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Application of Live and Non-metabolizing Cells of *Aspergillus flavus* Strain 44-1 as Biosorbent for the Removal of Lead from Solution

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Abstract: Study on the feasibility of using *Aspergillus flavus* cells as biosorbent to remove lead from solution was carried out using a 500mL shake flask. The effect of metal concentration, biosorbent concentration, temperature and pH on lead adsorption were investigated. The maximum uptake of lead by *Aspergillus flavus* cells was 144.5 mg lead/g dry cell. The highest uptake of lead by this biosorbent was occurred at pH 4 and temperature 40°C.

Key words: *Aspergillus flavus*, biosorption, bioremediation, biosorbent, lead

Introduction

Increasing industrialization brings with it the problems of removal of unwanted and possibly toxic metals from chemical process effluent. Several toxic metals, which present in the waste water, such as cadmium, lead, zinc and copper cannot be degraded by biological and chemical processes (Scragg, 1999). The discharge of non-essential nature of these metals into environment posses a severe threat to human and living creatures. In this condition, these metals are highly toxic non-essential elements that serve no known biological function (Ting and Teo, 1994). In addition, these metals tend to bioaccumulate and biomagnify in our food chain, which could be eventually consumed by human.

The outstanding physical properties of pure lead are its high density, softness, malleability, flexibility, low melting points, low strength and low elastic limit, high corrosion resistance are the basis of most applications of lead metal (Abel, 1973). The major consumers of lead are storage batteries, tetraethyl lead, cable covering, solder, ammunition, fuel for transportation vehicle and radiation shields. All compounds of lead are toxic (Abel, 1973). Serious lead intoxication is now most frequently encountered by inhalation of vapors or dusts of lead and lead compounds.

Heavy metal contaminants at low concentration and large volumes of liquor need to be handled are difficult to remove from aqueous solutions using traditional methods such as precipitation by liming, cementation or electrodeposition. Alternative methods based on biological systems, especially using microbial biomass, with the complexation abilities of large molecules, the use of novel membranes and selective precipitants that overcome previous solubility product barriers are constantly under development.

This study predominantly investigated the feasibility of using the fungal biomass of *Aspergillus flavus* strain 44-1 as biosorbent without any further pretreatment in lead-containing wastewater. Several physico-chemical parameters such as metal concentration, biosorbent concentration, pH and temperature were examined to investigate the lead sorption capability of different natures of *Aspergillus flavus* cells.

Materials and Methods

Microorganism: The fungus, *Aspergillus flavus* strain 44-1, was obtained from Fermentation Technology Unit, Enzyme and Microbial Technology Laboratory, Institute of Bioscience, University Putra Malaysia. This fungus was used in kojic acid fermentation. The cultivation of the fungus, preparation of biosorbent and the biosorption experiments were carried out at the Department of Biotechnology, Faculty of Food Science and Biotechnology, University Putra Malaysia. The fungus was cultivated in a 500mL shake flask containing 100mL medium and inoculated with 5mL of spore suspension (2×10^8 cells/mL). The medium employed for the cultivation of the fungus consists of 2.2 g/L glucose, 0.2 g/L yeast extract, 0.1 g/L KH_2PO_4 and 0.05 g/L $MgSO_4.7H_2O$. The inoculated flasks were incubated at 30°C, on an orbital shaker agitated at 200 rev/min for 24 hours. Cell biomass was harvested from the culture broth after 5-6 days of the cultivation.

Preparation of biosorbent: The cell biomass was washed with distilled water, rinsed twice with ultrapure water and filtered to obtain free living cells. The free living cells, without any further pretreatment, were kept at 4°C in an air-tight container prior to be used in biosorption experiments.

Biosorption experiment: Biosorption experiments were conducted to investigate the effect of lead concentration, biosorbent concentration, pH and temperature on the sorption ability of the fungal biomass. Synthetic solution of ultrapure water containing $Pb(NO_3)_2$ was used in all biosorption experiments. Sorption isotherm equilibrium experiment was carried out in batch process using 500 mL shake flask. A 0.1 g of cells was added to 100 mL lead-containing solutions. The flasks were agitated at 250 rev/min in an orbital shaker and the temperature was maintained at 30°C for 24 hours. The supernatant with free residual lead ion was separated from biosorbent using centrifugation at 3000 rev/min for 15 minutes. The concentration of lead in the supernatant was analyzed using atomic absorption spectrophotometer (Perkin Elmer 3300).

Results

Biosorbent and Pb^{2+} concentration: The amount of biosorbent added into the media was an important factor, which could affect the process of biosorption. The uptake of Pb^{2+} was decreased with increasing biosorbent concentration (Fig. 1) due to the shortage of Pb^{2+} in the solution, as lead concentration did not increase with increasing biosorbent concentration.

The performance of the overall biosorption process was influenced by the ratio of initial lead concentration to biosorbent concentration. The effective ratio between initial lead concentration and biosorbent can be achieved when a low amount of biosorbents was required to remove high concentration of the metal from the solution. In our study, we found that a minimum amount of 0.5 g biosorbent was sufficient to remove averagely 21.3 mg/L of maximum Pb^{2+} from 100 mg/L initial Pb^{2+} concentration (Figs. 1 and 2). Maximum uptake of Pb^{2+} (23.1 mg/L) was achieved at effective ratio of 200 (Fig. 2). Effective ratio of lead to biosorbent was obtained when the ratio between initial lead concentration and amount of biosorbent added was compared. The effective ratio could be significant in scaling up the contactor such as using stirred tank reactor and further treatment of industrial effluent (Hii, 1999).

The performance of biosorption process was also greatly influenced by the concentration of lead in the solution. The sorption of Pb^{2+} was increased with increasing Pb^{2+} concentration until the binding sites were saturated. The maximum Pb^{2+} uptake

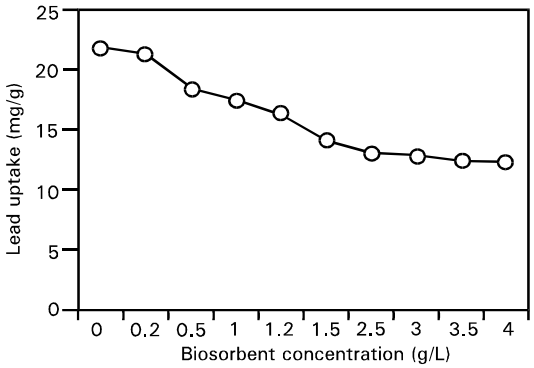

Fig. 1: Effect of biosorbent concentration on lead adsorption by *Aspergillus flavus* using different biosorbent concentrations (0.1 - 4 g/L). Experiment was conducted at 30°C for 24 hours in an orbital shaker at 250 rev/min with initial lead concentration, 100 mg/L.

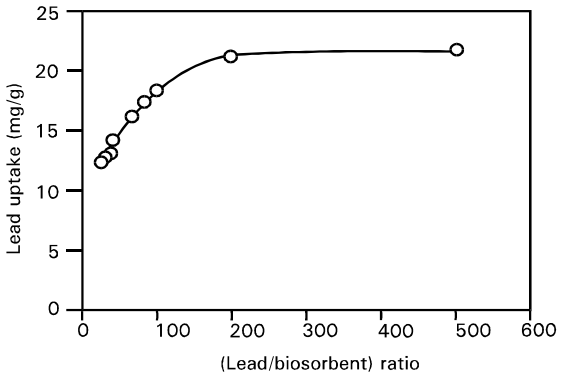

Fig. 2: Effect of [lead / biosorbent] ratio on lead adsorption by *Aspergillus flavus*. Experiment was conducted at 30°C for 24 hours in an orbital shaker at 250 rev/min with initial lead concentration, 100 mg/L.

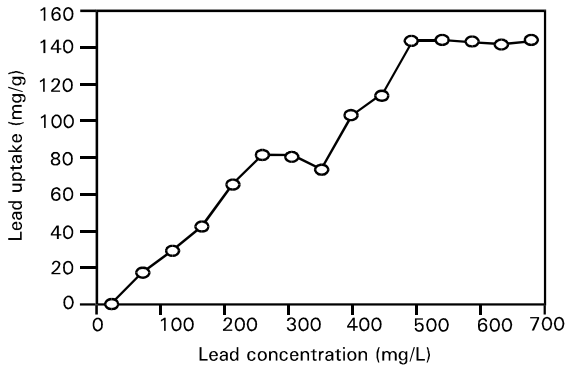

Fig. 3: Effect of lead concentration on lead adsorption by *Aspergillus flavus* using different initial lead concentration (0 - 480 mg/L) . Experiment was conducted at 30°C for 24 hours in an orbital shaker at 250 rev/min with 0.1 g of biosorbent added .

was 144.5 mg/g dry cell when the binding sites reached the saturation (Fig. 3).

Effect of pH: It is well known that the pH greatly affect the adsorption rate on microbial cells (Sag *et al.*, 1995). The Pb^{2+} uptake was generally increased with increasing the lead

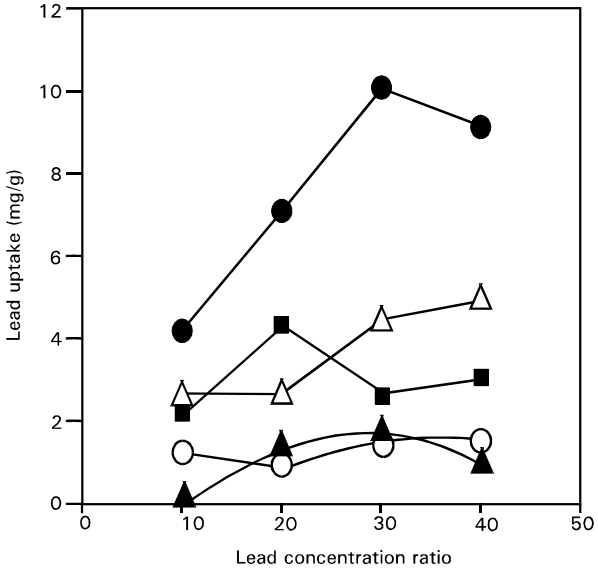

Fig. 4: Effect of pH on lead uptake on single lead system by *Aspergillus flavus*. Experiment was conducted at 30°C for 24 hours in an orbital shaker at 250 rev/min. Symbols represent: (Δ) pH 1, (o) pH 2, (▲) pH 3, (●) pH 4, (■) pH 5.

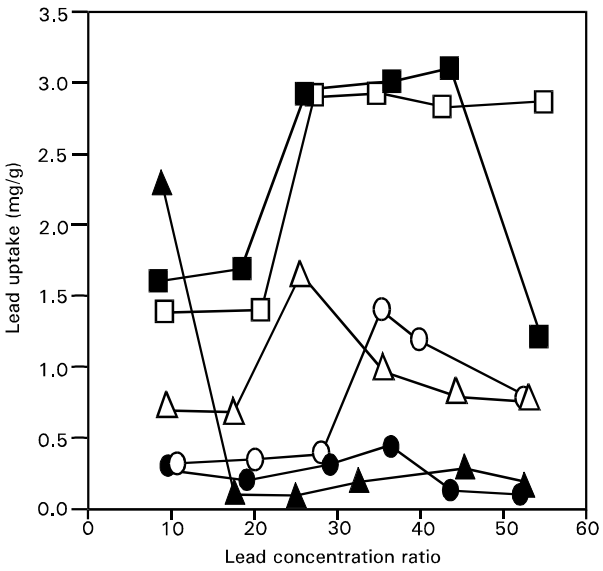

Fig. 5: Effect of temperature on lead adsorption by *Aspergillus flavus*. Experiment was conducted at 30°C for 24 hours in an orbital shaker at 250 rev/min. Symbols represent: (●) 15°C, (o) 25°C, (□) 30°C, (■) 40°C, (Δ) 50°C, (▲) 60°C.

concentration at pH 1, 2, 3 and 4 (Fig. 4). However, decrease in lead uptake at Pb^{2+} concentration 30 mg/L was observed at all the pH values investigated except pH 1, whereby the uptake became almost constant beyond 30 mg/L of lead. As for pH 5, the uptake was increased with increasing Pb^{2+} concentration up to 20 mg/L. At Pb^{2+} concentration, above 20.0 mg/L, decrease in the uptake of Pb^{2+} was observed. The experiment at pH higher than 5 was not conducted as Pb^{2+} precipitation was occurred at pH above 5.6.

Effect of temperature: The uptake of Pb^{2+} was generally increased

Table 1: Comparison of lead uptake capacity by various adsorbents

Microorganism	Nature of cell	Uptake capacities	References
Aspergillus flavus 44-1	Viable biomass	144.5 mg Pb/g adsorbent	This study
Arthrobacter sp.	-	130 mg Pb/g adsorbent	Veglio *et al.* (1997)
Streptomyces noursei	-	62.0% of removal	Mattuschka and Straube (1993)
Ecklonia radiata		282.0 mg Pb/g adsorbent	Matheickal and Yu (1996)
Lemna minor	Viable biomass	85-90% of removal	Rahmani and Sternberg (1999)
	Non-viable biomass	60-75% of removal	
Chlorella vulgaris	-	82.9 g Pb/g adsorbent	Aksu and Kutsal (1991)
Rhizopus oligosporus	-	98.6 mg Pb/g adsorbent	Ling (1996)
Aspergillus flavus	Oven-dried biomass	196.0 mg Pb/g adsorbent	Normala (1998)
	Freeze-dried biomass	251.62 mg Pb/g adsorbent	
	Live biomass	280.41 mg Pb/g adsorbent	
Rhizopus arrhizus	-	10.4% of dry weight	Tobin *et al.* (1984)
Rhizopus arrhizus	-	55.6 mg Pb /g adsorbent	Fourest and Roux (1992)

with increasing Pb^{2+} concentration up to 27 mg/L and 46 mg/L for the biosorption carried out at 15, 25 and 30°C and 40 and 50°C, respectively (Fig. 5). The Pb^{2+} uptake was the lowest at 60°C, though significantly higher uptake was observed at low Pb concentration (10 mg/L).

Discussion

Uptake of Pb^{2+} was decreased with increasing biosorbent concentration. Electrostatic interaction between cells may be a significant factor in the biomass dependence of metal adsorption (Rome and Gadd, 1987). Besides, with increasing biosorbent concentration, the equilibrium have altered between the free Pb^{2+}, free biosorbent and Pb^{2+} –loaded biosorbent, which led to the disruption in the formation of metal-biosorbent complexes.

Results obtained were usually in hyperbolic curve with the biosorbent uptake value levelly off when it reaches the complete saturation of the adsorbate at high concentration of the biosorbent (Volesky, 1990). Moreover, biosorption capacity of the microbial biomass increases when the initial metal concentration rises, as long as all multiple binding sites were still unsaturated. When the high affinity surface of the adsorbent begin to saturate, surface binding of low affinity surface will increase, thus the removal of metal decrease as the concentration increase (Fourest and Roux, 1992). This further explains that a possibility of obtaining a linear curve at low concentrations than at high concentrations of adsorbate in most of the biosorption processes.

Darnall *et al.* (1986) reported that at pH values above the isoelectric point of the cells, there is a net negative charge on the cells of *Chlorella vulgaris*. The ionic state of such ligands as carboxyl, phosphate, imidazole and amino groups will promote reaction with metal ions. At lower pH, however, the overall surface charge on the cells will become positive which will inhibit the approach of positively charged metal cations. It is likely that protons will then compete with metal ions for the ligands, thereby decreasing the interaction of metal ions with the algal cells. However, results obtained by Galun *et al.* (1987) showed that Cu^{2+} uptake using *Penicillium digitatum* was virtually pH-insensitive, which might involve neutral ligands as described by Tobin *et al.* (1984).

The alteration of pH values might affect the membrane potential of the biosorbent, which eventually affect the Pb^{2+} uptake. Moreover, the alteration of pH values in the solution will change the isoelectric point of the biosorbent . The ionic charge on the surface of the biosorbent will determine the amount of positively-charged Pb^{2+} being adsorbed.

The highest uptake of Pb^{2+} was achieved at 40°C as the enzymes involved in lead accumulation functions well in this optimum temperature. At higher temperature of 50°C and 60°C, the growth of *Aspergillus flavus* was affected as the enzymatic system was disrupted at these temperatures. Besides, formation of different species of lead may occur at higher temperature, which might reduce the formation of ligand–free Pb^{2+} complexes on the biosorbent in the solution.

Comparison of lead uptake capacity by the biomass of *Aspergillus flavus* with other adsorbents is summarized in Table 1. The lead uptake by *Aspergillus flavus* cells was higher than those obtained by other fungi cells such as *R. oligosporus* and *R. arrhizus* but lower than that obtained by *Ecklonia radiata*.

In conclusion the biomass of *Aspergillus flavus* strain 44-1 was found to be a potential biosorbent that can be used in treating the wastewater, which contained lead from industrial effluent. The optimum condition for the biosorption of lead by *Aspergillus flavus* cells was found at pH 4 and temperature 40°C.

Acknowledgment

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