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APPLICATION OF FUNGAL WASTE BIOMASS ORIGINATING FROM STEROID HORMONE MANUFACTURE FOR HEAVY METALS REMOVAL

Abstract: The biomass of *Curvularia lunata*, used previously for hydrocortisone production, was investigated as a heavy metal biosorbent. Removal of lead, zinc and cadmium ions was evaluated as a function of biosorbent dosages, initial ion concentrations, mode of mycelium modifications, initial pH of metal solutions and when these metals ions where presented in binary as well in ternary combinations. The results presented in this paper indicate the potential utility of *C. lunata* waste biomass for lead and, to a lower extent, for zinc and cadmium ions removal from acid solutions.

Key words: *Curvularia lunata,* heavy metals, hydrocortisone production, waste biomass

1. INTRODUCTION

Pollution of the environment by heavy metals is a serious ecological problem. The main sources of these contaminants are wastes from metallurgical and metal finishing industries, galvanizing, electroplating, battery and chemical manufacturing. After being released into the environment, toxic ions of heavy metals are bioaccumulated throughout food chain, which can cause ecological and health hazards (MALIK 2004; IQBAL, EDYVEAN 2004). There are two groups of methods for removing heavy metals from wastewaters: conventional and biological. Conventional processes include physico-chemical reactions such as filtration, precipitation, oxidation, reduction and ion exchange. The mentioned techniques appear to be costly and ineffective in case of effluents with low metal concentration, particularly in the range of 1-100 mg l^{-1} (SAEED *et al.* 2005). Due to this fact, biological methods, like biosorption, have focused a great deal of attention, as an attractive alternative for heavy metal removal (AHLUWALIA, GOYAL 2007).

Many studies demonstrated that diverse materials of biological origin such as plants, bacteria, algae, yeast and filamentous fungi can be successfully used to take up heavy metals from aqueous solutions (IQBAL, EDYVEAN 2004; SAEED *et al.* 2005; LO *et al.* 1999; SOARES *et al.* 2002; HAN *et al.* 2006; AKSU, DÖNMEZ 2006; MELGAR *et al.* 2007). Nevertheless, commercial application of sorbents originating from microorganisms may be limited due to the costs involved in the production of biomass. Filamentous fungi and yeast are widely used in a variety of industrial fermentation processes (e.g. production of antibiotics, organic acids, enzymes and in brewery industry). Additionally, since fungal biomass is an abundant by-product and is of little use, it may be an inexpensive source of biomaterial for heavy metal remediation processes. It is well documented that waste biomass derived from fungi of biotechnological importance (mainly species of *Aspergillus, Fusarium, Penicillium, Mucor* and *Saccharomyces*) can be used as heavy metal biosorbents (LO *et al.* 1999; KAPOOR *et al.* 1999; JIANLONG *et al.* 2001; GÖKSUNGUR *et al.* 2005; PARK *et al.* 2005; MUNGASAVALLI *et al.* 2007).

The 11β-hydroxylation of cortexolone is a transformation widely used in pharmaceutical industry. It is a direct way to obtain hydrocortisone, an effective anti-inflammatory drug as well as an important precursor of other pharmaceutical corticosteroids (FERNANDES *et al.* 2003). Filamentous fungus *Curvularia lunata* is one of the most promising cortexolone 11β-hydroxylators (PARASZKIEWICZ *et al.* 1998; LU *et al.* 2006). To our knowledge, no research has been carried out on the heavy metal removal by waste mycelium of *C. lunata* obtained after cortexolone conversion to hydrocortisone.

The aim of the presented study was to investigate the ability of dead biomass of *C. lunata* to remove heavy metals from aqueous solutions. For experiments was chosen the wild strain of *C. lunata* IM 2901 with the confirmed ability of 11β-hydroxylation of cortexolone (WILMAŃSKA *et al.* 1992; PARASZKIEWICZ *et al.* 1998; KANWAL *et al.* 2001). The basic characterization of heavy metals binding by growing mycelium of this fungus and by its emulsifier had been previously described (PARASZKIEWICZ *et al.* 2007). Microbial uptake of metals is significantly decreased in wastewaters of low pH. In experiments described in this paper pH values of metal solutions were from 4 to 6 because we were particularly interested in the possibility of heavy metal removal from acidic wastewaters, without preliminary pH adjustment.

2. MATERIALS AND METHODS

2.1. Microorganism and maintenance

The filamentous fungus *Curvularia lunata* (Wakker) Boedijn IM 2901 from the collection of the Department of Industrial Microbiology and Biotechnology, University of Lodz was used in the study. The fungal strain was maintained on ZT slants (PARASZKIEWICZ *et al.* 2002) at 4°C and transferred at 2–month intervals.

2.2. Mycelium growth conditions and cortexolone hydroxylation

Mycelia originating from 10–day–old cultures on ZT slants were suspended in PL-2 medium (PARASZKIEWICZ *et al.* 2002) and incubated at 28°C for 24 h on a rotatory shaker (180 rpm). First–step preculture (10%) was used as inoculum for the second-step of cultivation, conducted in conditions described above. According to the method developed by PARASZKIEWICZ and DŁUGOŃSKI (1998), steroid hydroxylase induction was carried out for 6 h by adding to the 18-h-old second-step preculture cortexolone dissolved in ethanol (final concentration of steroid 0.1g l^{-1} and ethanol in the culture 1% v/v). The achieved 24-h-old second-step culture (60 ml) was introduced into 2l-Erlenmeyer flasks containing 540 ml of the PL-2 medium and supplemented with cortexolone dissolved in ethanol (at final concentration of steroid 1.0 g l^{-1} and ethanol in the culture 1% (v/v)). Final cultures were incubated on a rotatory shaker in conditions described above for 48 hours.

2.3. Isolation and pretreatment of the fungal biomass

After fungal growth and cortexolone transformation to the whole 48-h-old culture a mixture of chloroform: acetone (9:1) was added. Steroid extraction was repeated three times. The collected waste biomass was washed several times with deionised water for culture broth and organic solvents removal and then lyophilised. In the next part of the work biomass prepared in this way was referred to as unmodified or waste biosorbent.

In some experiments mycelia modified (pretreated) with ethanol or sodium hydroxide (NaOH) were used. Ethanol treated samples of biosorbent were prepared by suspending 50 g of wet *C. lunata* biomass in 500 ml of 80% ethanol. The modification procedure was carried out at room temperature on a rotatory shaker (180 rpm) for 1 hour. After pretreatment procedure, the biomass samples were washed several times with deionised water and then lyophilised. Modification with NaOH was carried out according to the method described by KAPOOR *et al.* (1999). NaOH treated mycelium was prepared by boiling 50 g of wet biomass in 500 ml of 0.5 N NaOH solution for 15 min. Biomass pretreated with sodium hydroxide was washed with deionized water until the pH reached the near neutral range (pH 6.8-7.2) and then it was lyophilised.

2.4. Metal sorption studies

The standard stock solutions (0.2 M) were prepared by dissolving the respective nitrate salts of the metals in deionised water. The pH of working metal solutions (0.2 M) were adjusted to required values (4.0; 5.0 or 6.0) with 0.1 M HCl.

Sorption experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml of 0.2 or 5.0 mM single metal solution and lyophilized waste biomass (at a final concentration of 0.5 or 1.0 g l^{-1}). For studies on metal biosorption from binary and ternary solutions, the concentration of each metal ions in the solution was 0.2 mM. The samples were incubated on a rotatory shaker (180 rpm), at 28°C, for 2 hours. Then metal-loaded mycelium was separated by filtration (Millipore filters with 0.45 μm pore size) and dried at 105°C to constant weight. Metal concentration in the mycelium samples was analyzed in a Varian atomic absorption spectrometer (Spectra 300) according to the method described by SŁABA and DŁUGOŃSKI (2004). The efficiency of metal ions removal was calculated based on the weight of metal ions contained in the biomass per flask divided by the initial metal ions weight in the solution per 100%. All data represent the mean of three independent experiments. An average standard deviation was calculated.

3. RESULTS

3.1. Comparison of the effectiveness of different heavy metals removal by waste biomass of *C. lunata*

In the first part of the work the removal of seven various heavy metals was studied. Metal uptake was conducted by unmodified mycelium samples (at a concentration of 0.5 g l^{-1}) suspended in single metal solutions (0.2 mM), adjusted to pH 4. The removal efficiency for Pb^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Cr^{2+}, Ni^{2+} and Co^{2+} was found to be 46.2, 30.7, 27.0, 22.6, 18.9, 11.8 and 2.4%, respectively (Fig. 1). Because the highest removal efficiency occurred in Pb^{2+}, Zn^{2+} and Cd^{2+} solutions, these three metals were selected for further investigations.

3.2. Effect of the biosorbent dosage and the initial concentration of lead, zinc and cadmium on the removal efficiency

Efficiency of Pb^{2+}, Zn^{2+} and Cd^{2+} removal was examined as a function of *C. lunata* biomass dosage (0.5 and 1.0 g l^{-1}) as well as the initial concentration of heavy metal (0.2 and 5.0 mM). Metal uptake was conducted by unmodified biosorbent samples suspended in single Pb^{2+}, Zn^{2+} or Cd^{2+} solution, adjusted to pH 4. The removal efficiency of heavy metal ions was improved almost twice in the presence of a higher dose of biomass (Fig. 2). Moreover, as shown in Fig. 3, with the decrease in the initial concentration of heavy metal (from 5.0 to 0.2 mM) a significant increase in Pb^{2+}, Zn^{2+} and Cd^{2+} removal efficiency was achieved (from 7.0, 3.8 and 3.2% to 81.8, 55.9 and 47.4%, respectively). In conclusion, conditions: 1 g l^{-1} of fungal biomass and heavy metal ions at the initial concentration of 0.2 mM, promoting the removal of lead, zinc and cadmium were used in the next parts of the study.

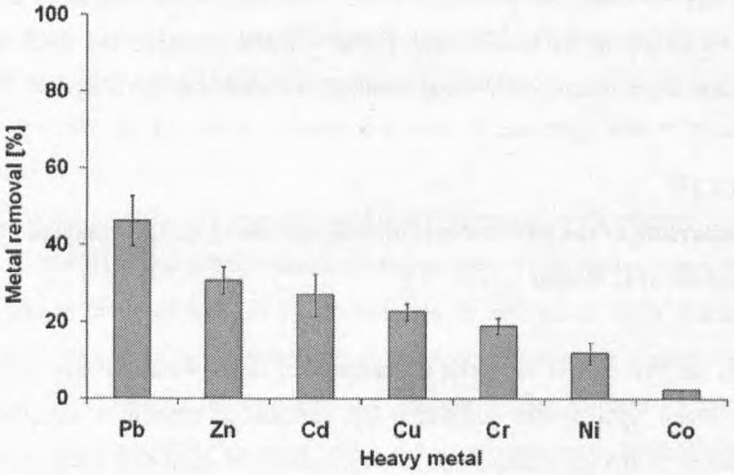

Fig. 1: Removal efficiency of Pb, Zn, Cd, Cu, Cr, Ni and Co from single metal solutions at pH 4 by waste biomass of *C. lunata* at a concentration of 0.5 g l^{-1}, suspended in single metal solutions (0.2 mM). Data are mean ± SD (n=3)

3.3. Effect of waste biomass modification on lead, zinc and cadmium removal efficiency

Effectiveness of heavy metal removal (from single metal solutions, at pH 4) by unmodified, NaOH pretreated and ethanol pretreated biomass samples of *C. lunata* reached for Pb^{2+}: 81.8; 30.5 and 3.7%, for Zn^{2+}: 55.9; 54.5 and 50.7% and for

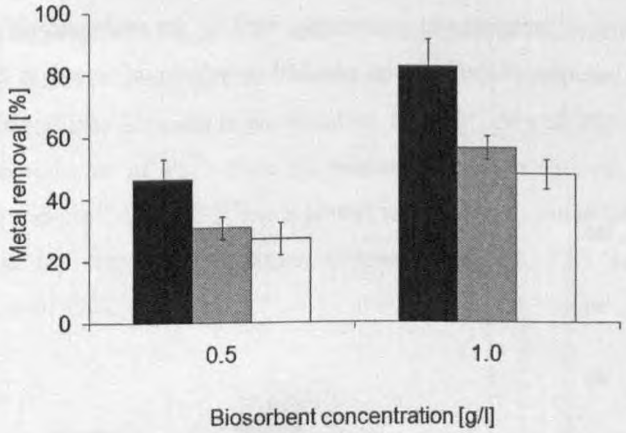

Fig. 2: The influence of biosorbent dosage on Pb^{2+}, Zn^{2+} and Cd^{2+} removal efficiency from single metal solutions (0.2 mM) at pH 4. Black bars – Pb; grey bars – Zn; white bars – Cd. Data are mean ± SD (n=3)

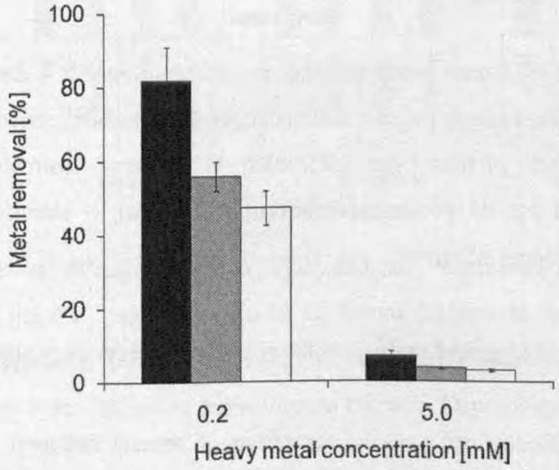

Fig. 3: The influence of the initial concentration of Pb^{2+}, Zn^{2+} and Cd^{2+} on these metal ions removal efficiency from single metal solutions at pH 4, by waste biomass of *C. lunata* at a concentration of 1 g l^{-1}. Black bars – Pb; grey bars – Zn; white bars – Cd. Data are mean ± SD (n=3)

Cd^{2+}: 47.4, 45.2 and 45%, respectively (Fig. 4). The data mentioned above revealed that both chemical pretreatment procedures used in the study strongly decreased Pb^{2+} sorption capacity of the biomass and had only little influence on Zn^{2+} or Cd^{2+} removal.

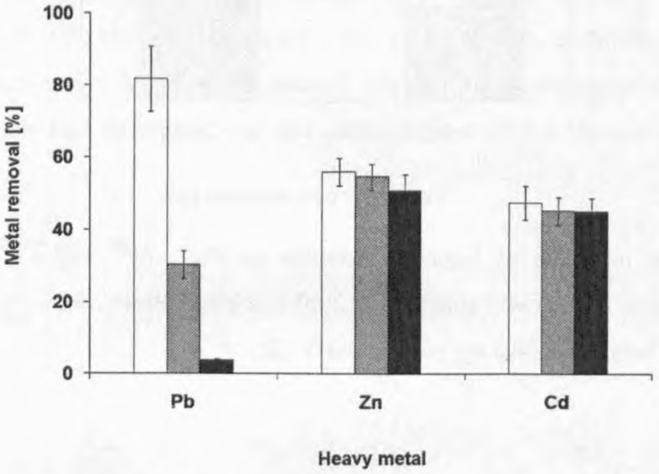

Fig. 4: Influence of *C. lunata* waste biomass modification on Pb^{2+}, Zn^{2+} and Cd^{2+} removal efficiency from single metal solutions at pH 4 (biosorbent concentration 1 g l^{-1}; initial concentration of heavy metal 0.2 mM). White bars – untreated biomass; grey bars – 0.2 M NaOH pretreated biomass; black bars – ethanol pretreated biomass. Data are mean ± SD (n=3)

3.4. Removal of lead, zinc and cadmium from binary and ternary solutions

Industrial effluents are usually composed of several different metal ions. Therefore removal of Pb^{2+}, Zn^{2+} and Cd^{2+} from single metal solutions was compared with the efficiency of this process recorded for binary as well as ternary metal combinations. Results presented in Fig. 5 revealed that biosorption capacity of zinc and cadmium ions decreased approximately by 25 and 30%, respectively when these two metals were presented together. The presence of Pb^{2+} significantly interfered in the sorption of zinc and cadmium ions. About 3-fold decrease in zinc removal efficiency was detected from binary (Zn^{2+}+Pb^{2+}) and ternary (Zn^{2+}+Pb^{2+}+Cd^{2+}) systems as compared with single Zn^{2+} solution (respectively 17.2, 19.5% contrasted with 55.9%). Similar strong decline of removal efficiency was noted for cadmium when these metal ions occurred in combination with Pb^{2+} or with Pb^{2+} plus Zn^{2+}. In contrast, the removal of Pb^{2+} from all examined metal solutions: (Pb^{2+}+Zn^{2+}), (Pb^{2+}+Cd^{2+}) and (Pb^{2+}+Zn^{2+}+Cd^{2+}) was almost as efficient as noted for the sorption conducted in the single Pb^{2+} solution (respectively 79.2, 72.1 and 80.6% in comparison to 81.9%).

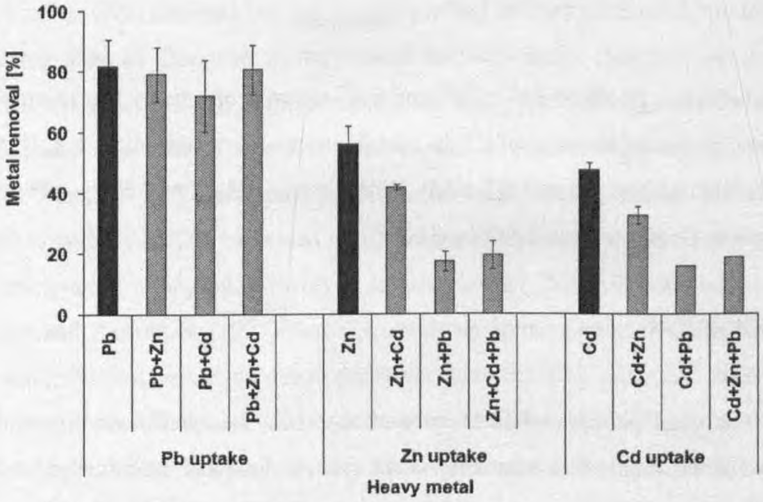

Fig. 5: Removal efficiency of Pb^{2+}, Zn^{2+} and Cd^{2+} from binary and ternary metal solutions at pH 4 by waste biomass of *C. lunata* (biosorbent concentration 1 g l^{-1}; initial concentration of each heavy metal 0.2 mM). Black bars – single metal solution; grey bars – binary or ternary metal mixture. Data are mean ± SD (n=3)

3.5. The effect of pH on lead, zinc and cadmium removal

As shown in Fig. 6 the uptake of Pb^{2+}, Zn^{2+} and Cd^{2+} conducted in single metal solutions occurred to be pH dependent. The increase in metal solution pH value from 4 to 6 accelerated the removal efficiency of Pb^{2+}, Zn^{2+} and Cd^{2+} from 81.9, 55.9 and 47.4% respectively to 94.0 75.4 and 62.6%.

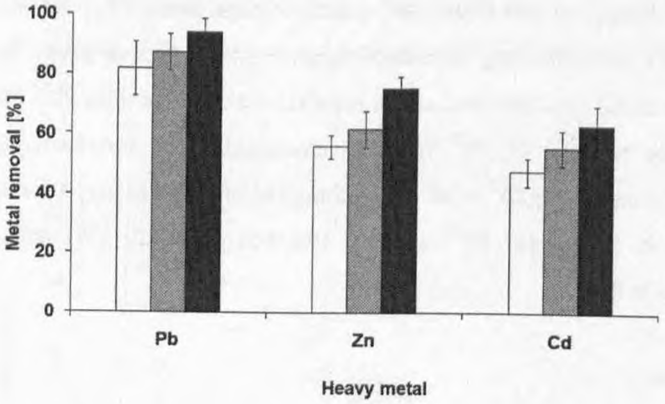

Fig. 6: Influence of pH on Pb^{2+}, Zn^{2+} and Cd^{2+} removal efficiency from single metal solutions by waste biomass of *C. lunata* (biosorbent concentration 1 g l^{-1}; initial concentration of heavy metal 0.2 mM). White bars – pH 4; grey bars – pH 5; black bars – pH 6. Data are mean ± SD (n=3)

4. DISCUSSION

The dead biomass of filamentous fungus *Curvularia lunata* obtained after hydrocortisone production via cortexolone conversion was examined as a heavy metal biosorbent. Among seven heavy metals (Pb, Zn, Cd, Cu, Cr, Ni and Co) tested, the mycelial biomass exhibited the highest removal efficiency for Pb^{2+}, Zn^{2+} and Cd^{2+}. A similar order of uptake intensity (Pb>Zn>Cd>Ni) was reported by YAN and VIRARAGHAVAN (2003) for dead mycelium of *Mucor rouxii* as a heavy metal biosorbent. The higher affinity of *C. lunata* mycelium to lead than to other heavy metal ions is in line with many studies on diverse fungal biosorbents (IQBAL, EDYVEAN 2004; MELGAR *et al.* 2007; KAPOOR *et al.* 1999; GÖKSUNGUR *et al.* 2005; SŁABA, DŁUGOŃSKI 2004). Three strains of filamentous fungi: *Curvularia lunata* IM 2901 (the same as used in this work), *Curvularia tuberculata* IM 4417 and *Paecilomyces marquandii* IM 6003 previously tested by us, during the growth in liquid medium also accumulated lead with higher efficiency than zinc and cadmium

(PARASZKIEWICZ *et al.* 2007). According to ZOUBOULIS *et al.* (1999) a greater sorption of Pb^{2+} than of many other heavy metals (e.g. Zn^{2+}, Cd^{2+}, Ni^{2+} or Cu^{2+}) may be partially explained by a high stability constant for Pb^{2+} binding to the ligands of fungal surface.

The possibility to enhance heavy metals removal as a result of physical or chemical pretreatment of plant and microbial biosorbents is pointed out by many researchers (SAEED *et al.* 2005; KAPOOR *et al.* 1999; GÖKSUNGUR *et al.* 2005; MUNGASAVALLI *et al.* 2007). The improvement of metal binding properties of modified biosorbents could be explained by the increase in metal ions access to the metal binding sites, attributed to the cleansing effect of the agents used, modification of binding sites or changing in the overall surface charge (KAPOOR *et al.* 1999; GÖKSUNGUR *et al.* 2005). Data presented in this paper revealed that additional modifications of *C. lunata* mycelium with NaOH or ethanol did not enhance either Pb^{2+} Zn^{2+} or Cd^{2+} sorption capacity. The same, experimental results indicate that ethanol as well as NaOH treatment of *C. lunata* biomass caused the removal or at least inactivation of the sites involved in lead uptake. Nevertheless, according to PURANIK and PAKNIKAR (1997) findings, while using the modified biosorbent on a large scale, the cost escalation due to pretreatment needs to be taken into account.

Sorption of lead, zinc and cadmium by *C. lunata* waste biomass was also examined by us when heavy metals ions where presented in binary as well in ternary combinations. Efficiency of the mycelium to remove lead ions from binary (Pb^{2+}+Zn^{2+} or Pb^{2+}+Cd^{2+}) as well as from ternary (Pb^{2+}+Zn^{2+}+Cd^{2+}) metal solutions occurred to be similar to the one achieved when Pb^{2+} was presented alone. Nevertheless, the presence of lead ions significantly decreased the sorption of Zn^{2+} and Cd^{2+}. Our results are in agreement with those obtained by KAPOOR *et al.* (1999) and indicate that antagonic interactions between various heavy metals may occur in multiple metal solutions. The drop in the sorption of Zn^{2+} and Cd^{2+} from two- and three-metal combinations indicates a competition among Zn, Cd and Pb ions for the same binding sites presented on the surface of *C. lunata* biomass. Greater affinity of lead than of two other examined heavy metals to the biosorbent was probably caused by a larger ion size of Pb^{2+} in comparison to Zn^{2+} and Cd^{2+} (0.112, 0.074 and 0.097nm, respectively) (ANGYAL 1989).

In the present studies the uptake of heavy metals by *C. lunata* mycelium was found to be influenced by the initial pH of metal solution and rose due to the increase in pH value. The removal of Pb^{2+}, Zn^{2+} and Cd^{2+} from single metal solutions reached at pH 6 the highest levels of 94.0, 75.4 and 62.6%, respectively. According to PURANIK and PAKNIKAR (1997) lead and zinc uptake by waste biomass of *S. cinnamoneum* was also influenced by pH of the metal solution. The pH range (5.0-6.0) established in that study as the optimum for zinc uptake fits well with our results, but maximum the lead removal determined at pH 4.5 occured to be lower than for *C. lunata* biosorbent. Nevertheless, the pH of the lead solution increased from 4.0 to 6.5 during the adsorption process conducted by *S. cinnamoneum*. At pH 6 the maximum capacity of lead uptake was estimated with mycelium of *Mucor rouxii* as a metal biosorbent (LO *et al.* 1999). SAY *et al.* (2001) used biomass of filamentous fungus *Phanerochaete chrysosporium* for Pb^{2+} removal and also determined the optimum pH value for biosorption as 6.0. It has been commonly agreed that pH value of metal solution can strongly influence metal uptake intensity of biosobents (IQBAL, EDYVEAN 2004; MELGAR *et al.* 2007; GÖKSUNGUR *et al.* 2005; MUNGASAVALLI *et al.* 2007). Due to the increased hydrogen (H^+) and hydronium (H_3O^+) concentrations at the high acidic pH solution, these ions compete effectively with metal ions in binding to negatively charged groups on the biosorbent surface. Consequently, the increase in heavy metal uptake with increasing pH could be attributed to less ionic competition (PURANIK, PAKNIKAR 1997).

From the results presented in this study it can be concluded that *C. lunata* biomass, a potential waste by-product from hydrocortisone manufacture, could be considered as an inexpensive material for lead and, to a lower extent, zinc and cadmium ions removal from aqueous effluents.

Acknowledgements

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