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Biosorption of lead (Pb(II)) ions by active and inactive biomass of heavy metal tolerant fungal biomass isolated from the polluted sites

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ABSTRACT

Heavy metals impose a severe environmental and public health hazard because of their toxic effects and their ability to incorporate in terrestrial and aquatic food chains. Biosorption has many disadvantages over conventional technologies in significant metal removal performance from large volumes of effluents. Fungal biosorption can effectively be used for the removal of metals from contaminated water and soil. Heavy metal tolerant fungal species were isolated from the polluted sites and the most tolerant fungal strain FI-01 was selected. The FI-01 strain was identified as *Penicillium chrysogenum* on the basis of morphological and microscopic characterization. The live (active) and dead (inactive) biomass was prepared for biosorption assay. The operating parameters viz., pH, temperature and initial metal ion concentration was optimized to 5.0, 35°C and 100 mg/l for maximum biosorption. The dead biomass has shown 23.2 percent more biosorption capacity. The biosorption data of dead biomass shows good fit with the Freundlich adsorption isotherm.

Key words : *Biosorption, Heavy metal, Fungal biosorbent*

Introduction

The technological significance of heavy metals lead to the great emphasis on their use (Kumar *et al.*, 2014; Rao *et al.*, 2014; Saleem *et al.*, 2014) in industries resulting in generation of heavy metal laden waste contaminating the environment with metal pollution (Gautam *et al.*, 2014). Several studies have indicated the metal pollution through Industrial waste (Wang and Ren, 2014). Metal-containing wastewater is continuously produced, posing a major hazard to the environment and public health. This is primarily owing to their bioaccumulation and penetration into food systems (Malik, 2004; Chuah *et al.*, 2005). Because of their hazardous behaviour in the environment, industrial effluents loaded with cadmium, chromium, copper, lead, mercury, nickel, palladium, and zinc are of special importance for treatment (Barakat, 2011).

Lead, one of the most extensively used heavy metals, is primarily utilized in the production of electric batteries, paint, lead melting, internal combusting engines, fueled aviation engines, and explosives. Lead is extremely hazardous, and exposure to high levels can result in encephalopathy, hepatitis, and nephritic syndrome (Ezzouhri *et al.*, 2010). The need of treating and removing heavy metals from such effluents to allowable levels before releasing into natural streams, rivers, and seas is quickly spreading over the world. Several conventional

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wastewater treatments have been developed and are being used successfully on a broad scale to reduce hazardous component concentrations in wastewaters in this approach. But these conventional treatment technologies are not economical for large scale use. These technologies need further input of certain chemicals making them less eco-friendly. Therefore a technique for waste water should be explored having properties like ease in-operation, effective, cost-effective and eco-friendly.

Fungi are considered as one of the agents that possess the capability of removal of heavy metals (Parmeswari *et al.*, 2010). Fungi has different mechanisms like valance transformation, active uptake, intracellular or intercellular precipitation and biosorption, to tolerate and detoxify the metal ions (Yan and Viraraghavan, 2003). Uptake of metal ions by living or dead biomass via binding of metal ion on the cell wall and extracellular material is known as biosorption (Bishnoi and Garima, 2003). Given the mechanisms of metal resistance in fungi, it's possible that screening metal-tolerant fungus will result in strains that have better metal accumulation. Therefore, the isolation and identification of lead (Pb) tolerant indigenous fungi with biosorption potential from the waste dumpsite of Mandideep, industrial area and outer surroundings of UCIL factory site in Bhopal, Madhya Pradesh. Lead is one of the common environmental pollutants that are frequently encountered together in sewage and industrial wastewaters. Hence the metal was chosen for this study.

Materials and Methods

Collection of samples

Soil samples were collected from different regions of selected polluted sites of Bhopal Madhya. The polluted site selected were discharge site in Mandideep industrial area and the different regions surrounding the UCIL factory. After removing surface contaminants, soil samples were taken from the 05–15 cm depth. Metal utensils were not used for sampling; instead, a plastic spatula was used. The samples were gathered in self-closing polythene bags and double-bagged. Five replicates of soil samples were collected at every sampling point. Three sampling points were taken into consideration in the site. These points are at least 25 meters apart from each other. The samples collected from each sampling point were thoroughly mixed for uniform distribution of the fungal cells. Collected samples were analyzed within 48 hours.

Isolation of Lead tolerant fungi

The glassware was acid washed and sterilized before using them for the experimental procedure so that unwanted contamination could be avoided. Ten gram of composite soil mixture was mixed with 90 ml of sterilized distill water and the mixture was shaken for 30 minutes. This soil suspension was serially diluted from 10^{-1} to 10^{-6}. Further spread plate technique was performed to isolate the fungi from the diluted samples. Plates containing potato dextrose agar amended with 10 ppm of lead nitrate ($Pb(NO_3)_2$) was inoculated with 0.1 ml of soil suspension and L-shaped spreader was used for spreading the suspension onto the plate. The plates were allowed to incubate at 28 °C± 2 for 72 hours. The colonies that were morphologically distinct from each other were considered as lead tolerant fungi. The colonies were picked and purified on plates and slants of potato dextrose agar (PDA). These pure cultures were further used as a source of inoculums for the tolerance studies, minimum inhibitory concentration and the biosorption assay.

Biosorption Assay

The fungal isolates were allowed to grow in a potato dextrose broth (PDB) at pH 6.0 and temperature of 30 °C for a week before performing the biosorption assay. The obtained fungal isolate was filtered and collected for the biosorption assay of lead ions. The biosorption assay was performed by using two types of biomass i.e. live biomass and dead/inactive biomass. For biosorption assay with live biomass, the filtered biomass of fungal isolate was collected and washed with deionizer water and subjected to biosorption assay. While to get dead/inactive biomass, fungal isolates were deactivated by autoclaving at 1.5 atm pressure and the temperature of 121 °C for 5 minute of time duration. After autoclaving the biomass was filtered and dried at 50 °C for 24 hours. Both type of live and dead biomass was further utilized for biosorption of lead ion from metal solution. Live biomass was used immediately after washing the filtered biomass. The inactive or dried biomass was stored in vacuum desiccators till its use.

Effect of pH on biosorption of lead

The biosorption of lead ions by live and inactive biomass of isolated and selected fungal isolates was assessed under varying pH range of 3.0 to 10.0. The 150 ml synthetic metal solution of 100 mg/l concentration was inoculated with 1g of live and inactive biomass separately at 30 °C temperature. The contact time for biosorption of metal ion was kept 120 minutes.

Effect of initial lead ion concentration on biosorption process

Lead solution of different concentration was prepared by dissolving lead nitrate $Pb(NO_3)_2$ in distilled water. The biosorption was assessed under different initial Pb(II) ions concentrations of 25, 50, 75, 100 and 125 mg l^{-1}, under pH of 5.5 and 30 °C temperature, with 1 g of biomass in 150 ml of metal solution. The filtrates were collected after 120 minutes for analysis of residual metal ions in the artificial metal solution.

Effect of temperature on biosorption

The metal solution of 100 mg/l was inoculated with 1g of both live and dead biomass in 150 ml of metal solution and exposed to different temperatures ranging from 25 to 45 °C for the time duration of 120 minutes. The residual metal ion was estimated from each solution exposed to different temperature conditions.

Determination of metal ions biosorption

The filtrate from each biosorption study was collected and concentration of residual lead ion was measured by using atomic absorption spectrophotometer. The biosorption in percentage was estimated by using the formula given below:

$$Biosorption\,\% = \frac{C_i - C_f}{B} \times 100$$

Where, C_i is the initial concentration of lead ions in artificial metal solution mg/l, C_f is the residual Pb(II) ion concentration in the solution mg/l after completion of biosorption process and B is referred to the biosorbent dose in grams.

Adsorption isotherm modeling

The biosorption data was generated by the experimentation performed with optimized parameters using dead or inactive biomass. The data of biosorption was analyzed by Langmuir and Freundlich adsorption isotherm models, widely used for biosorption for water and wastewater treatment processes. The mathematical expression of Langmuir isotherm models is as follows:

$$1/q_e = {}^{1}/_{q_{max}} + {}^{1}/_{b} \times q_{max} \times C_e$$

Where q_e is the quantity of metal ions adsorbed under equilibrium conditions (mg/g), C_e is referred to as the equilibrium concentration (mg/l) in the metal ion solution, b is the equilibrium adsorption constant giving information about the affinity of binding sites (l/mg), q_m is the mass of adsorbed solute completely required to saturate a unit mass of adsorbent (mg g-1). The Freundlich model is given below:

$$\ln q_e = lnK_f + \frac{1}{n} lnC_e$$

Where, K_f (dm^3/g) and *n* are Freundlich isotherm constants, being indicative of the extent of the biosorption and the degree of nonlinearity between solution concentration and biosorption, respectively. Freundlich isotherm constants were determined from the plot of ln q_e versus ln C_e.

Results and Discussion

Isolation of lead tolerant fungi

In the present study, total twenty three isolates of fungal isolates were isolated from the polluted sites. All the isolated fungal isolates were picked and pure colonies were isolated. The fungal isolates were assigned a nomenclature from FI-01 to FI-23. Based on tolerance studies, FI-01 fungal strain was further selected for biosorption assay. On the basis of morphological and microscopic characteristics the fungal isolate, FI-01 was identified as *Penicillium chrysogenum*.

Biosoprion assay

The selected fungal species of *Penicillium chrysogenum* was further selected for biosorption of metal ion from artificial metal solution. For biosorption studies, live and inactive/dead biomass of *Penicillium chrysogenum*, was assessed separately for uptake of metal ions.

Effect of pH on biosorption

The effect of pH on biosorption of Pb(II) ion was compared for both live and dead biomass of *Penicillium chrysogenum*. Both live and dead biomass of *P. chrysogenum* showed higest removal efficiency at pH

5.0 as evidenced from Fig. 1. For live biomass, the *P. chrysogenum* has shown highest uptake of 55.98 percent at pH 5.0, while 70.47 percent of lead removal was observed with dead biomass at pH 5.0. Under acidic pH the lead ion removal was low because, metal ions and protons compete for the binding sites on biosorbent (Dhankar and Hooda, 2011). While at higher pH values, the metal ions precipitates in the solution thus stability of metal ions get decreased, and metal ion removal is also decreased (Amini *et al.*, 2008).

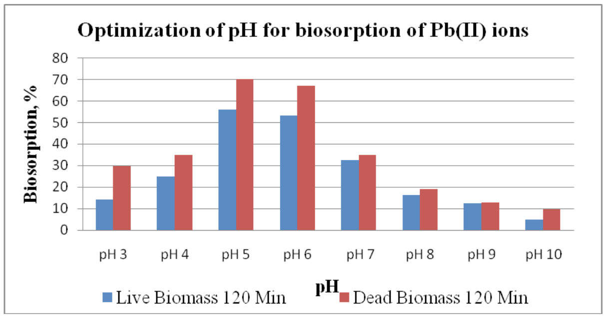

Fig. 1. Effect of pH on biosorption of lead ions by live and dead biomass of *P. chrysogenum.*

Effect of initial metal ion concentration

The metal ion uptake capacity of both live and dead/inactive biomass was found to be increases with increase in the initial concentration of Pb(II) ions. The highest uptake of metal ion was observed at 100 mg/l with metal uptake of 6.34 mg/g and 8.26 mg/g of live and dead biomass respectively. From the Fig. 2 it can be assessed that the metal ion uptake capacity increases with increasing metal concentration while the biosorption percentage decreases as the initial concentration of metal ion increases. At higher initial metal ion concentration, the incidence of collision rate between the metal ions present in the aqueous solution and fungal biosorbents increases resulting in the increase in metal uptake capacity with increased metal ion concentration (Aksu, 1992).

Effect of temperature on biosorption of Pb(II) ions

Temperature affects the enzymatic system, solubility of metal ions, cell wall stability and composition and configuration of cell wall. As depicted from the Fig. 3, the highest biosorption percentage of 62.87 and 80.17 percent was observed at the temperature of 35°C for live and dead biomass of fungal isolate *P. chrysogenum* respectively. Moderate temperature range i.e., 25 to 35°C shows minor effect on biosorption but at higher temperatures the integrity of cell membranes is affected and which in turn hinders the metal ion binding to active sites of

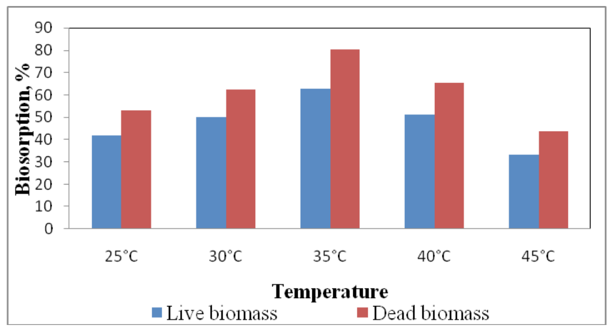

Fig. 3. Effect of different temperature on biosorption capacity of Pb(II) metal ions by live and dead biomass of *P. chrysogenum.*

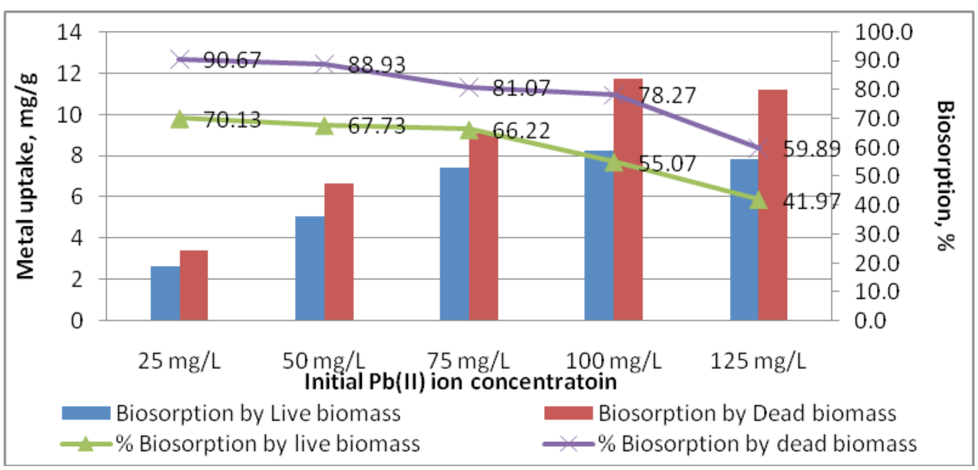

Fig. 2. Effect of initial metal ion concentration on biosorption of lead ion by live and dead biomass of *P. chrysogenum.*

biosorbent leading to reduction in biosorption potential at higher temperatures.

The biosorption capacity of both live and dead biomass was compared through all the optimizing parameters. The metal ion removal capacity of all the fungal isolates was found to be more than that of live or active biomass. This may be attributed to the fact that active cellular metabolism of live biomass gets hindered due to unfavorable conditions which in turn leads to low biosorption efficiency (Smily and Sumithra, 2017). Therefore, the dead or inactive biomass was selected as potential candidate for metal ion removal from aqueous solutions.

Adsorption isotherm modeling

Langmuir and Freundlich adsorption isotherm models are two common types of model that are frequently used. Sorption isotherms reflect about the concentration dependent equilibrium distribution of metal ions between aqueous and solid phases. Figure 4 and 5 shows the Langmuir and Freundlich isotherm for Pb(II) biosorption on dead/ inactive biomass of *P. chrsogenum* at 35 ºC temperature and pH 5.5 from aqueous solution of Pb(II) ion of concentration of 150mg/l. Table 1 shows the results of Langmuir and Freundlich isotherm models and lead adsorption constants on *P.chrysogenum*.

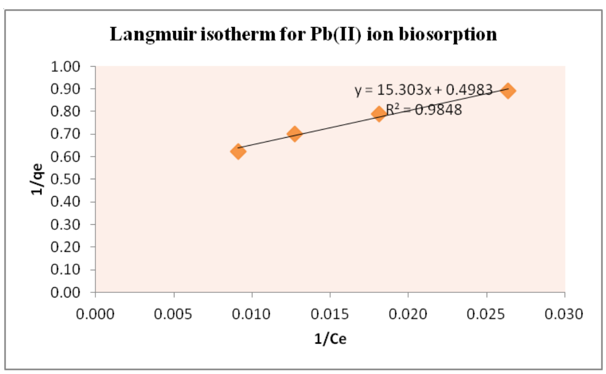

Fig. 4. Langmuir adsorption isotherm model

In Langmuir adsorption isotherm, the value of q_{max} and constant b was observed to be 2.007mg/g and 0.0325 mg/l respectively. The R^2 correlation factor obtained was 0.9848. The low value of Langmuir constant b shows the low binding affinity between the biosorbent and Pb(II) metal ions that may result in less stable adsorption product.

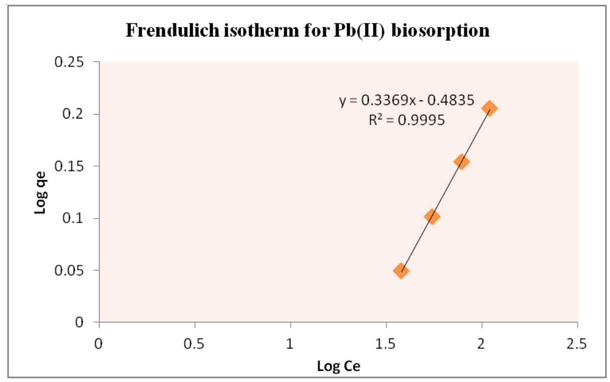

Fig. 5. Freundlich adsorption isotherm model

The Freundlich adsoption model fits well for Pb(II) ion biosorption on to the fungal isolate *P.chrysogenum*. In Freundlich isotherm, the value of 1/n and K_F was determined to be 0.3369 mg/l and 3.045mg/g respectively. If the value of '1/n' is found to be less than 1, the increase in adsorption capacity was observed due to modification of the adsorbent. As this value becomes greated than 1, the adsorption bond becomes weak and the q_e changes significantly. The K_F in the freundlich equation is mainly related to the adsorbent capacity for adsorption of metal ions; and larger the value of K_F, more is the capacity of biosorbent.

Conclusion

It was concluded from this investigation that the dead biomass of *Pencillium chrysogenum* can be potential candidate adsorbing lead metal ions and could be applied for bioremediation of soil and industrial effluent. Passive adsorption process on cell surface was performed using inactivated (dead) fungal biomass and active sorption process was also performed using live biomass (Sag, 2001). Dead biomass was considered to be superior to live ones. The initial solution pH, temperature and initial Pb(II) ion concentration significantly influence the uptake of

Table 1. Adsorption parameters calculated using the Langmuir and Freundlich isotherms

Biomass	Langmuir model			Freundlich model		
	q_{max} (mg/g)	K_L or b	R^2	1/n	K_F	R^2
Inactive biomass of *P.chrysogenum*	2.007	0.0325	0.9848	0.3369	3.045	0.9995

lead ion while maximum adsorption was found to be at 5.0, 35 °C and 100 mg/l, respectively. The Freundlich adsorption model was used for the mathematical expression of the biosorption of lead ions. These fungal isolates may be used in for metal remediation from wastewater and heavy metal contaminated soils.

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