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Biosorption of Copper and Lead by Heavy Metal Resistant Fungal Isolates

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Abstract- Microorganisms play a significant role in bioremediation of heavy metal contaminated soil and wastewater. In this study heavy metal resistant fungi were isolated from the waste water treated soil samples of Hudiara drain, Lahore. The optimum pH and temperature conditions for heavy metal removal were determined for highly tolerant isolates of *Aspergillus* species along with the initial metal concentration and contact time. Biosorption capacity of *Aspergillus flavus* and *Aspergillus niger* was checked against Cu (II) and Pb (II) respectively. The optimal pH was 8-9 for A. flavus and 4-5.4 for *A. niger*, whereas the optimal temperature was 26°C and 37°C respectively. Moreover, the biosorption capacity of *A. flavus* was 20.75-93.65 mg/g for Cu (II) with initial concentration 200-1400 ppm. On the other hand the biosorption capacity of A. niger for Pb (II) ranged from 3.25-172.25 mg/g with the same range of initial metal concentration. It was also found that equilibrium was maintained after maximum adsorption. The adsorption data was then fitted to Langmuir model with a coefficient of determination greater than 0.90. The knowledge of the present study would be helpful for further research with reference to bioremediation of polluted soils.

Index Terms- Aspergillus; Biosorption, Copper; Heavy Metals; Lead.

I. Introduction

Soil is a crucial component of rural and urban environments, and in both places land management is the key to soil quality. Excessive accumulation of heavy metal in soil is toxic to human beings and animals. World over the agricultural soil contamination with heavy metals is a major problem on defense related and industrial sites (Parameswari *et al.,* 2010). Human actions like mining industries and metal manufacturing with storage, transportation and disposal problems are the main sources of pollution (Zhang *et al.,* 2005). Heavy metals get accumulated into soils and plants and could have a negative influence on physiological activities of plants and cause for the reductions in plant growth, dry matter accumulation and yield (Suciu *et al.,* 2008).

As heavy metal pollution is a serious environmental problem with severe health effects, its remediation is essential. The physical and chemical methods of remediation are time consuming and expensive, hence a biological approach provides an alternative solution of the problem. Microbes are naturally capable of degrading wastes and have a capability to survive under stress conditions. Recently microbes like bacteria, fungus and algae have been effectively used as adsorbing agents for heavy metals removal (Munoz *et al.,* 2006). In metal polluted environments, microbial populations adapt to high concentrations of heavy metals and become resistant to toxic concentration (Prasenjit and Sumathi, 2005). Different species of *Aspergillus* have been reported as efficient nickel and chromium reducers (Yan and Viraraghavan, 2003). Recently, several filamentous fungi (FF) species are found to be useful for biological treatment of the sludge (settling, dewatering, organic compound degradation) under controlled operating conditions (Lacina *et al.,* 2003). For removal of heavy metals from solutions fungi have identified as potential biomass that belong to the genera *Penicillium* and *Rhizopus* (Volesky and Holan, 1995). Huang and Huang (1996) studied that *Aspergillus oryzae* can remove copper and cadmium ions from aqueous solutions. In the same way, Zafar *et al.* (2006) reported promising biosorption for Cd and Cr by two filamentous fungi, *Aspergillus* sp. and *Rhizopus* sp., isolated from metal-contaminated agricultural soil.

The response of microorganisms towards toxic heavy metals is of importance in view of their interest in the reclamation of polluted sites. The main purpose of the present investigation was to evaluate the ability of isolated fungal strains towards the remediation of copper and lead by characterizing the bioaccumulation of heavy metals.

II. Materials and Methods

Samples collection and isolation of fungi

Soil samples were collected from peri-urban agricultural areas along the Hudiara drain, Lahore. From selected soil samples *Aspergillus flavus* and *Aspergillus niger* were isolated by soil dilution method and preserved in laboratory for further heavy metal biosorption analysis. In the collected soil samples, the copper and lead mean concentration was 94.5 and 68.4 mg/kg respectively.

Biosorption analyses

Aspergillus flavus and *Aspergillus niger* biomass was prepared in Potato Dextrose Broth (PD broth). To prepare 100 ml of the Potato broth, 30 ml of potato extract and 2 gm of glucose was added in conical flask and filled up to100 ml by distilled water. Flask was closed with cotton plug and aluminum foil and autoclaved for 30 minutes. After that the flask was opened in laminar flow and *Aspergillus* sp. were introduced in it. The flasks were agitated on a shaker for 3-4 days at 150rpm and at 30˚C. After 3-4 days thick bed of fungal biomass was developed which was further used for biosorption experiments. To explore the biosorption of the *Aspergillus* isolates to the heavy metals, varying initial heavy metal concentrations with optimal culture conditions were used. Each growth medium was amended with

$CuSO_4$ and Pb $(NO_3)_2$ with different concentrations ranging from 200-1400ppm.

Effect of pH and temperature on fungal biomass adsorption

In order to check the effect of pH on adsorption capacity of *Aspergillus* sp., the fungal isolates were conditioned at different pH ranging in 4-9. To simultaneously search for optimal temperature for each pH the represented cultures were incubated at different temperature ranges (22-37˚C) with time interval of 20 hours on rotary shaker at 150rpm. After 20 hours, the fungal biomasses were filtered in order to separate the biomasses from media. The biomasses were separated from media and the mixture was filtered through Whattman filter paper. The biomass on the filter paper was then dried by the process of drying on hot plate in order to absorb the moisture content at temperature of 120˚C for about 3-4 days.

Removal of Cu and Pb at different concentrations

Dried and powdered dead biomass (0.5g) was inoculated into 100ml of metal solution containing 200-1400 ppm $CuSO_4$ and Pb $(NO_3)_2$ in distilled water for single metal system. The flasks were kept on the rotary shaker at 150rpm at 30°C for 50 hours. The content of supernatant was determined by the use of atomic absorption spectrophotometer. The experiment was done in duplicate and the amount of metallic ion biosorbed per gram of biomass (q) and the efficiency of biosorption (E) were calculated using following equations (Javaid *et al.*, 2010):

$$q = \left(\frac{C_i - C_f}{m} \right) V \quad \textbf{(1)}$$

Where $\boldsymbol{q}$ = mg of metal ions uptake per gram biomass (mg/g), $\boldsymbol{C_i}$ = initial concentration of the metallic ions (mg/L); $\boldsymbol{C_f}$ = final concentration of metallic ions (mg/L); $\boldsymbol{m}$ = dried mass of the biosorbent in the reaction mixture (g) and $\boldsymbol{V}$ = volume of reaction mixture (ml).

$$E = \left(\frac{C_i - C_f}{C_i} \right) \times 100 \quad \textbf{(2)}$$

Isotherm assessment

The uptake of heavy metals ion by inactive cell of fungi were analyzed by the adsorption isotherms Langmiur models and the amount of metal bound by the biosorbents was calculated as follows (equation 3).

$$q_e = \frac{bQ_m C_{eq}}{1 + bC_{eq}} \quad \textbf{(3)}$$

Where, $\boldsymbol{q}$ = metallic ions adsorbed per unit of weight of adsorbents (mg/g), $\boldsymbol{Q_m}$ = maximum possible amount of metallic ions adsorbed per unit of weight of adsorbents (mg/g); $\boldsymbol{b}$ = constant related to the affinity of binding sites for metal ions, $\boldsymbol{C_{eq}}$ = equilibrium concentration (mg/L)

III. Results and Discussion

Effect of pH

Biomass of *Aspergillus flavus and Aspergillus niger* were exposed to Cu (II) and Pb (II) respectively. *Aspergillus flavus* followed an increasing trend and exhibited maximum sorption capacity for the Cu (II) in the pH range 8-9 with maximum efficiency 97% while *Aspergillus niger* exhibited maximum sorption capacity for Pb (II) at pH range 4-5.4 with biosorption efficiency 21.5%, above this pH substantial decline in metal uptake was evidenced which represents the pH factor being highly sensitizing element (Fig. 1). Hasan *et al.* (2000) reported maximum removal of nickel in the pH range of 4.5-5.5. The variation of adsorption of nickel at various pH is on the basis of metal chemistry in solution and the surface chemistry of the sorbent. Low pH limits the biosorption of Cu (II) ions on fungal biomass surfaces, probably due to the ion exchange between metallic species and competition effects with oxonium (Hydronium) ion to some extent in the biosorption mechanism (Yin *et al.*, 1999). In similar findings by earlier investigators it has been attributed to protonation or poor ionization of acidic functional group of cell wall at low pH, inducing a weak complex affinity between the cell wall and the metal ions (Chergui *et al.*, 2007). The reduction in metal ions uptake by fungus at higher pH can be explained on the basis that at higher pH values the metal ions may accumulate in the cells or the intra-fibular capillarity of the cell walls by a combined sorption micro precipitation mechanism therefore, biosorption experiments are meaningless at higher pH (Beveridge, 1986).

Effect of temperature

Fig. 2 shows that the effect of temperature on the biosorption of heavy metal ions was significant. The heavy metal causes a decline in the uptake of Cu (II) by *A. flavus*. The maximum biosorption capacity was 81.6 mg/g and efficiency 40.8% at 26°C, while *A. niger* followed the declining trend with maximum biosorption capacity and efficiency of Pb (II) 91mg/g and 45.5% respectively at 37°C. Temperature is known to affect the stability of the cell wall, its configuration and can also cause ionization of chemical moieties. These factors may simultaneously affect the binding sites on isolated fungal and bacterial species causing reduction in heavy metal removal. Energy independent mechanisms are less likely to be affected by temperature since the processes responsible for removal are largely physiochemical in nature (Gulay *et al.*, 2003). Similar results have been reported in the bioaccumulation of Cr (VI) by *S. equisimilis* and *A. niger* (Goyal and Banerjee, 2003).

Effect of contact time

Time course profiles for the adsorption of Cu (II) and Pb (II) by *A. flavus* and *A. niger* revealed that almost 83% and 82% removal of heavy metal ions and saturation level of total biomass was recorded during first 10 minutes (Fig. 3). The adsorption equilibrium or plateau level gradually reached within 15-20 minutes for both Cu (II) and Pb (II) ions. The Cu (II) uptake by *A. flavus* kept on increasing gradually after 30 minutes while adsorption of Pb (II) followed a gradual declining trend after 30 minutes. The findings verify two phases of biosorption, an initial rapid uptake due to surface adsorption and subsequent slow uptake due to membrane transport of metal ions into cytoplasm of cell or slow intracellular diffusion or reduced permeability of cell wall (Saglam *et al.*, 2002). Similar results were obtained by

Chatterjee *et al.* (2010), while in some other studies single-step uptake has been suggested for different biosorbent (Huang *et al.*, 1990).

Effect of initial concentration of metal ions

The graphical presentation for the effect of initial metal ion concentrations on biosorption capacity of the test fungi is depicted in fig. 4. The temperature was maintained at about 30˚C.The batch was carried out for more than 50 hours. The biosorption capacity of *A. flavus* was 20.75-93.65 mg/g with initial concentration 200-1400 ppm. On the other hand the biosorsorption capacity of *A. niger* for Pb ranged from 3.25-172.25 mg/g with the same range of initial metal concentration mentioned above.

The results of present findings clearly indicate that the sorption capacity increased and reached a saturation value as the metal ion concentration increased in aqueous medium. This assessment is in line with previously reported data on metal ion sorption by many other similar studies (Sheng and Ting, (2007). There is an evidence that at high metal ion concentration the number of ions adsorbed is more than at low metal concentration, where more binding sites were free for interaction (Mukhopadhyay *et al.*, 2007).

Isotherm assessment

Isotherm studies are basic requirement to design biosorption application procedures. For this purpose the empirical models i.e. Langmuir (1916) for single solute system was employed to describe the biosorption equilibria of the test fungus. The parameter resulted from the Langmuir plots for Cu (II) and Pb (II) ions by the test fungus are presented in table 1. The plot of $1/q_e$ vs $1/C_{eq}$ in various initial concentrations range (200-1400 ppm) of metal ions were found to be linear indicating the applicability of classical Langmuir adsorption isotherm to single metal ion solution (Fig. 5 A and B). The coefficients of determination (R^2) are more or less greater than 0.90, indicating that models adequately describe the experimental data of all metal ions biosorption. The maximum capacity (q_m) was determined from the Langmuir isotherm point calculated by the model in function of the experimental values of q_e shows a linear tendency among the observed and predicted values. The Langmuir isotherm "*b*" the stability complex formed between metals ions and fungal cell wall under specific experimental conditions clearly demonstrated the small values, which means greater affinity of biomass for copper and lead ions. A good metal sorbent in general should have a high q_m as well as low b value. Javaid *et al.* (2010) also observed a classical linear Langmuir adsorption isothem with value of coefficient of determination greater than 0.90 for four different heavy metals. Similar results have been reported by Sa *et al.* (2002).

In biosorption analysis, it has been commonly observed that pH, temperature, contact time and initial concentration of metal solution strongly influence the fungal biosorption process cumulatively (Wang and Chen, 2009). Several researchers have also investigated the effect of these parameters on biosorption of heavy metals by using different biomass and found similar results with the present study. According to previous researches and the present study, it is estimated that this data is appropriate for environmental studies. The biosorption is an efficient process to remove the contaminants from the environment. Ability of *Aspergillus* sp. to adsorb the metal ions with excellent mechanical properties provides an opportunity to utilize such organisms for number of purposes for the remediation of waste from particular source.

It was concluded from this investigation that *Aspergillus* sp. are good adsorbing medium for metal ions having high adsorption yields for the treatment of soils containing copper and lead ions. These strains have a remarkable metal adsorption capacity over a wide range of temperature and basic pH and may be employed in future for metal remediation from waste water and heavy metals contaminated soils.

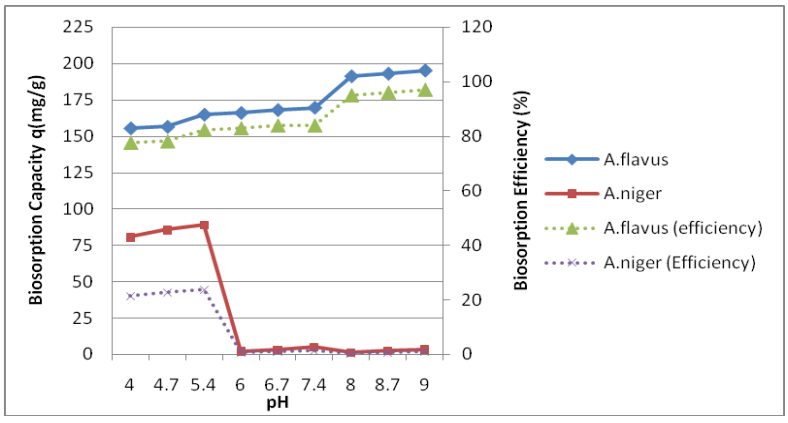

Fig. 1. Effect of pH on the biosorption of Cu (II) and Pb (II).

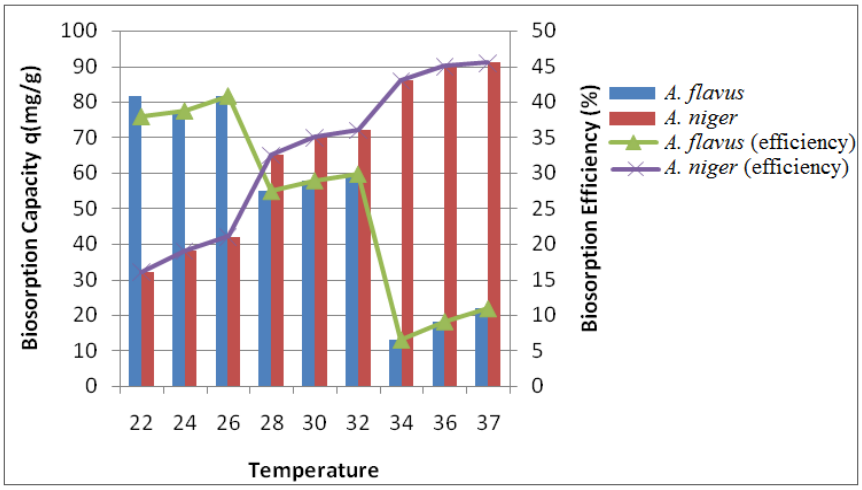

Fig. 2. Effect of temperature on the biosorption of Cu (II) and Pb (II).

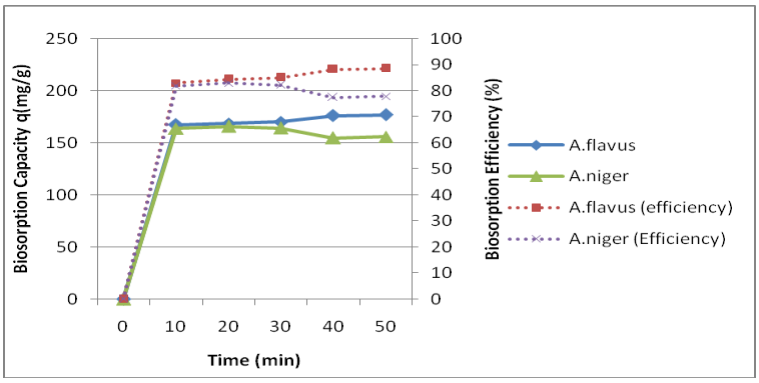

Fig. 3. Effect of contact time on the biosorption of Cu (II) and Pb (II) with efficiency.

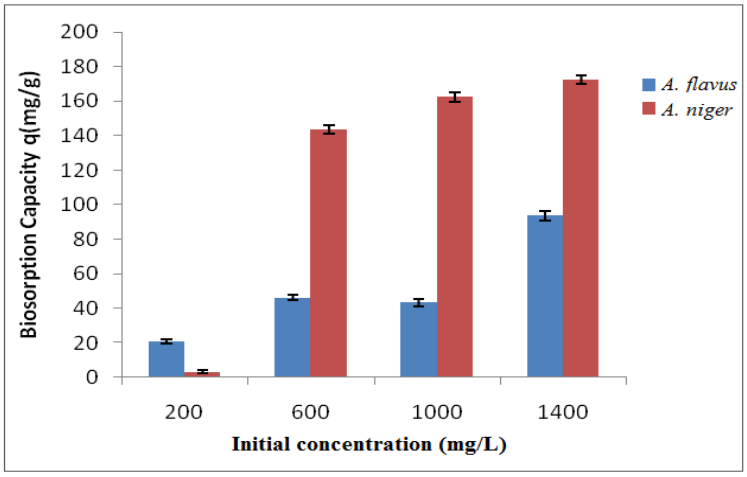

Fig. 4. Effect of different metal concentrations on biosorption capacity of *A. flavus* and *A. niger*.

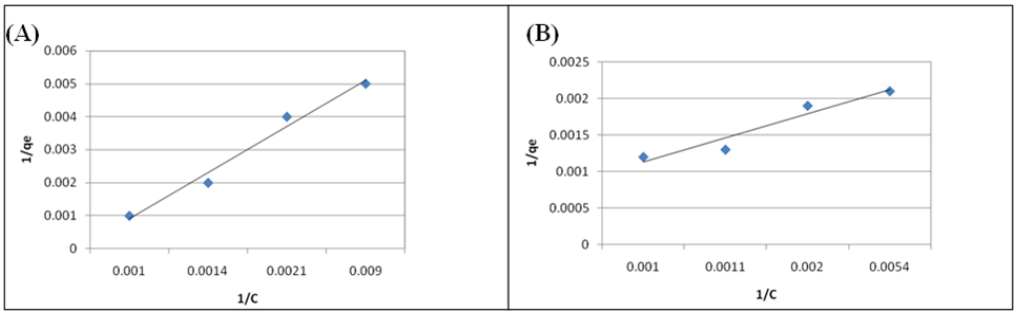

Fig. 5. Linearized langmuir adsorption isotherm of (A) Cu (II) by *A. flavus* and (B) Pb (II) by *A. niger*.

Table 1. Isotherm model parameters for the biosorption of metal ions.

Metal ions	q_m (mg/g)	b (mg/l)	R^2
Cu (II)	963	0.002	0.98
Pb (II)	1142	0.001	0.926

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