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Removal of Cadmium, Copper and Lead from Tertiary Metals System Using Biomass of *Aspergillus flavus* 44-1

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Abstract: Batch sorption equilibrium experiments using biomass of *Aspergillus flavus* as biosorbent were conducted using shake flask experiment. Experiments were carried out to investigate the effect of metal concentration, biosorbent concentration, pH and temperature on biosorption of cadmium, copper and lead in tri-metals system. Results showed the presence of other cations in the solution that affected the uptake of the selected metal by the biosorbent. It has been suggested that direct ionic competition occurred within the same class of divalent cations and alteration of the solution chemistry.

Key words: *Aspergillus flavus*, biomass, biosorption, cadmium, copper, equilibrium, fungi, lead, live free cell, tertiary

Introduction

The microbiological remediation of contaminated sites can be performed either through the stimulation of indigenous microbiological populations or through the introduction of microorganisms which possess the specific traits necessary for clean-up (Jacobsen and Gayazov, 1997). The bioremediation of soils, sludges, sediments and wastes polluted with heavy metals generally involves the active microbiological processes of leaching, precipitation or sequestration, it may also involve the process of biosorption onto living or dead biomass (Mergeay, 1997). Numerous interactions may have occurred between the soil microorganisms and the environment. These interactions may lead to an adaptation of the microbes allowing survival of them in local environments containing toxic concentrations of heavy metals; due to biological requirements for essential heavy metals or due to the need to derive energy in harsh environments (Mergeay, 1997). These adaptations and many others have led to various processes of bioaccumulation, bioleaching and bioprecipitation, which can be exploited for biotechnological purposes to increase or decrease the bioavailability of metals.

Presence of single metallic cations in actual industrial effluents rarely occurred. Actual industrial effluents consist of many other organic and inorganic pollutants, dissolved solids, particulates and man-made xenobiotics. While single metal existing in the solution is easier to be studied, however the presence of multielement cations is more complex. Apart from physico-chemical parameters, such as pH, temperature, metal and biosorbent concentration, multi component biosorption also depends on the number of metals competing for binding sites, metal combination, levels of metal concentration, order of metal addition, residence time (Ting *et al.*, 1991; Pascucci and Sneddon, 1993; Pascucci, 1993). There are possible interaction effects between different species in solution and potential interactions on the surface, in particular depending on the sorption mechanism and reversibility versus irreversibility (Sag and Kutsal, 1998). Competition between different metal ion species for the surface sites will certainly occur and will depend on several of the ionic characteristics (Hu *et al.*, 1996; McKay and Porter, 1997).

This study was conducted to investigate the performance of the cadmium, copper and lead biosorption by biomass of *Aspergillus flavus* at various sorption conditions in the tri-metals system.

Materials and Methods

Microorganism: The fungal strain of *Aspergillus flavus* 44-1, obtained from Fermentation Technology Center, Institute of

Bioscience, University Putra Malaysia, was used to produce the fungal biomass, which was eventually reprocessed into biosorbent.

Medium of growth: The cells were cultivated in a medium, which consist of glucose (22.0g/L), yeast extract (2.0g/L), KH₂PO₄ (1.0g/L) and MgSO₄.7H₂O (0.5g/L). Cells were cultivated in a 500 mL shake flask containing 250 mL medium, inoculated with 5.0 mL spore suspension and was incubated at 30°C, 250 rev/min on an orbital shaker for 24 hours. Cultures were harvested after 5-6 days of incubation.

Biosorbent preparation: The cell biomass was washed with distilled water, rinsed with ultrapure water twice and filtered to obtain free living cells. The biomass was kept at 4°C for subsequent use in all the biosorption experiments.

Biosorption experiment: Synthetic solutions of ultrapure water containing CdCl₂.H₂O (97% pure), CuSO₄.5H₂O (99.9% pure) and Pb(NO₃)₂ (99.5% pure) were used in all of the biosorption experiments. The solutions, which contain the desired concentration of Cd²⁺, Cu²⁺ and Pb²⁺ were prepared by diluting the above stock solutions. Sorption isotherm equilibrium experiment was carried out in batch process using 500 mL shake flask. Cells (0.1g) were added to 100mL metal-containing solutions. All of the metals in the solution were added simultaneously in equal concentration. The shake flasks were agitated in an orbital shaker at 250 rev/min and 30°C for 24 hours. The supernatant with free unadsorbed metal ion was separated from biosorbent using centrifugation at 3000 rev/min for 15 minutes. The concentration of cadmium, copper and lead in the supernatant was analyzed using atomic absorption spectrophotometer (Perkin Elmer 3300).

Results

Biosorbent: Amount of biosorbent added into the solution will determine the amount of binding site available for adsorption. Similar to dual metals system (results not included here), presence of other cations will interfere the uptake of the ions. In the tri-metals system of Cd-Cu-Pb, the highest uptake was observed in copper. On the average, uptake of Cu was increased with increasing biosorbent concentration (Fig. 1). We suggested that the fluctuation was observed in copper uptake since the presence of both cadmium and lead in the solution possess a direct competition among the three divalent ions. The uptake of lead

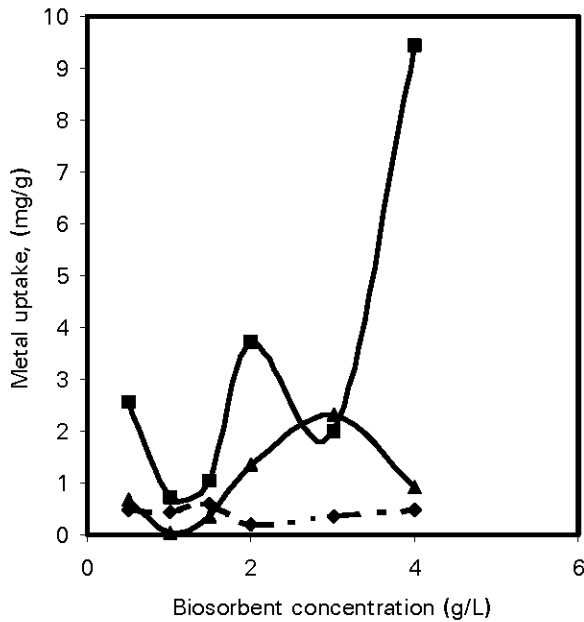


Fig. 1: Effect of biosorbent concentration (0.1-4.0g/L) on cadmium (◆), copper (■) and lead (▲) biosorption by *Aspergillus flavus* in tri-metals system. Experiment was conducted at 30°C for 24 hours in an orbital shaker at 250 rev/min. All experiments were carried out in triplicate.

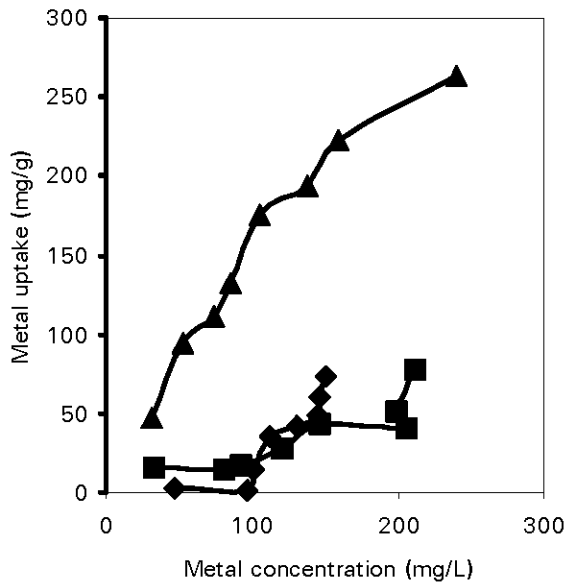


Fig. 2: Effect of metal concentration (0–300mg/L) on cadmium (◆), copper (■) and lead (▲) biosorption by *Aspergillus flavus* in tri-metals system. Experiment was conducted at 30°C for 24 hours in an orbital shaker at 250 rev/min. All experiments were carried out in triplicate.

was increased with increasing biosorbent concentration until biosorbent concentration of 3.0g/L. Then the uptake of lead was gradually decreased (Fig. 1). The lowest uptake of metal was cadmium, averagely 0.45mg/g throughout the range of biosorbent concentration (Fig. 1).

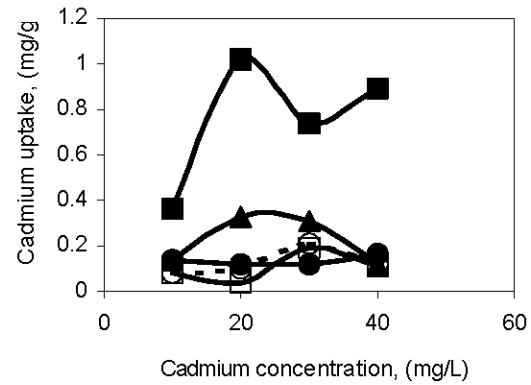


Fig. 3a: Effect of pH on cadmium biosorption by *Aspergillus flavus* in tri-metals system. Experiment was conducted at 30°C for 24 hours in an orbital shaker at 250 rev/min using 0.1 g biosorbent. All experiments were carried out in triplicate. Symbols represent: pH 1 (■), pH 2 (▲), pH 3 (□), pH 4 (○) and pH 5 (●).

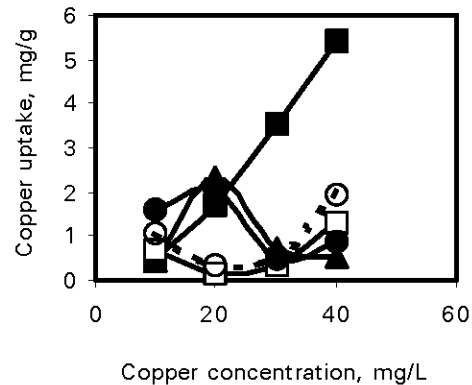


Fig. 3b: Effect of pH on copper biosorption by *Aspergillus flavus* in tri-metals system. Experiment was conducted at 30°C for 24 hours in an orbital shaker at 250 rev/min using 0.1 g biosorbent. All experiments were carried out in triplicate. Symbols represent: pH 1 (■), pH 2 (▲), pH 3 (□), pH 4 (○) and pH 5 (●).

Metals (Cd^{2+} , Cu^{2+} and Pb^{2+}): The effect of metal concentration on Cd^{2+} , Cu^{2+} and Pb^{2+} biosorption by *A. flavus* in tri-metals system is given in Fig. 2. Lead was the highest metal uptake by biosorbent, followed by copper and cadmium. It can be observed from the graphs that there was direct competition for the binding site between cadmium and copper. Copper uptake overtook the uptake of cadmium until 100mg/L. Cadmium uptake increased over copper after 100mg/L as copper uptake became saturated. Uptake of cadmium and copper began to increase gradually after 150 and 200mg/L, respectively. The uptake of lead was not affected by the presence of cadmium and copper in solution. Uptake of lead increased drastically, compared to other two metals, with increasing lead concentration.

pH: Effect of pH on Cd^{2+} , Cu^{2+} and Pb^{2+} uptake by *A. flavus* in tri-metals system is shown in Fig. 3a, b and c, respectively. The uptake of Cd^{2+} was generally increased at higher concentrations of 30 and 40mg/L for pH 1 to 4. The uptake of cadmium was almost constant at pH 5. The uptake of cadmium was highest at pH 1, followed by pH 2, 4 and 3. Uptake of cadmium at pH 5 was between the uptake values of pH 4 and 3 (Fig. 3a).

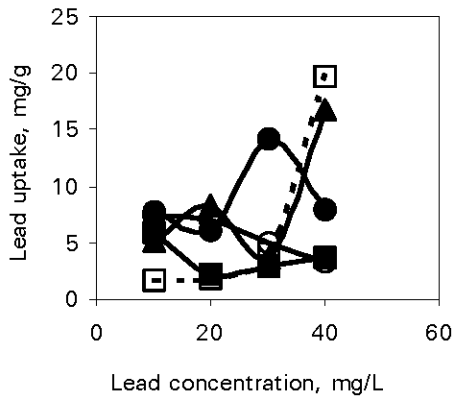


Fig. 3c: Effect of pH on lead biosorption by *Aspergillus flavus* in tri-metals system. Experiment was conducted at 30°C for 24 hours in an orbital shaker at 250 rev/min using 0.1 g biosorbent. All experiments were carried out in triplicate. Symbols represent: pH 1 (■), pH 2 (▲), pH 3 (□), pH 4 (○) and pH 5 (●).

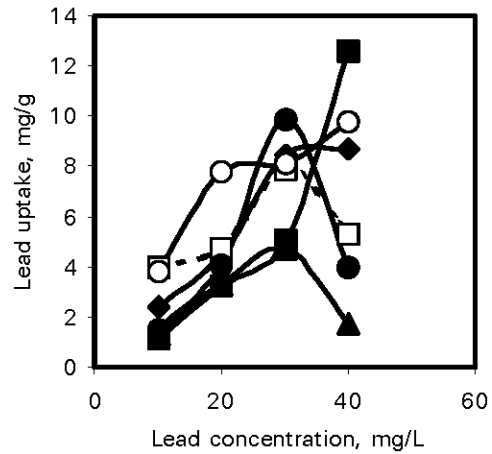


Fig. 4b: Effect of temperature on copper biosorption by *Aspergillus flavus* in tri-metals system. Experiment was conducted for 24 hours in an orbital shaker at 250 rev/min using 0.1 g biosorbent. All experiments were carried out in triplicate. Symbols represent: 15°C (◆), 25°C (■), 30°C (▲), 40°C (□), 50°C (○) and 60°C (●).

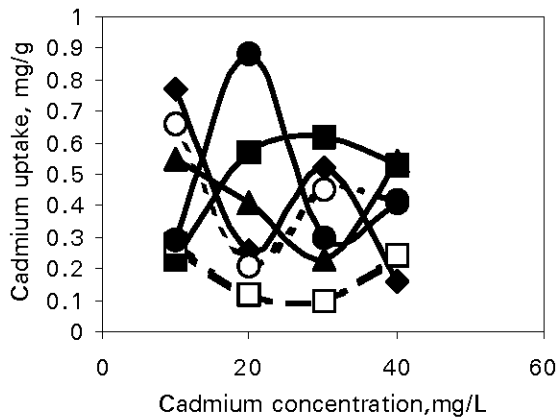


Fig. 4a: Effect of temperature on cadmium biosorption by *Aspergillus flavus* in tri-metals system. Experiment was conducted for 24 hours in an orbital shaker at 250 rev/min using 0.1 g biosorbent. All experiments were carried out in triplicate. Symbols represent: 15°C (◆), 25°C (■), 30°C (▲), 40°C (□), 50°C (○) and 60°C (●).

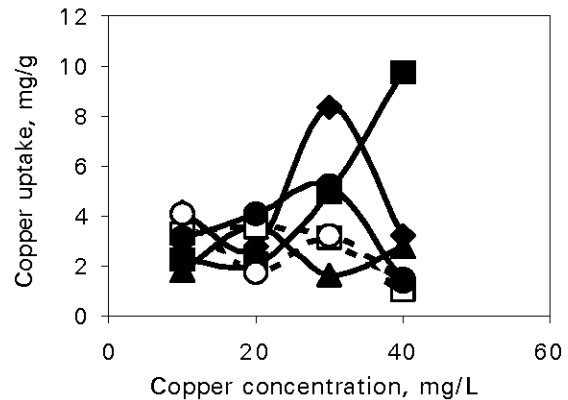


Fig. 4c: Effect of temperature on lead biosorption by *Aspergillus flavus* in tri-metals system. Experiment was conducted for 24 hours in an orbital shaker at 250 rev/min using 0.1 g biosorbent. All experiments were carried out in triplicate. Symbols represent: 15°C (◆), 25°C (■), 30°C (▲), 40°C (□), 50°C (○) and 60°C (●).

The uptake of Cu^{2+} increased with increasing copper concentration at pH 1, 3 and 4. At pH 2 and 5, the uptake of Cu^{2+} was increased before gradually decreased after 20.0mg/L of copper concentration. Uptake of Cu^{2+} was observed to be highest at pH 1 (Fig. 3b).

The uptake of lead was observed to be higher at pH 2 and 3. At pH 2 and 3, the uptake of copper increased with increasing copper concentration. The uptake of lead was decreased with increasing lead concentration at pH 1 and 4. At pH 5, the lead uptake was increased before decreased with increasing lead concentration (Fig. 3c).

Temperature: Effect of temperature on Cd^{2+} , Cu^{2+} and Pb^{2+} uptake by *A. flavus* in tri-metals system is shown in Fig. 4a, b and c. In the uptake of Cd^{2+} , highest uptake was observed to occur at 60°C. Uptake of cadmium decreased at 15, 30 and 50°C during early process of adsorption before increased at the higher concentration of metal. At 40°C, the cadmium uptake was averagely increased with the increasing cadmium concentration.

Uptake of cadmium increased before gradually decreased after 20.0mg/L at 60°C (Fig. 4a).

In the uptake of Cu^{2+} , the uptake pattern was shown to be increased with the increasing copper at temperature values of 15, 25, 30 and 60°C. At 40°C, the uptake of Cu^{2+} was decreased with increasing copper concentration. On the other hand, uptake of copper at 50°C was averagely decreased. Uptake of copper at lower temperature of 15 and 25°C were higher than other temperature values (Fig. 4b).

In the uptake of Pb^{2+} , highest uptake was occurred at 25 °C. At 15°C, uptake of lead was increased and reached saturation at the end. Uptake of lead was generally increased at 25 and 50°C. While at of 30, 40 and 60°C, the uptake of lead at early stage was increased before being decreased at the later stage (Fig. 4c).

Discussion

Biosorbent: In single metal systems of cadmium, copper and lead, the uptake of metals was decreased with increasing biosorbent concentration (results not included here). On the other hand, the uptake of metals was increased with increasing biosorbent

concentration except in the binary system of Cd-Cu (results not included here). Similar to the dual metal systems, the uptake of cadmium, copper and lead in tri-metals systems were observed to be increased with increasing biosorbent concentration. This may be due to the presence of direct competition between the three divalent ionic species, which belong to the same group of ions, classified as borderline ion by Nieboer and Richardson (1980). Variation in sorption order could also be attributed to differences in their electronegativity (Puranik and Paknikar, 1999). The sorption of Pb^{2+} was less preferred in comparison with Cu^{2+} , which may be due to its higher atomic weight. Sorption might occur through chelation and Cu^{2+} have a higher affinity than Cd^{2+} in metal binding by chelation (Cabral, 1992). We also believe that the presence of other co-cations interrupted the equilibrium established between the free metal ions, free adsorbents and metal-loaded adsorbents.

Metals (Cd^{2+} , Cu^{2+} and Pb^{2+}): Compared to single cadmium and copper system, the uptake of cadmium and copper in tertiary system was reduced in the presence of lead. In the presence of lead, uptake of cadmium was reduced two times and uptake of copper was slightly reduced. However, presence of cadmium and copper in the solution did not inhibit uptake of lead. Lead uptake was observed to increase to nearly 250mg/L, compared with 140 mg/L single lead system (results not included here).

Sorption isotherm indicated the equilibrium distribution of cadmium, copper and lead between the aqueous and the biosorbent phase versus Cd^{2+} , Cu^{2+} and Pb^{2+} concentration. The resulting relationship between Cd^{2+} , Cu^{2+} and Pb^{2+} uptake and their initial metal concentration is usually represented by the hyperbolic curve (Volesky, 1990; Hii, 1999). The ideal adsorption or favorable adsorption can be represented by a non-linear but concave towards abscissa for biosorption isotherm (Weber, 1972).

The adsorption equilibrium curve for copper and lead was considered as Type II isotherm curve by Basmadjian (1997). This curve was one of the inflecting curve of favourable adsorption curve. It is generally associated with multi layer adsorption and capillary condensation. It arises in moisture uptake by sorbents and by natural products; such as, wool and grain and is generally seen in the sorption of vapours near their boiling point. It also makes its appearance in the form of effective equilibrium curves in adiabatic sorption (Basmadjian, 1997). In the tertiary mixtures containing various combinations of lower concentrations of metal ions, however, increases in total metal ion concentration resulted in higher initial adsorption rates compared with the single metal system, owing to the large difference in concentration between the adsorbent surface and multimetal solution and the competition between the metal ions to bind to the active sites of the fungus (Sag and Kutsal, 1998). Since a fixed cell biomass affects a finite number of surface binding sites, the equilibrium uptake would be expected to show saturation kinetics at high total metal ion concentrations. It is important to note that this non-linearity does not result only from the saturation of the biomass but is also mainly related to the interferences between concentrated components that compete for the adsorption sites (Sag and Kutsal, 1998).

pH: From our observations, the highest uptake of metal have shifted to lower pH values in the presence of other cations, compared with single metal systems. We suggested that iso-electric point of biosorbent was altered in the presence of other cations. At pH values above iso-electric point, there was a net negative charge on the cells and the ionic state of ligands such as carboxyl, phosphate and amino groups will be such as to promote the reaction with Cd^{2+} , Cu^{2+} and Pb^{2+} . As the pH is lowered, however, the overall surface charge on the cells will become positive, which will inhibit the approach of positively charged cations. It is likely that protons will then compete with metal ions for the ligands and thereby decrease the interaction of metal ions with the cells (Sag *et al.*, 1995). Optimum pH for heavy metal uptake is organism-dependent because of adsorptive sites within a single strain. Different metals have different pH

optima, possibly due to different solution chemistry of metal ions (Sag *et al.*, 1995). However, the equilibrium established in the optima pH for the particular metals and biosorbent may be altered in the presence of interfering ions.

Temperature: Although information about the temperature effect on biosorption process is lacking, we believe that temperature affects the biosorption process through cell growth and cell metabolism; e.g. enzymatic system and species forms of metals in the solution.

For biosorption using live microbial cells, intracellular accumulation may occur and enzyme such as reductase may play an important role. It has been suggested by Karim (2000) that microbial oxidation of compounds is coupled to metal reduction and involved enzyme reductase involved. Oxidation of complex organic matter such as sugars, acids, acetate, fatty acids, etc. use metals as electron acceptor by reducing them. For instance, Fe^{3+} is reduced by Ferum reductase using NADH as electron donor and metal as electron acceptor and reducing them.

Uptake of copper and lead was higher at lower temperature of 15 and 25°C. It might be related to the activity of enzyme reductase and explained by the above statements. Presence of other metal species in the solution may alter the optimum pH and temperature for enzyme activity.

Uptake of cadmium was the highest at 60°C probably due to metal chemical forms of the cadmium species which may be easily taken up by the biosorbent. Coombs (1979) reported that there is a distinct difference in chemical forms of metals in different water sources. In seawater, cadmium was present in the form of chloride compounds for more than 90% at 25°C. Increase in temperature usually resulted in an increase in rate of cadmium uptake although there are exceptions in some cases (Coombs, 1979). When salinity and temperature changes are combined, their effects tend to be additive (Coombs, 1979). In river water, cadmium was almost entirely in the form of carbonate compounds. In estuarine water, the metal species are in between varying mixture. Although we were unable to determine the cadmium species in our synthetic medium, change in temperature will change the chemical form of cadmium in the solution, which eventually affect the adsorption process.

The sorption equilibrium curve between free Cd^{2+} and cadmium-loaded biosorbent is usually represented by hyperbolic curve. However, there was some curve whereby the uptake of metal was increased very slowly after reaching saturation. It may be due to chemisorption as described by Sag and Kutsal (1998). Most of predominant processes in adsorption occurred physically. When the temperature increased, the weak binding between metal species and ligands on the biosorbent will be loosen. They may knock out easily by highly motile proton, water molecules and free metal ions in the solution which have higher kinetic energy. Desorption rate was higher than adsorption rate of the metal on biosorbent and the equilibrium in the system will be re-established. In certain cases, the metal species were concentrated at certain surface area of the biosorbent, which contained various multiple binding sites for adsorption. Both of the phenomena may explain the reasons for the fluctuation of the curve obtained.

In conclusion, presence of other cations in the solution may inhibit or enhance the adsorption rate of the selected metal ion, in addition to the effect of several physico-chemical parameters, such as; metal concentration, biosorbent concentration, pH, temperature and viscosity. We hope that this study will serve as a preliminary model study in biosorption of mixture of metals. In actual industrial effluents, there are many other factors, which affect the efficiency in biosorption performance.

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