorption of lead (see Materials and Methods for type of treatment).

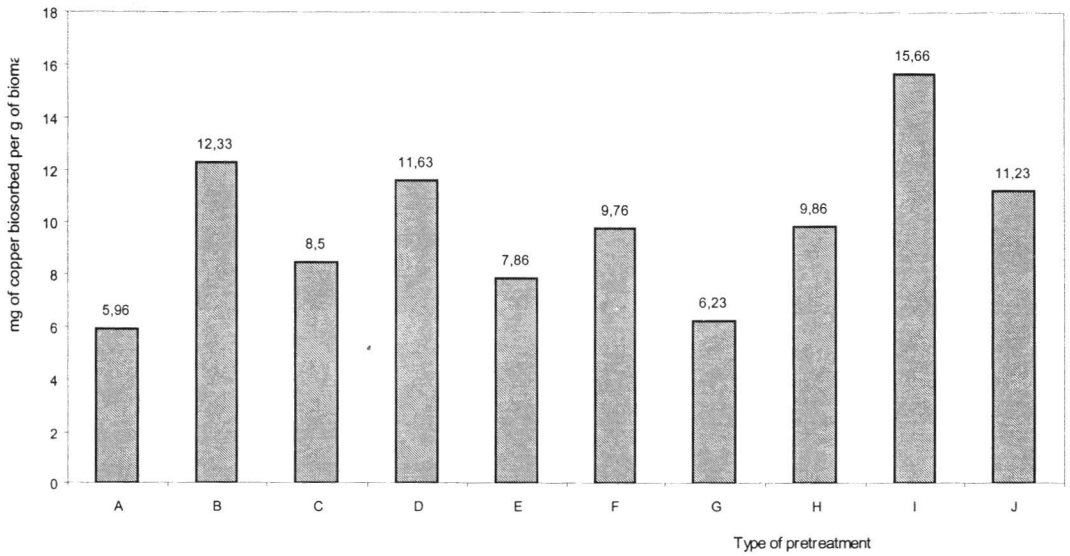

Fig 2. The effect of pretreatment on biosorption of copper (see Materials and Methods for type of treatment)

The data presented shows that *P. lanosa coeruleum* biomass pretreated by certain methods can substantially increase biosorption of lead, copper and nickel ions. The *P. lanosa coeruleum* biomasses only dried at 60°C for overnight (type B) and autoclaved for 15 min at 121°C and 15 psi (type C) increased copper biosorption while decreased nickel biosorption. In addition, drying pretreatment improved (27%) while otoclaving decreased (51%) lead biosorption. So we can say that biosorption mechanisms could vary depending on metal species. Siegel et al reported that after heat treatment of a *Penicillium* sp. the rate of lead uptake was increased but the ultimate capacity was unchanged (Gadd, 1988). Galun et al (1987) reported that *Penicillium* biomass pretreatment at 100°C for 5 min increased the bioadsorption of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} and the increase was attributed to the exposure of latent binding sites after pretreatment. Microorganisms can take up nickel intracellularly (Gadd, 1988). Therefore, Yan and Viraraghavan (2000) reported that the reduction of bioadsorption ca-

pacity of autoclaved *Mucor rouxii* biomass, in comparison with living biomass, may be attributed to the loss of intracellular uptake.

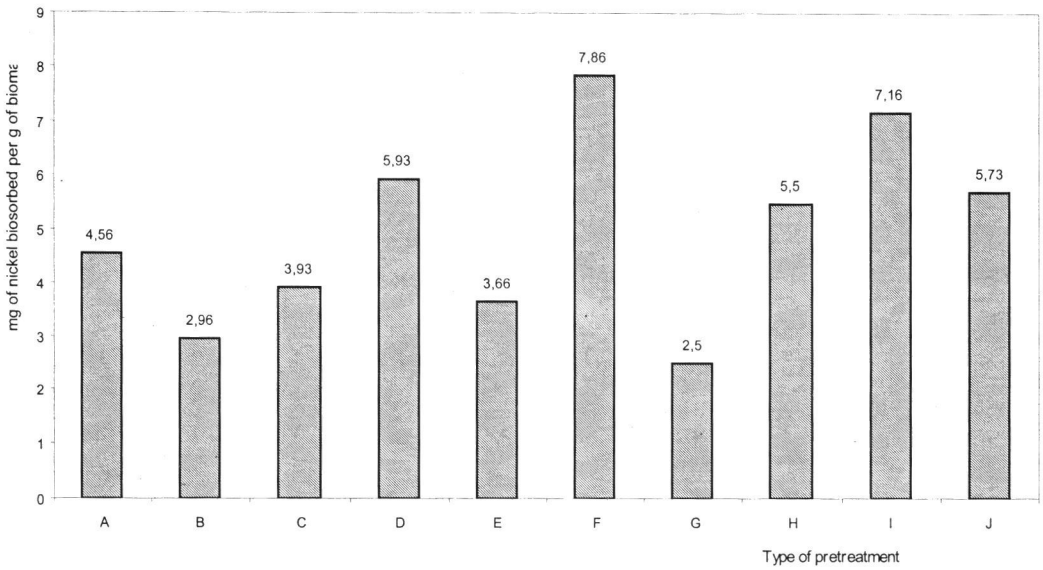

Fig 3. The effect of pretreatment on biosorption of nickel (see Materials and Methods for type of treatment).

Sodium hydroxide and detergent significantly improved biosorption of lead (27% for both), copper (95%, 162%) and nickel (30%, 57%). As a result of sodium hydroxide treatment, the number of protein amino groups that can be engaged in metallic ion binding is markedly decreased. Deproteination should, theoretically, decrease metal retention. But, the current results were opposite. The increase may be due to an exposure of active-metal binding sites embedded in the cell wall or chemical modifications of the cell wall components. In a study Galun et al. (1987), NaOH treated *Penicillium digitatum* also showed enhancement in bioadsorption of Ni^{2+}. Removal of surface impurities, rupture of cell membrane and exposure of available binding sites for nickel bioadsorption after pretreatment may be the reason for the increase in nickel bioadsorption. Huang and Huang (1996) suggested that increase in metal biosorption after pretreating the biomass could be due to removal of surface impurities and exposure of available binding sites for metal biosorption. Luef et al (1991) tried to explain the increase in zinc uptake by *A. niger* after sodium hydroxide treatment and they suggested that the removal of certain polysaccharides from the cell wall by alkali treatment generates more accessible spaces within the β-glucan chitin skeleton, thus allowing more zinc ions to be sequestered by this structure. Similar results were reported by Ashkenazy *et al.* (1997), who showed that lead biosorption by *Saccharomyces uvarum* was more efficient after sodium hydroxide treatment. The explanation they offered is that the increase in the metal uptake after the protein removal steps is brought about by unmasking of some of the cellular groups, which cannot participate in the sorption process without the treatment with alkali.

Kapoor and Viraraghavan (1998) reported that lead, cadmium and copper biosorption by *Aspergillus niger* was more efficient after formaldehyde treatment. But, in this study, the formaldehyde pretreatment increased only copper biosorption while decreased lead and nickel biosorption. Huang and Huang (1996) suggested that when biomass pretreated with formaldehyte, methylation of amine groups present in the cell wall significantly decreased the biosorption capacity of copper, which suggests that amine groups play an important role in biosorption of copper ions. It should be noted that in their work, living biomass during formaldehyde pretreatment was only mixed, not boiled. The difference in results may be due to a specific pretreatment. In addition, the cell wall composition can be characteristic of the fungal species. Analyses of cell walls of certain Fungi Imperfecti, which probably are or were derived from Ascomycetes, reveal additional differences in composition. The isolated cell wall of *Aspergillus niger*, for instance, consists chiefly of neutral carbohydrate (73 to 83%) and hexoamine (9 to 13%), with smaller amounts of lipid (2 to 7%) and protein (0.15 to 2.5%). Phosphorous constituted less than 0.1% of wall weight. The acetyl content was 3 to 3.4%, which corresponded to 1 mol/mol

stituted less than 0.1% of wall weight. The acetyl content was 3 to 3.4%, which corresponded to 1 mol/mol of hexosamine. Purified cell wall preparations of the imperfect fungus *Penicillium chrysogenum* consisted of at least two layers and contained the largest proportion of glucose, then glucosamine, galactose, and mannose in molar ratios of 9: 4.5: 3:1. Approximately 2% of the wall was protein (Volesky, 1990b).

Glutaraldehyte, detergent, sodium hydroxide, dimethyl sufoxide and hydrogen peroxide pretreatment increased biosorption capacity for nickel in comparison with living biomass, 72%, 57%, 30%, 26% and 20% respectively. However, Kapoor and Viraraghavan (1997) reported that pretreatment of *A. niger* decreased nickel biosorption. They suggested that nickel removal could be better by living cells due to intracellular nickel uptake or the presence of chelating ligands that may be present on the cell surface in trace amounts, even after washing the biomass thoroughly before using in biosorption experiments. It needs to be pointed out that glutaraldehyte pretreatment was the best for nickel removal (7.86 mg g^{-1}). At present it is not very clear how pretreated biomass was able to remove nickel ions. The effect of detergent and dimethyl sulfoxide pretreatments on biosorption capacity may be similar to that of sodium hydroxide. More detailed studies are required to understand why enhancement or reduction in adsorption capacity occurs under specific pretreatment conditions.

Acid pretreatment significantly decreased biosorption of lead and nickel, 66%, 46% respectively, in comparison with living biomass, which is in agreement with the observation of Kapoor and Viraraghavan (1998) in the case *A. niger*. However, this pretreatment insufficiently increased copper biosorption capacity of *P. lanosa coeruleum* in comparison with living biomass (5%). The polymeric structure of biomass surface exhibits a negative charge due to the ionization of organic groups and inorganic groups. The higher the biomass electronegativity is the greater the attraction and adsorption of heavy metal cations. Thus, the remaining H^+ ions on the acidic pretreated *P. lanosa coeruleum* biomass may change the biomass electronegativity, resulting in a reduction in bioadsorption capacity. On the contrary, Huang and Huang (1996) reported that acid pretreatment can strongly enhance the adsorption capacity of *A. oryzae* biomass. It should be noted that in their work, living biomass after acid pretreatment was directly used in bioadsorption of copper ions instead of being autoclaved and dried. 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 it is used in biosorption of metal ions.

Conclusion

The biosorption properties of the biomass of filamentous fungus, *P. lanosa coeruleum* were studied for lead, copper and nickel ions. According to the results, it is obvious that *P. lanosa-coeruleum* biomass pretreated with some chemicals is enable to use in order to remove heavy metals from industrial effluents. If the removal of especially lead and copper ions from aqueous solutions is required, then it may be advantageous to use the novel fungal strain, *P. lanosa-coeruleum*, and to pretreat with one of heat, sodium hydroxide and detergent. If the removal of especially nickel ions is required, then it may be used *P. lanosa-coeruleum* biomass pretreated with glutaraldehyde. Thus the fungal biomass of *P. lanosa-coeruleum* may be applied to develop potentially cost effective biosorbent for removing lead, copper and nickel from effluents.

Since the chemical composition of fungal cell walls can vary considerably between different species, there may be considerable differences in adsorption capacities between species, strains, and even different cell types of the same organisms. We must continue the fundamental research into the better understanding of the mechanism of biosorption on what drives the selectivity of biosorptive and bioaccumulatory process.

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