Bio sorption of Cadmium (II) from Aqueous Solutions by Free and Immobilized Biomass of Penicillium Citrinum

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Abstract – In recent years removal of heavy metals from wastewater streams is of worldwide concern. Studies revealed that biosorption seems to be more efficient and cost effective process inspite of conventional chemical based removal methods. The present study explores the ability and to evaluate the comparative potential of free and immobilized biomass of Penicillium citrinum for Cd (II) removal from its aqueous solution of concentration 20 ppm in batch mode. Experiments were conducted to optimize the various parameters like pH, biosorbent dose, contact time and initial metal ion concentration in batch mode with the help of free and immobilized biomass of selected fungus. Biosorption of Cd(II) was found to be pH dependent and maximum biosorption was observed at pH 6 by both free and immobilized biomass. Immobilised biomass was found to have more biosorption efficiency in comparison to free biomass. This is due to sorption capacity of alginate beads. The biosorption of Cd (II) was best fitted with the pseudo second order kinetic model with regression coefficient more than 0.99. Langmuir and Freundlich isotherms model fits well with the experimental data. All these observations indicate that the immobilized biomass of Penicillum citrinum could be used as a suitable biosorbent and serve as baseline data for desingning a treatment process for removal of Cd (II) from wastewater.

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OVERVIEW

Over the past few decades rapid urbanization and swift growth of chemical industries has resulted in contamination of environment due to toxic waste effluents mainly heavy metals, which are known to be toxic and non biodegradable, causing health hazards to biota has become a serious issue. Heavy metals enter into the environment by both natural and anthropogenic sources. Industrial processes, agricultural activities, sewage treatment and mining operations, all are responsible for producing large quantities of metal containing wastewaters. In addition, domestic effluents, landfill leachate, agricultural runoff and acid rain also contribute to heavy metals in water (Aksu and Kutsal, 1990). Removal of heavy metals from industrial wastewater is of primary importance because they are not only causing contamination of natural water bodies but are also toxic to many life forms. In this context a cost effective treatment for metal contaminated waste water is required, as environmental laws become stringent (Kadirvelu *et al.,* 2002). Decontamination or effective disposal of wastewater has always been challenging with a thrust on cost-effective treatment technologies showing minimal side effects. For more than a decade, researchers have been looking for cheaper and more effective methods to remediate heavy metal-contaminated waters and reduce the growing public health risk. Although heavy metal removal from inorganic effluent can be achieved by conventional methods such as chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction, each treatment has its inherent limitation. Most of these are ineffective or excessively expensive when the metal concentrations are less than 100 mg/L (Ahluwalia and Goyal, 2007).

Biosorption is proven to be quite effective at removing metal ions from contaminated solution in a low-cost and environment-friendly manner. Metal uptake by non-living biomass has some advantages; e.g. the process is not subjected to physiological constraints such as metal toxicity and thermal inactivation of the microorganism. The major advantages of biosorption over conventional treatment methods include low cost, high efficiency of metal removal from dilute solution and possibility of metal recovery. The biosorption process involves a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions) and a solid phase (sorbent or biosorbent; biological material) on which it is sorbed.

Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases. Algae, bacteria, fungi and yeast have proved to be potential metal biosorbents (Volesky, 1986). Fungi in particular have demonstrated unique metal absorption characteristics and are easy to cultivate (Gadd, 1988).

The present study deals with the aim of biosorption of Cd(II) by free and immobilized biomass of *Penicillium citrinum*. Cadmium is one of the most toxic heavy metals and considered to be carcinogenic. Cadmium in humans can cause serious damage to kidney and bones (Rajendran *et al*, 2003). Other effects of Cd are diarrhoea, growth retardation, bone deformation, anaemia, hypertension and liver injury. Cadmium is introduced into water bodies from smelting, metal plating, cadmium - nickel batteries, phosphate, fertilizer, mining, pigments, stabilizers, alloy industries and sewage sludge.

The main objective of this work is to investigate the comparative biosorption capacity of low-cost absorbent non-living free and immobilized biomass of *Penicilium citrinum* for the removal of Cd(II) from aqueous solution.

MATERIALS AND METHODS

Biomass preparation and immobilization

The fungal strain used in the present study was *Penicillium citrinum*, isolated from metal-contaminated soil of steel industry, situated in Haryana. Fungal isolation was done using solidified Rose Bengal culture medium (2% agar). For experimental purpose, fungal species were cultivated in liquid phase using a shaking incubator. The spores were transferred to 250 ml Erlenmeyer flasks filled with 100 ml of a culture medium composed of the following (g/L): Dextrose, 10 gm; Peptone, 5.0 gm; $MgSO_4.7H_2O$, 0.5 gm; KH_2PO_4, 1.0 gm and Streptomycin, 0.033 gm. The liquid phase pH was adjusted to 5 by the use of 1 N HCl. Once inoculated, the flasks were shaken on a rotatory shaker at 130 rpm for five days at 30 ± 2^0C. The biomass was collected and washed with generous amount of distilled water. The dead biomass was prepared by giving autoclave treatment (15 lb, 121 °C for 20 min) to cultured biomass. After that, the biomass was dried at 60°C for a period of 16 h and powdered. Then the powdered biomass was sieved through standard sieve to obtain particles of size upto 0.3 mm. 50% of biomass was used as such while rest of 50% biomass was immobilized by entrapment in polymer matrix of Na-alginate. 3% (w/v) slurry of sodium alginate was prepared in distilled water. After cooling, 3% (w/v) of biomass was added and stirred on magnetic stirrer. The alginate biomass slurry was introduced into 0.1 M $CaCl_2.2H_2O$ for polymerization and bead formation using 5 ml syringe. The resultant beads were of ~4 mm diameter. The fungus entrapped beads were cured in this solution for 1 h and then washed twice with 200 ml of sterile distilled water (Bai and Abraham, 2002).

Experimental procedure

The batch biosorption experiments were conducted using 250ml Erlenmeyer flasks at constant temperature (25^0C) and agitation speed (150 rpm). For optimization of different parameters such as pH, biosorbent dose, contact time and initial metal ion concentration, the batch experiments were performed using 20 mg/L (except during the optimization of initial metal ion concentration) synthetic solution of Cd(II). To study the effect of pH on removal of Cd (II), experiments were conducted by keeping biosorbent dose, contact time and initial metal ion concentrations constant, while pH was varied. Similarly effect of other parameters on removal of Cd (II) was studied. The pH of solution was maintained at a constant value by adding either 0.1 M HCl or NaOH. Each flask was removed after 60 minutes contact period (except during the optimization of contact time) and the solution was filtered using Whatman filter paper. The residual concentration of Cd (II) in filtrate was determined by Atomic Absorption Spectrophotometer (Shimadzu 6300, Japan). All experiments were done in triplicate. The removal percentage (*R* %) of zinc was calculated for each run by following expression:

$$R (\%) = [(C_i – C_e)/ C_i] \times 100$$

Where C_i and C_e were the initial and final concentration of cadmium in the solution. The uptake capacity of the biosorbent for each concentration of Cd(II) at equilibrium was calculated using the equation:

$$q_e (mg\ g^{-1}) = [(C_i – C_e)/ M] \times V$$

Where, C_i and C_e were the initial and final concentration of Cadmium ions (mg L^{-1}) in the test solution respectively. *V* is the volume of solution (L) and *M* is the mass of biosorbent (gm) used.

Adsorption isotherms:

The biosorption data obtaied for the Zn (II) was analyzed using Langmuir and Freundlich isotherms

Langmuir's isotherm model is valid for monolayer adsorption onto a surface containing a finite number of identical sites, which is represented as equation:

$$\frac{Ce}{qe} = \frac{1}{Qob} + \frac{Ce}{Qo}$$

Where qe is the amount adsorbed at equilibrium (mg/g); Ce is the equilibrium concentration (mg/l); Qo and b are the Langmuir constants.

The Freundlich equation (Bai and Abraham, 1998) proposes an empirical model that is based on the sorption on heterogeneous surface and has the form:

Log x/m = log k + 1/nlog Ce

Where k (mg/g) and n are Freundlich isotherms constants; Ce is the equilibrium concentration (mg/l); x/m is the amount adsorbed (mg/g) and m is the adsorbent dose (g/l).

RESULTS AND DISCUSSIONS

Batch biosorption studies Effect of pH

Experiments were conducted to study the effect of pH at constant initial Cd(II) concentration-20 mg/L, biosorbent dose (0.2 gm/50 ml for free biomass and 1.0 gm/50 ml for immobilized biomass) and contact time-60 minutes, shaking speed-150 rpm, temperature 25^{0}C while pH was varied from 2 to 7 for both the immobilized biomass and free biomass.

The removal of Cd(II) by *P.Citinum* at different pH is shown in the Fig. 1. The maximum removal of Cd(II) i.e. 58.61% for free and 67.71% for immobilized biomass was observed at pH 6.0.

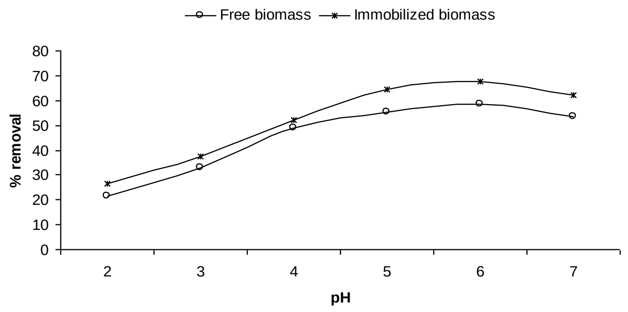

Fig1. Effect of solution pH on Cd(II) removal by free and immobilised biomass of *Penicillium citrinum*

Similar pH values for maximum Cd(II) removal have been reported for fungal biomass *Phanerochaete chrysosporium* (Say et al., 2001) and with *Jypnea valentiae* (Aravindhan et al.,2010) biomass at pH 6.0. This may be due to the fact that at low pH (<1.0) the biosorption capacity for all metal ions is very low, because large quantity of hydrogen ions competes with metal ions at sorption sites. As the pH increases, more negatively charged cell surface become available thus facilitating greater metal uptake (Ahuja et al,1999). However, metal precipitates at high pH values (>7.0) which inhibit the contact of metal with the most fungal biomass (Fan et al, 2008).

Effect of biosorbent dose:

The Effect of biosorbent dose on biosorption of Cd(II) was investigated by varying biosorbents dosages in the range of 0.2g/50ml to 0.7g/50ml for free biomass and 0.5gm/50ml to 0.3gm/50ml (wet basis) for immobilized biomass. While others parameters such as optimum pH (6.0), initial metal ion concentration (20 mg/l), contact time (60 min.), temperature (25^{0}C) and stirring speed (150 rpm) were kept constant.

As evident from the results, the percentage removal of Cd(II) increased with increase in biosorbent dose upto some extent, thereafter no appreciable increase in percentage removal was observed with further increase in biosorbent dose. The Cd(II) percent removal was found to be increased from 58.43% to 78.88% for free biomass and 61.46% to 77.32% for immobilized biomass. The optimum dose for Cd(II) removal was found to be 1.0gm/50 ml with 66.42% removal for immobilized biomass and 0.2 gm/50 ml with 58.43% removal in case of free biomass of *Penicillium citrinum*.

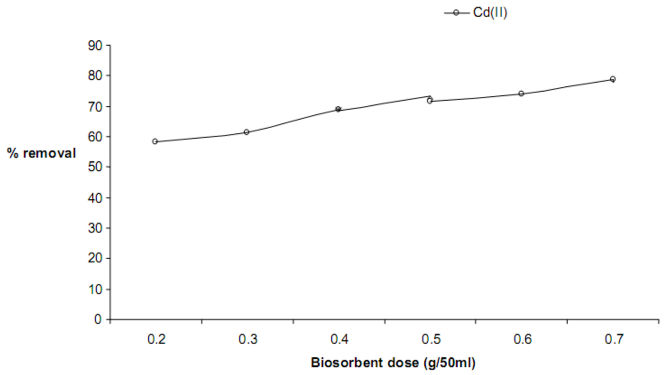

Fig.2 Effect of biosorbant dose on Cd(II) removal by free biomass of *Penicillium Citrinum*

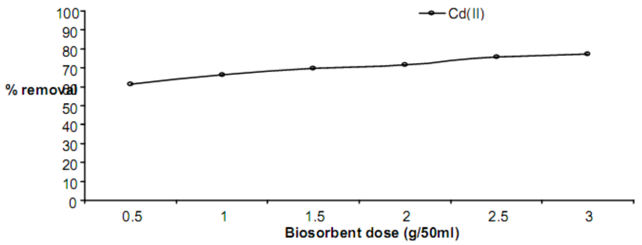

Fig.3 Effect of biosorbant dose on Cd(II) removal by immobilized biomass of *Penicillium Citrinum*

Effect of Contact time

Experiments were conducted at optimized pH 6 for both the immobilized and free biomass and optimized biosorbent dose for Cd(II) i.e. 1.0gm/50ml and 0.2gm/50ml (for immobilized and free biosorbent respectivily) and initial metal ion concentration of 20mg/L, temperature 25^0C and stirring speed 150rpm. Contact time was varied from 15 minutes to 180 minutes.

The results indicated that Cd(II) removal increased with increasing contact time. The removal for immobilized biomass increased from 52.71% to 81.64 % and removal for free biomass from 37.05% to 74.94%. The optimum time observed was 60 minutes for free and immobilized biomass after which metals ions concentration in the test solution became constant. This may be explained by the fact that initially a large number of vacant sites may be available for absorption and after some time, the remaining vacant surface sites may be difficult to occupy due to repulsive forces between the solute molecules of the solid and bulk phase (Chand *et al,* 1994 and Saravanane *et al*, 2002). Tsekova *et al*. (2010) observed similar trend for Cu(II) and Cd(II) by free and immobilized biomass of *Aspergillus niger.*

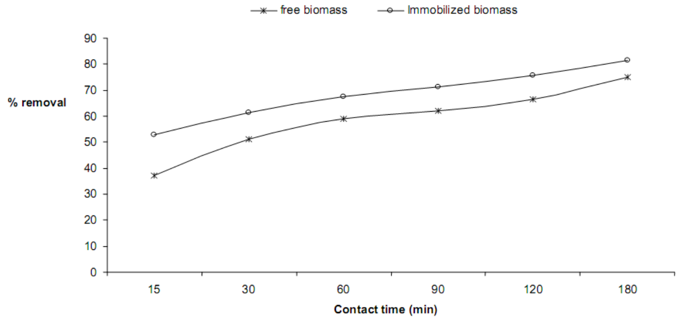

Fig. 4 Effect of Contact time on Cd(II) removal by free and immobilized biomass of *Penicillium citrinum*

Effect of Initial metal ion concentration

The effect of initial metal ion concentration on removal of Cd (II) was investigated by conducting experiments at optimized pH 6.0, contact time 60 min and biosorbent dose (1.0gm/50ml for immobilized and 0.2gm/50 ml free biomass of *P. citrinum*). The initial metal ion concentration was varied from 10mg/L to 200mg/L.

From the results fig 4, it was observed that the percentage biosorption of Cd(II) ions decreased as initial concentration of metal ions was increased from 10mg/L to 200mg/L for both the biosorbents. This was due to the increase in number of metal ions competing for available binding sites in the biomass and also due to lack of binding sites in the biomass for the complexation of ions at higher concentration levels (Akar and Tunali, 2006). On the other hand at lower concentrations of metal ions, almost all the metal ions present in the solution interact with binding sites and facilitate maximum biosorption at 10mg/L i.e.75.08% and 66.72% for immobilized biomass and free biomass of *Penicillium citrinum* respectively.

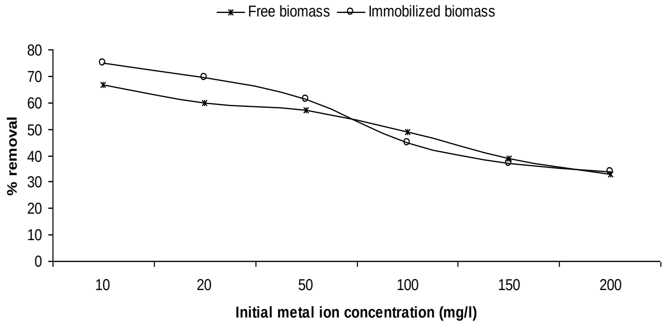

Fig.5 Effect of initial metal ion concentration on Cd (II) removal by free and immobilized biomass of *P.citrinum*

Adsorption Isotherms

The Freundlich and Langmuir Models were used for mathematical description of the biosorption of Cd(II) ions and isotherms constants were determined to compare the adsorption capacity of free and immobilized biomass of *P.citrinum.* The data have a high value of correlation coefficient (r2>0.90) (Table 3.1). Higher value of correlation coefficients indicated that adsorption data are best fitted in both Freundlich and Langmuir Model.

Table 3.1: Isotherm model constant and correlation coefficient for biosorption of Cd(II).

Biosorbent form	**Langmuir constant (mg/gm)**			**Freundlich constant (mg/gm)**		
	Q_0	b	R^2	K	n	R^2
Penicillium citrinum (Immobilized biomass)	39.21	0.032	0.9775	2.54	1.84	0.9858
Penicillium citrinum (Free biomass)	22.02	0.022	0.9945	1.2	1.55	0.9788

The value of Freundlich constants k & n, i.e. 2.54 mg/gm & 1.84 L/mg for immobilised biomass of *P.citrinum* and free biomass of *P.citrinum*, i.e. 1.2mg/gm & 1.55 mg/gm. The higher value of k indicates a higher adsorption capacity. Higher the n (n>1) value, higher is the intensity of adsorption (Sharma & Forester, 1994).

CONCLUSION

Based on the present study the biosorbent *Penicillium citrinum* was found to be suitable biosorbent for removal of Cd(II) from synthetic solution. The biosorption of Cd(II) was found to be pH, biosorbent dose, contact time and initial metal ion concentration. Maximum biosorption of Cd(II) was observed at pH 6.0., biosorbent dose (0.2g/50ml for free biomass and 0.1g/50ml for immobilized biomass of *Penicillium citrinum*) initial metal ion concentration 10 mg/l in 60 minutes of contact time. The rate of biosorption increased with increasing biosorbent dose and contact time up to certain limit after which it attained saturation. From the batch studies it was observed that immobilized biomass has greater biosorption efficiency than free biomass for Cd(II). This is due to sorption capacity of alginate beads. The biosorption pattern of the biosorbent studied followed the Langmuir isotherm and Freundlich isotherm for biosorption of Cd(II).

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