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Research Article

Study of lead (II) biosorption using *Aspergillus niger* and its optimization studies

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Abstract

An extensive attention has been paid on the management and bioremediation of heavy metals in the soil and water polluted by different industries. Of the number of methods suggested, biosorption was represented as one of the innovative and cost effective tools for the removal of heavy metals from the polluted environment. The present study provides a better understanding and exploring of lead biosorption using dead biomass of *Aspergillus niger* MTCC 281. Above 4 mg/mL concentration, lead was found to inhibit the growth of fungal strains. The dead biomass of fungi was evaluated and optimized for lead biosorption which showed maximum adsorption at acidic pH of 3.0 and optimal temperature at 40 ºC with metal ion concentration of 1 mg/mL. The lead biosorption was assessed under optimized conditions and showed 65.28 % of total biosorption. Thus, the fungal strain, *Aspergillus niger* MTCC 281 can serve as a potential biosorbent for the lead biosorption and offer its application in environmental cleanup.

Keywords: Biomass, Biosorption, Lead, Scanning Electron Microscope (SEM)

Introduction

The rapid industrialization in the last few decades led to the increase in the synthesis and accumulation of various heavy metals directly or indirectly in the environment through pesticides, fertilizers, leather processing effluents etc. It is well known that these heavy metals incur serious ill-effects on living organisms including humans even at minimal concentrations. Hence, the accumulation of heavy metal in the environment exists as serious issue and has to be removed for the wellbeing of living organisms associated with such environment (Parvathi and Nagendran, 2007). Even though various physical and chemical methods are available for the removal of heavy metals, each method has its own merits as well as demerits. For example, below 50 mg/L concentrations of heavy metals, the electrochemical and chemical methods were found to be ineffective. But heavy metals are found to be lethal even at certain lower concentrations. The search of novel, effective technologies for heavy metal removal led the researchers in the side of biosorption, using bacteria, fungi, algae and yeast as biosorbents due to their metal sequestration properties (Nilanjana et al., 2008). The biosorption stood ahead of other conventional methods in its high efficiency, low cost, recovery and reusability of the biosorbent etc.

Among the other biosorbents, fungi have been proved as one of the efficient biosorbents for the sorption of toxic heavy metals from effluents (Ahmad et al., 2006). The fungi also offer advantages over other biosorbents including high cell surface area which imports them strong metal binding efficiency over others. Also the antibiotic nature of fungi assists in the removal of heavy metals and provides an ecofriendly environment. Both living and non-living cells of fungi can be employed for the biosorption method. The dead biomass of fungi possess several advantages over the

live cells which includes their wider acceptability, less toxicity, no media requirement, easy recovery of metals, reusability, etc. (Preetha and Viruthagiri, 2005). Hence, in the present investigation, non-living cells of *Aspergillus niger* MTCC 281 was used for the sorption of heavy metal lead from aqueous suspension and its process optimization. *A. niger* has been reported to be having ability to remove several heavy metals including cadmium, copper, lead etc. (Kapoor and Viraraghavan, 1997).

Materials and Methods

Microorganisms

For the present study, *Aspergillus niger* MTCC 281 was procured from IMTECH, Chandigarh, India was used as a test strain throughout the investigation. The strain was maintained on PDA (potato dextrose agar) slants at 4°C and sub-cultured every month till use.

Lead tolerance test

To determine the ability of fungi to grow on the heavy metal, lead, the screening method proposed by Rani et al. (2007) was followed. The study was conducted in potato dextrose agar (PDA) amended with 1 mg/ml concentration of lead nitrate, $Pb(NO_3)_2$ as the source of lead. The PDA plate without lead source was considered as control. Inoculation was done using fungal agar plug of 7 mm diameter spotted at the center of the lead amended screening plate. The plates were incubated for 7 days at 28°C and Metal Tolerance Index (Ti) was determined using the formula:

$$Ti = \frac{Dt}{Du}$$

Where,

Dt = Radial extension of fungi grown in Pb amended medium (cm)
Du = Radial extension of fungi grown in control (cm)

Minimum Inhibitory concentration test

The minimum inhibitory concentration (MIC) of lead against the fungi was evaluated by determining the maximum tolerance of *Aspergillus niger* MTCC 281 against Pb using the method of Rani et al. (2007). PDA plates were prepared with different concentrations of Pb ranging between 1 mg/ml to 5 mg/ml concentration. Inoculation was carried out as described earlier and the plates were incubated at 28°C for 7 days. The plates were observed for visible fungal growth and MIC was determined based on the least concentration of Pb that inhibited the growth of fungi (Akhtar et al., 2013).

Fungal biosorbent preparation

The fungal biosorbent was prepared by inoculating *Aspergillus niger* MTCC 281 in potato dextrose broth and incubated for 5 days for the development of fungal mat. The fungal mat developed was removed from the broth and viable fungal spores were killed by adding 0.5N NaOH and kept in boiling water bath for 15mins. After attenuating, the mat was washed with tap water and followed by distilled water until pH reached 7.0. The biomass was then air dried at 80°C, ground and stored for further studies.

Biosorption studies

The biosorption study was carried out by batch contact method described by Bajpai and Rai (2010). Initially, the lead solution was prepared by dissolving 10 mg of $Pb(NO_3)_2$ in 10 ml of distilled water (1 mg/mL concentration). The lead solution was added with 20 mg of ground biomass as adsorbent. The suspension was incubated in shaken condition for about four hrs to attain equilibrium adsorption. The suspension was then centrifuged and the amount of lead in supernatant was assayed spectrophotometrically. The biosorption efficiency (%) of the fungal biosorbent was calculated using formula:

$$E.(\%) = Ci\frac{Cf}{Ci} \times 100$$

Where,

E = Biosorption efficiency (%)
Ci = initial metal ion concentration, mg/L
Cf = final metal ion concentration, mg/L

Optimization of Lead biosorption

In order to optimize the environmental and cultural parameters for the maximum biosorption of lead by fungal biosorbent, the parameters such as incubation

time, temperature, pH, initial biomass concentration and lead concentration were optimized. The suitable incubation time for lead biosorption was optimized by analyzing the amount of lead present in the reaction mixture at regular intervals (6, 12, 18, 24, 30 and 36 hrs) of incubation. Subsequently, the optimum temperature for the biosorption efficiency was determined by incubating the flasks at different temperatures such as 23, 27, 30, 37 and 40°C. The effect of initial pH on lead biosorption was evaluated by adjusting the pH of reaction mixture between the pH ranges of 1 and 10 using 0.1N NaOH and 0.1N HCl. The effect of initial metal (Pb) concentration and biosorbent concentration were also determined by altering their concentrations from 1 to 10 mg/ml concentration. The estimation of residual concentration of lead was carried out in each parameter optimized studies using cell free supernatant collected through centrifugation.

Scanning Electron Microscopic Analysis

The surface modifications of the biosorbent before and after encounter with lead were analyzed using Scanning Electron Microscope (SEM) analysis. The biosorbents were mounted on aluminum slabs with gold coating for better visualization of micrographs.

Results and Discussion

In the present investigation, the feasibility of dead fungal biomass (*Aspergillus niger* MTCC 281) was tested for biosorption of lead from the synthetic solutions. The efficiency of dead biomass can be less than, equivalent to or greater than that of the live biomass. Microorganisms mediated biosorption of heavy metals is one of the most promising method for the environmental cleanup (Konopka *et al.,* 1999). Among them, fungi avails more advantages over other organisms due to the ease they offer for removal from liquid substrates and hence was selected for the present study. The heavy metal tolerance of fungal strain *Aspergillus niger* MTCC 281 was determined by inoculating in lead amended plates. *A. niger* also showed the highest resistance up to 4 mg/ml lead concentration, which made it to be a successful candidate for lead biosorption. The development of clear halo zone formed around the fungi in Pb amended medium indicated the metal solubilization. *A. niger* is reported as an efficient biosorbent for the absorption of various heavy metals (Sayer et al., 1995; Fomina et al., 2005).

The fungal cells can be killed for biosorption by using several physical and chemical methods (Kapoor and Viraraghavan, 1995). The sequestering of the metal ions by the fungi is facilitated by the presence of chitin and chitosan present in their cell walls (Muzzarelli, 1972; Tsezos, 1986). The structural polysaccharides of the cell wall were the main sites of metal interaction and the metal uptake was carried out through ion exchange (Muralidharan et al., 1994).

The efficacy of the biosorption of any heavy metals depends the surface properties of biosorbent which may also influenced by various physico-chemical properties of metal ion solution. Hence, the effect of incubation time, temperature, pH, initial metal ion and biomass concentration on the adsorption of lead was investigated further. These findings are of greater interest in scale up of the process to optimize the amount of biosorbent to be used for maximal metal ion removal in industrial effluent purification.

Biosorption of lead by dead biomass of *Aspergillus niger* MTCC 281 was investigated in batch experiments at different pH ranging from 1 to 10. The dead biomass showed maximum biosorption percentage of 57.33 at pH 3 (Fig.1). There was no significant change in pH of the solution after completion of adsorption. The biosorption efficacy was decreased gradually with the increase in pH after 3. The pH influences the electrostatic binding of ions to corresponding functional groups hence, plays an important role in adsorption of metal ions. Another most important role played by pH is altering the active binding sites of sorbents to facilitate the biosorption in acidic conditions (Nah et al., 2003). The results revealed that acidic pH was best for lead adsorption. Iram et al. (2013) also evidenced the same by observing pH 5 to be optimum for the biosorption of lead and other heavy metals by *Aspergillus fumigatus*.

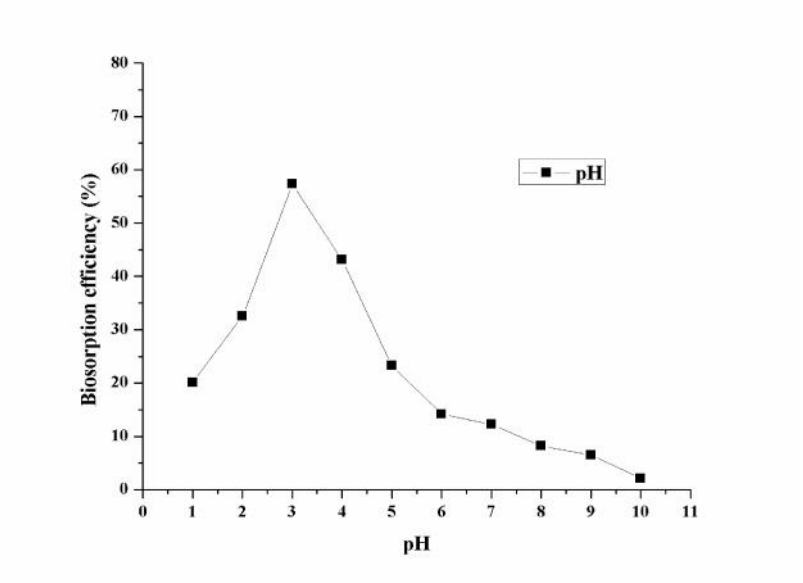

Fig. 1: Effect of pH on lead biosorption

Time course for biosorption of lead by dead biomass (biosorbent) from synthetic metal solution in batch culture was investigated. Figure 2 shows the time course biosorption efficiency of *Aspergillus niger* MTCC 281. The results revealed that the lead removal rate initiated in the 6th hr of contact time reached maximum of 58.35 % during 24th hr of incubation with a continuous agitation (Fig.2). After 24th hr, there existed a steady phase of biosorption till 36th hr of contact time. Rani et al (2007) observed the maximum removal of lead using live *A. niger* after 72 hrs of incubation.

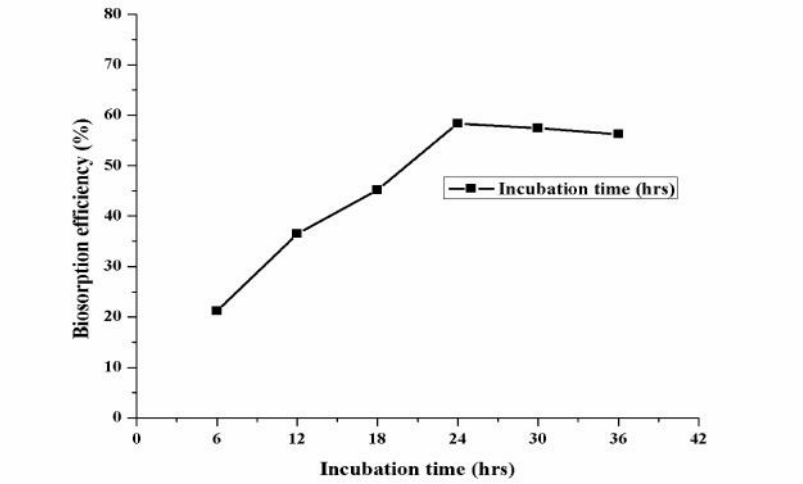

Fig. 2: Effect of incubation time on lead biosorption

Temperature (°C) also plays a prominent role in biosorption of lead. The results reveal that maximum lead removal was observed at 30ºC with biosorption percentage of 60.12 (Fig.3), whereas less biosorption activity was observed when incubated at 27ºC or beyond 37ºC. The reported studies also correlates with the present findings as the optimal temperature for the lead biosorption by live *A. niger* was evaluated by Rani et al. (2007) who found maximum lead adsorption at temperature ranging from 28 to 40ºC. Sağ and Kutsal (1996) also found maximum biosorption of heavy metals between 25-45ºC using *R. arrhizus* as biosorbent.

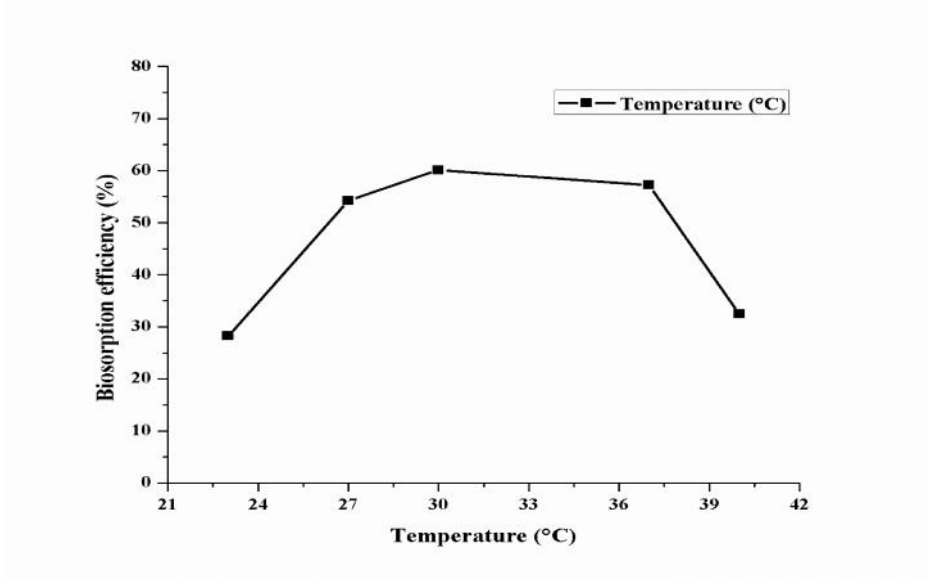

Fig. 3: Effect of temperature on lead biosorption

Absorption capacity of *Aspergillus niger* MTCC 281 was determined at different initial lead concentrations. Higher lead removal was observed at lower concentrations of lead in the medium. There exists a complete saturation of biosorption sites with all lead ions at lower metal concentration. The metal sorption capacity of dead biomass of *Aspergillus niger* MTCC 281 attains its peak at 2 mg/ml concentration with a biosorption percentage of 65.28 (Fig.4). The sorption of the metal is enhanced by the availability of the more number of binding sites in the biomass (Bai and Abraham, 2001). The residual metal decreases with the increase in initial metal concentration. The decrease in metal absorption with respect to increase in metal concentration is attributed due to the lack of equilibrium with respect to the biomass shortage in the solution. Hence, it is not feasible to increase the metal concentration beyond 1 mg/ml concentration for biosorption studies using *Aspergillus niger* MTCC 281.

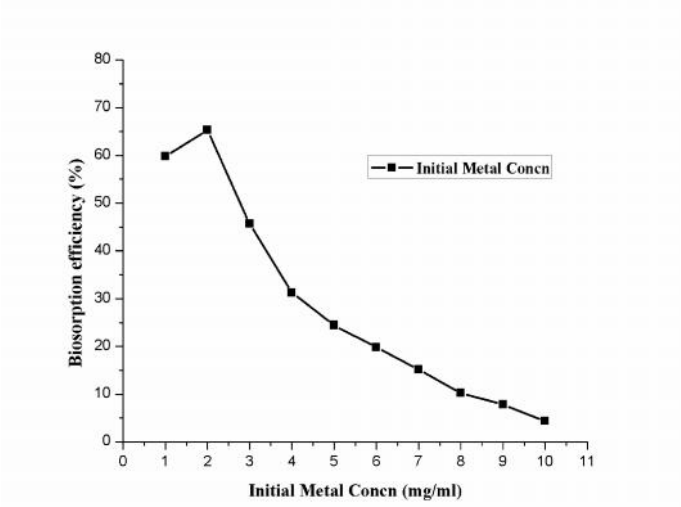

Fig. 4: Effect of initial metal concentration on lead biosorption

The initial fungal biomass concentration required for the better biosorption of lead was optimized further. The lead biosorption by the biomass was initiated from 1 mg/ml concentration (9.65 %) and attained maximum at the biomass concentration of 3 mg/ml with 72.25 % of biosorption. From the observations,

it is well understood that the biomass concentration greatly influenced the lead biosorption. There exists a lack of equilibrium between biosorbent and metal concentration before and after optimum concentrations. In a similar lead biosorption study carried out by Yoonaiwong et al. (2011), they employed 2 mg/mL concentration of dead biomass of *Utricularia aurea* for the biosorption of lead. Santos and Lenzi (2000) also reported 2 mg/ml concentration of biosorbent was found to be was highly efficient for the biosorption of lead, after 48 hours of incubation

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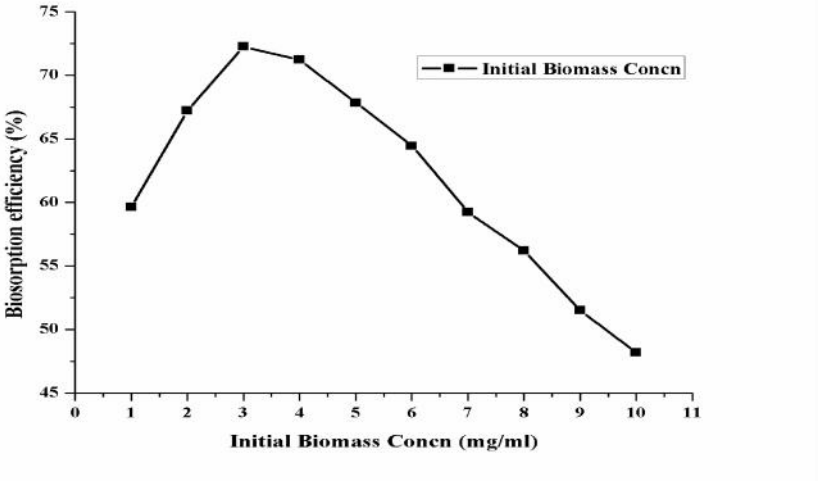
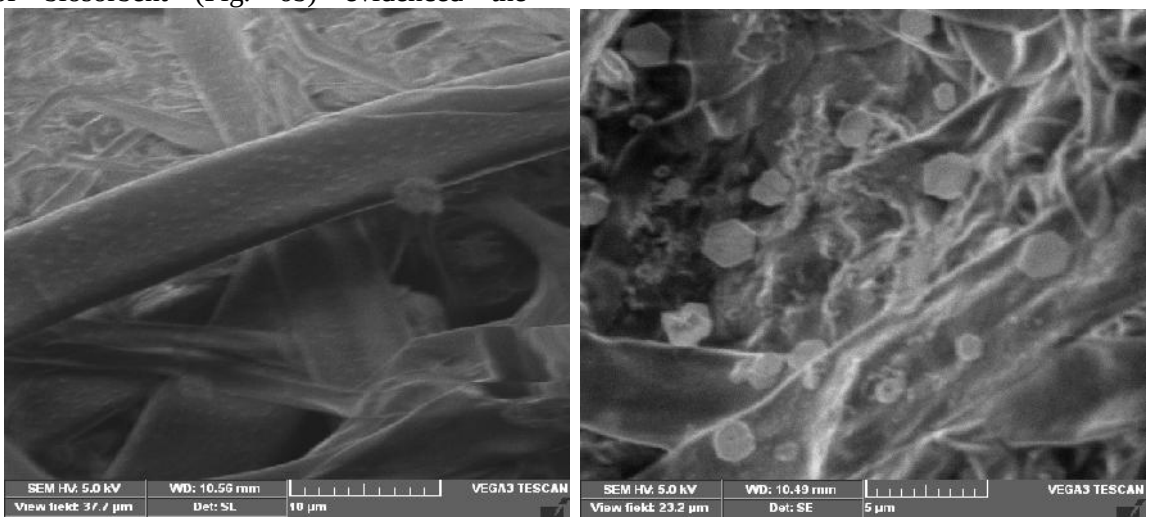


Fig. 5: Effect of initial biomass concentration on lead biosorption

The SEM micrographs of treated and untreated biosorbent were compared to understand the conformational changes that occurred in the surface of biosorbent against the adsorption of lead ions. There observed visible changes between the treated and untreated biosorbent in terms of surface pores, morphological disintegration and cylindrical structures. The presence of lead crystals over the surface of biosorbent (Fig. 6b) evidenced the biosorption potential of dead fungal biomass. The present study found in accordance with the results of Yoonaiwong et al. (2011) who analyzed biosorption potential of *Utricularia aurea* against lead. Similarly, dead biomass of *Aspergillus flavus* was evaluated for lead biosorption by Mahmooda et al. (2014) and visualized the conformational changes that occurred on surface of biosorbent.



(a) (b)

Fig. 6: SEM micrograph of untreated biosorbent (a) and treated biosorbent (b)

Conclusion

The usage of live as well as dead biomass as biosorbents was found to be a cost effective technology for the bioremediation of heavy metal from contaminated regions. Another added advantage of using fungal biomass is the regeneration/ recovering of biomass as well as metals which can be reused further. The remarkable properties of *Aspergillus niger* MTCC 281 as biosorbent in removal of lead suggest their application in the bioremediation of wastewater and soils contaminated with heavy metals.

Acknowledgments

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