RESEARCH PAPER
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Biosorption of lead using immobilized cells of *Aspergillus niger*

ANISHA LAPASIYA AND LOLLY JAIN

Department of Microbiology, K. J. Somaiya College of Science and Commerce, Vidyavihar, MUMBAI (M.S.) INDIA
Email : anisha_libra@yahoo.com; jain_lolly@yahoo.com

Fungal strain- *Aspergillus niger* was isolated from effluent of chemical and pharmaceutical industry using SDA agar. Pretreatment of live cells of *Aspergillus niger* was carried out. This dried biomass was immobilized in Ca-alginate beads. Ability of Pb biosorption with immobilized *Aspergillus niger* biomass was premeditated in the present study. Effect of Initial metal ion concentration, concentration of adsorbent and contact time doses on Lead (Pb) removal was determined. The concentration of lead in the filtrate was then analyzed by atomic absorption spectrophotometer. Suitable conditions for *Aspergillus niger* to take up Pb were shown to be at 50ppm lead as initial metal ion concentration using 2.4 grams of fungal beads as the adsorbent. The optimum contact time was found to be 150 minutes. Based on optimization results, biosorption and desorption processes were carried out. Biosorption experiment revealed that *Aspergillus niger* showed 74.61 per cent Pb removal. Desorption using EDTA retrieved 77.78 per cent Pb from the beads. Immobilized biomass offers many advantages including better reusability; high biomass loading and minimal clogging, increased substrate uptake and yield improvement, easier product recovery, thus reducing cost for equipment and energy demands.

Key words : Biosorption, Lead, Aspergillus niger, Atomic absorption spectrophotometer, Desorption

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INTRODUCTION

Heavy metals are widespread pollutants of great environmental concern as they are non-degradable and thus persistent. The presence of heavy metal ions from the transition series, *viz.*, Cu, Fe, Ni, Pb, etc. in the environment is of major concern due to their toxicity to many life forms. Unlike organic pollutants, the majority of which are susceptible to biological degradation, metal ions degrade into harmful end products.

In developing countries like India, wastewater treatment is of utmost importance. The degree of treatment may range from a main process for seriously polluted industrial waste to a polishing process for removing the trace concentrations which remain after the main treatment. New technologies are required that can reduce heavy metal concentration to environmentally acceptable level at affordable costs. Therefore, much attention has been given to the removal of metal ions by microorganisms due to its applications in environment protection and recovery of toxic or strategic heavy metals.

Mobilization of heavy metals in the environment due to industrial activities is a serious concern due to the toxicity of these metals in humans and other life forms. Removal of toxic heavy metals from industrial waste waters is essential from the stand point of environmental pollution control. Among the toxic heavy metals, mercury, lead and cadmium called the big three are in the limelight due to their major impact on the environment (Saleh *et al.,* 2009).

Alternative process is biosorption, which utilizes various certain natural materials of biological origin, including bacteria, fungi, yeast, algae, etc. These biosorbents possess metal-sequestering property and can be used to decrease the concentration of heavy metal ions in solution from ppm to ppb level. It can effectively sequester dissolved metal ions out of dilute complex systems with high efficiency and quickly, therefore it is an ideal candidate for the treatment of high volume and low concentration complex wastewaters (Wang and Chen, 2009).

Dead biomass in industrial application offers certain advantages over live cells. Systems using live cells are likely to be more sensitive to metal ion concentration (toxicity effects) and adverse operating conditions (pH and temp). Constant nutrient supply is required for systems using living cells and recovery of metals is more complicated from living cells. Dead biomass can be procured of industrial sources as a waste product from fermentation processes (Bishnoi *et al.,* 2005).

One of the important industrial applications of biosorption is recovery of loaded pollutants (especially valuable metals) from the biosorbent and simultaneous regeneration of the biosorbent for reuse. In fact, the usefulness of a specific biomass as a biosorbent depends not only on its biosorptive capacity, but also on the ease of its regeneration and reuse (Viraraghavan and Yun, 2008).

The aim of this study was to investigate the lead (Pb) biosorption efficiency with immobilized *Aspergillus niger* biomass. And also to check the effect of different doses of initial metal ion concentration, amount of absorbent and contact time. The desorption activity of EDTA, NH_4Cl, and HCl was tested.

RESEARCH METHODOLOGY

Biomass harvestation and pretreatment of live biomass:

Fungal strain-*Aspergillus niger* was isolated from effluent of chemical and pharmaceutical industry using SDA agar. Spores of 6-7 days old culture grown in Sabourauds agar plate was inoculated in SAB broth. Culture was grown on shaker to form spherical pellets. After 3-4 days the harvested broth was washed with deionised water. Live harvested mycelial biomass was treated with 0.5N NaOH for 30 mins. It was followed by washing with adequate amount of distilled water until the pH reached to neutral range (pH 6.8-7.0). It was then autoclaved at 15 lb/inch^2 for 20 min. The pretreated biomass was dried at 60^{o}C for 24 hr in hot air oven and converted into powder form by grinding in mortar or pestle.

Immobilization of cells:

The powdered biomass was immobilized by entrapment in polymer matrix of Na-alginate. 2 per cent (w/v) slurry of Na-alginate was prepared in D/W. After cooling, 5 per cent (w/v) of biomass was added. The alginate biomass slurry was added into 0.1M $CaCl_2.2H_2O$ for polymerization and bead formation using pipette. Resultant beads were 4mm in diameter. The fungus entrapped beads were cured in 0.1M$CaCl_2.2H_2O$ for 1 hr. Washed twice with 200ml St. D/W and stored at 4^{o}C in 5mM of $CaCl_2$ solution until use.

Optimization of various parameters for biosorption:

0.4 g of beads were inoculated into 100 ml metal solution containing (10, 25, 50, 100, 150, 200 ppm) lead nitrate in deionised water. The flasks were kept on rotary shaker for 10 mins at 30^{o}C. Solutions were analyzed after proper digestion and dilution by atomic absorption spectrophotometer. The experiments were repeated by using various adsorbent concentrations. (0.4, 0.8, 1.2, 1.6, 2.0, 2.4 g) and contact time. (10, 30, 60, 90, 120, 150 mins).

Biosorption experiment:

Based on the optimized results, biosorption experiment was performed in triplicate. Solutions were analysed using atomic absorption spectrophotometer.

Desorption experiment:

Beads from the biosorption experiment were subjected to desorption using: NH_4Cl- 0.01M/100 ml, HCl- 0.05M/100 ml, EDTA- 0.002M/100ml. Solutions were analysed using atomic absorption spectrophotometer.

Application:

Lead contaminated water sample from Valkeshwar was subjected to biosorption.

RESEARCH FINDINGS AND ANALYSIS

The isolates of *Aspergillus niger* on Sabourauds dextrose agar.

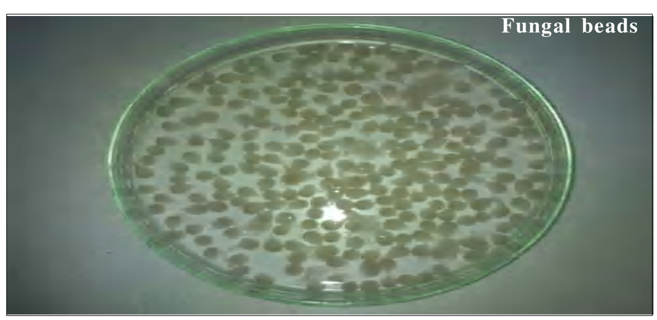
***Aspergillus niger* on SDA**

Large amount of biomass of *Aspergillus niger* was harvested from Sabourauds broth. Pretreatment of the cells by alkali treatment using NaOH and autoclaving yielded dead and dried biomass. This dead biomass was used for the further processes in carrying out optimization.

Powdered biomass

Immobilization of cells:

These beads were used in optimization and biosorption experiments.



Fungal beads

Optimization :

Based on these observations, the optimization was repeated with increasing the parameters gradient. Also all the parameters were done in triplicates to get a statistical result.

Table 1: Results for optimization

Beads concentration		
Grams	Lead absorbed (ppm)	Removal %
0.4	8	17.77
0.8	12	26.67
1.2	17	37.78
Initial metal ion concentration		
Conc. (ppm)	Lead absorbed (ppm)	Removal %
50	17	37.78
100	30.78	30.78
150	40.58	27.05
200	48.86	30.01
Contact time		
Minutes	Lead absorbed (ppm)	Removal %
30	27	60
60	28	62.22
90	31	68.89

Contact time :

For the same metal different time intervals showed different removal rates. This experiment showed that the removal rate was increasing gradually and then attaining stability at 120 and 150 minutes.

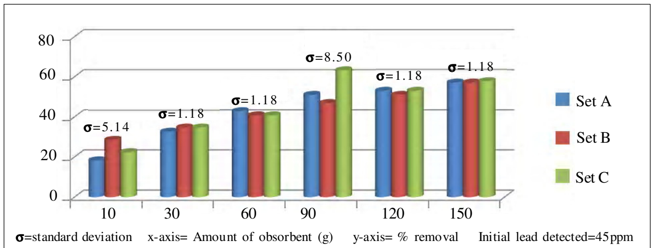


Fig. 1: Removal for different contact time (%)

Amount of absorbent (Beads) :

The experiment on metal uptake reveals that the metal uptake increased when biomass concentration rises. Therefore, it is useful to increase the biomass as according to the amount of metal solution to be treated.

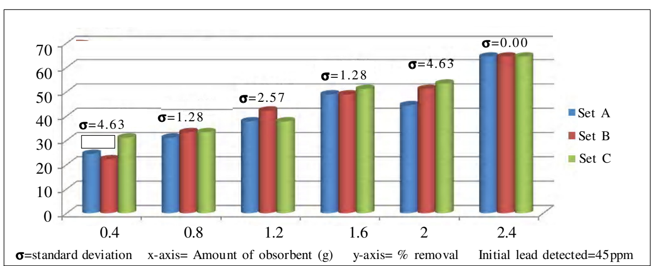


Fig. 2: Removal for different amount of absorbent (%)

Initial metal ions conc. :

The biosorption of Pb(II) ions by *A. niger* decreased with increasing initial concentration of metal ions. This indicates that the beads have a certain limit of metal absorption. Initial metal concentration plays an important role in determining the biosorptive capacity of the absorbent. The initial metal ion concentration used was 50 ppm.

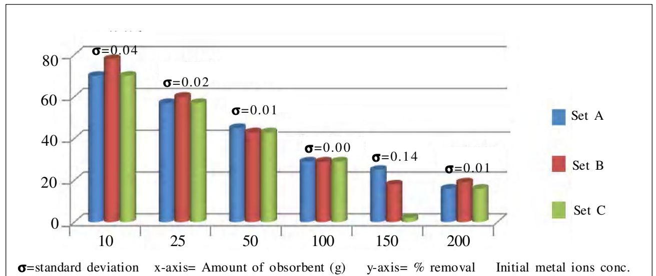


Fig. 3: Removal for different initial metal ion concentration (%)

Formula used to calculate standard deviation:

$$\text{Standard deviation}: \sqrt{\sum (x - \text{mean})^2 / n}$$

Where,

$\sum$ means 'the sum of'.

n means 'the number of values'.

x refers to 'the values given in the question'.

Biosorption experiment:

Based on the optimized results, the biosorption experiment was performed in triplicate using:

Beads concentration - 2.4 g.

Initial metal ion concentration - 50 mg/l.

Contact time - 150 minutes.

Table 2: Results of biosorption experiment

Sample	0 hr	Lead detected (ppm)	Lead absorbed (ppm)	Removal (%)
BE_1	50	12	38	76
BE_2	62	12	40	64.5
BE_3	54	9	45	83.33
Average				74.61

From the results obtained, it is clear that by increasing the parameter gradient, the biosorption capacity of lead was increased from 40.7 per cent to 74.61 per cent.

Desorption experiment:

Beads from the biosorption experiment were subjected

Table 3: Results of desorption experiment				
Sample	Chemical used	0 hr	Lead detected (ppm)	Removal (%)
DE1	NH_4Cl	38	2	5
DE2	HCl	40	28	70
DE3	EDTA	45	35	77.78

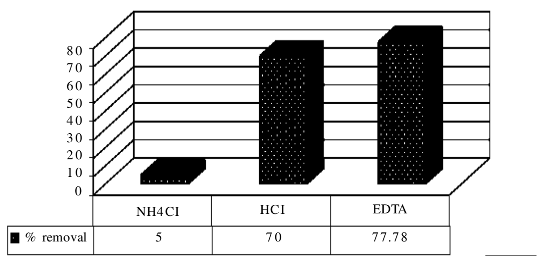


Fig. 3: Removal for different initial metal ion concentration (%)

to:

NH_4Cl, HCl, EDTA for 1 hour on Shaker conditions.

It was found that EDTA gave maximum desorption followed by HCl.

NH_4Cl gave as little as 5 per cent removal.

Lead contaminated water sample from Valkeshwar was subjected to biosorption.

70 per cent removal of lead from the water sample showed that the technique-IMMOBILIZATION is effective for biosorption of heavy metal-lead.

Table 4: Application of lead contaminated water sample				
	0 hr	Lead detected	Lead absorbed	Removal (%)
Test	10	3	7	70

Thus the current study suggests that the fungal strains may be used in future for bioremediation of wastewater and heavy metal contaminated soils.

Conclusion:

The present study thus focuses on ability of pre-treated and dead biomass of fungi to bind to metal like lead which was analyzed using atomic absorption spectrophotometer. Thus we can conclude that dead cells can be preferred over live cells as it has advantages with regards to no toxicity, nutrient requirements and other maintenance conditions. Biosorption appears to be suitable as secondary or polishing applications for metal removal from dilute waste streams, which would be competitive with ion-exchange resin based on final cost-effective analysis.

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LITERATURE CITED

Bishnoi, Narsi R., and Garima (2005). Fungus- An alternative of heavy metal containing wastewater: A review. *J. Scientific & Industrial Res.*, **64:** 93-100.

Saleh, M., Garni, Al, Ghanem, K. and Bahobail, A. (2009). Biosorption characteristics of Aspergillus fumigatus in removal of cadmium from an aqueous solution, *African J. Biotechnol.*, **8**(17):4163-4172.

Vijayaraghavan, K. and Yun, Y.S. (2008). Bacterial biosrbents and biosorption, *Biotechnol. Advances*, **26**:266–291.

Wang, Jianlong and Chen, Can. (2009). Biosorption for heavy metals removal and their future**.** *Biotechnol. Advances*, **27** : 195-226.