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Studies on Biosorption of Heavy Metals Using Pretreated Biomass of Fungal Species

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Abstract

Water pollution and contamination are the most serious problems being faced by the society today. Many processes are extensively studied to treat the polluted waters and wastewaters. One of the promising methods is biosorption. Biosorption is an alternative technique to conventional treatment methods of wastewaters and Industrial effluents. It uses the capability of cell surface to adsorb high quantities of metal ions by physical– chemical interaction with functional groups on the surface. In this study the potential of the pretreated biomass of Aspergillus Niger in biosorption of heavy metals like lead, Copper and Nickel from single metal solutions is evaluated. A.Niger biomass is pretreated with 0.5N NaOH for 30min and autoclaved at 121^0C for 20min. Efficacy of the biosorbent is tested in batch experiments for $Pb^{2+}$and Ni^{2+} in single metal solution under control led experimental conditions. Metal ions were analyzed by atomic absorption spectrophotometer using specific lamps at a specific wavelength. The equilibrium adsorptive quantity was determined to be a function of the solution pH, contact time, biosorbent dose and initial concentration of metal ions. The maximum removal of lead was observed around 75-80% at pH 6-7 with maximum adsorbent dose of 0.2g/ml. Nickel was observed to have maximum biosorption of around 50-60% at pH 5-8. The results showed that fungal biosorption has very good potential to be used in removal of metal ions.

1. Introduction

In recent years, there has been increasing interest in the use of biomass from microbial sources, particularly the microalgae, to absorb multimetal ions in the waste water. The other types of treatment like ion exchange, evaporation, precipitation, membrane separation etc. are too expensive to treat low levels of heavy metal in wastewater. Therefore a low cost biosorption process using algae as an adsorbent has lately been introduced as an alternative.Another advantage of this type of biomass is the wide range of functional groups present. Biosorption can be a solution to clean the environment contaminated by heavy metals. New biosorbents can be manipulated for better efficiency and multiple re-use to increase their economic attractiveness. Although actual wastewater treatment systems often have to deal with a mixture of heavymetals, most research work still only focuses on a single metal sorption. Lead and Nickel are released from different sources like plating, fertilizers and paper industries. The fungal cell walls can be considered as a two phase system consisting of chitin framework embedded on an amorphous polysaccharide matrix (Yan and Viraraghavan, 2000). The cell walls are rich in polysaccharides and glycoprotein's such as glycans [β -1-6 and β -1-3 linked D-glucose residues), chitin (β-1-4 linked N-acetyl-D- glucosamine), chitosan (β -1-4 linked D-glucosamine), mannans (β -1-4 linked mannose) and phosphormannans (phosphorylated mannans). Various metal binding groups, viz amine,imidazole, phosphate, sulphate, sulfhydryl and hydroxylare present in the polymers (Crist et al., 1981).

Non-living waste biomass of A. niger attached to wheat bran was used as a biosorbent for removal of Zn and Cu from aqueous solution and metal uptake was found to be a function of the initial metal concentration, biomass loading and pH. Metal uptake decreased in the presence of coions, (Modak et al., 1996). Alkali treated biomass of A. niger referred to as 'Biosorb', was found to sequester Cd^{2+}, Cu^{2+} Zn^{2+} Ni^{2+} and Co^{2+} efficiently up to 10% of its weight (w/w) and exhibited higher metal binding capacity as compared to Neurospora, Fusarium and Penicillium (Akthar et al.,1996).

2. Experimental

Sterilization of glass ware: The sterilization of glass wares such as sampling bottles, flasks, Petri dishes and test tubes after washing with detergent was sterilized using autoclave at 121^0C 15psi for 20 min (Autoclaving glassware catalogue).

Isolation of soil sample: The fungus *A. niger* was isolated from soil by serial dilution methods. Soil samples are passed through 2mm mesh sieve and mix thoroughly. Weigh out a 10 gm portion of soil into the dilution bottle containing 95ml of distilled water .Glass beads are added to this dilution blank to facilitate mixing. Cap the bottle on the mechanical shaker and shake for 10 min. Serial dilution is performed until a dilution of 10^{-7} reached. Subsequent pour plate of 0.1ml aliquot of this dilution onto the media. Pour plate method using potato dextrose Agar medium plates and media was weighed out and prepared according to the manufacture's specification, with respect to the given instructions and directions. The plates were incubated at 37^oC for 48 hrs. Pure cultures were obtained and Fungi are identified by morphological structures observed by lactophenol staining under 100X lens.

Media preparation: For experimental purposes, a liquid medium potato dextrose broth (PDB) with a pH value adjusted to 5 was prepared, which comprises (in g·1-1) the following: potato infusions 200, dextrose 20. Suspend 24 grams in 1000 ml distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Flask (250 ml capacity) containing 50 ml sterile medium were inoculated by o.1ml of fungal spore suspension prepared from 3-5days old culture fungal mycelium and incubated at 25°C for 7 days in a rotary shaker at 150rpm The fungi grew in a filamentous (mold like) form under air, with fragmentation of some hyphae into spherical cells. (Khalil I. Bander 2008).

Preparation of biosorbent: The fungal cell mass was determined by filtering the culture medium through weighed Whatmann filter paper no. (44). Mycelium was thoroughly washed with generous amounts of deionized water, re suspended and washed again. The biomass thus obtained was suspended in 0.5N Sodium Hydroxide solution for 15 min. The pretreated biomass was washed with deionized water until the pH of the solution was in a near neutral range (pH 6.8-7.2). This was autoclaved for 15min at 121°C the biosorbent is dried in an oven at 80ºC for 24 hours. When the biomass is dry it is powdered and sieved to size 0.125mm. (Ahmet 2004)

Preparation of adsorbate Solutions: Stock lead and Nickel solutions are prepared using Nickel Nitrate and Lead Nitrate

Batch Studies on Biosorption: Batch adsorption studies for individual metal compounds were carried out to investigate the effect of different parameters such as adsorbate concentration, adsorbent dose, agitation time and pH Different concentrations of biomass were combined with 100 ml of metal solution in 250 ml Erlenmeyer flask. The flasks were placed on a shaker with a constant speed of 150 rpm and left to equilibrate. Samples were collected at predefined time intervals. . The adsorbate was decanted and separated from the adsorbent using Whatman No.1 filter paper. To avoid the adsorption of adsorbate on the container walls, the containers were pretreated with the respective adsorbate for 24 hours. (Tarangini, K 2009)

3. Results and Discussion

3.1 Effect of pH

The most important single parameter influencing the sorption capacity is the pH of the adsorption medium. (Goyal et al., 2003). The influence of pH on the percentage sorption of lead is shown in the table 7 below. Little or no biosorption of metal ions was observed for pH less than 3.0. The heavy metal removal capacity increased with an increase in pH of 3.0 to 4.5. A sudden increase in sorption with a slight increase in pH is often referred to as an “adsorption edge”. Such an effect of pH on adsorption of heavy metals had also been observed for activated carbon (huang and Ostovic, 1978). Beyond pH 4.0 the metal ions removal increased with increase pH . The low metal biosorption at pH 3.0 has been attributed to the competition that metal ions face from hydrogen ions for the available biosorption sites (Huang et al 1988).The final pH values of the reaction mixture increased with an increase in initial pH values. The data indicated that biosorption started to increase for samples with final pH value of 4.0

which corresponded to an initial pH value of 3.2. The results below showed that pH is an important parameter effecting the biosorption of heavy metals at low pH. Heavy metal removal was inhibited, possibly as a result of a positive charge density on metal binding sites due to a high concentration of protons in solution. With an increase in pH, the negative charge density on the cell surface increase due to deprotonation of the metal binding sited and thus increases biosorption.

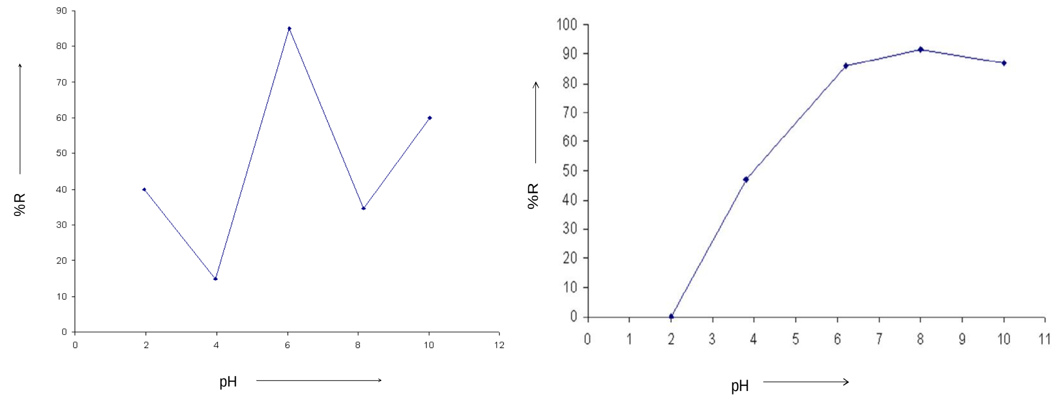

Figure 1: Effect of pH on Nickel and lead.

Effect of Sorption Time: A metal solution concentration of 10mg/ml is adjusted to the optimal pH 7 for lead the and biosorbent dose of 0.1mg is maintained .The filtrate is collected by using Whattmann filter at different time intervals of 5, 15, 30 45, 60, 90, 120, 150, 180, 210, 240 min. The plot below shows adsorption rates were high for the initial 10 minutes of contact, decreasing gradually to approach equilibrium. The equilibrium was observed at 5hr for lead and 8hr for Nickel. At the equilibrium the removal rates of adsorption for Lead and Nickel are observed as 70% and 60% respectively.

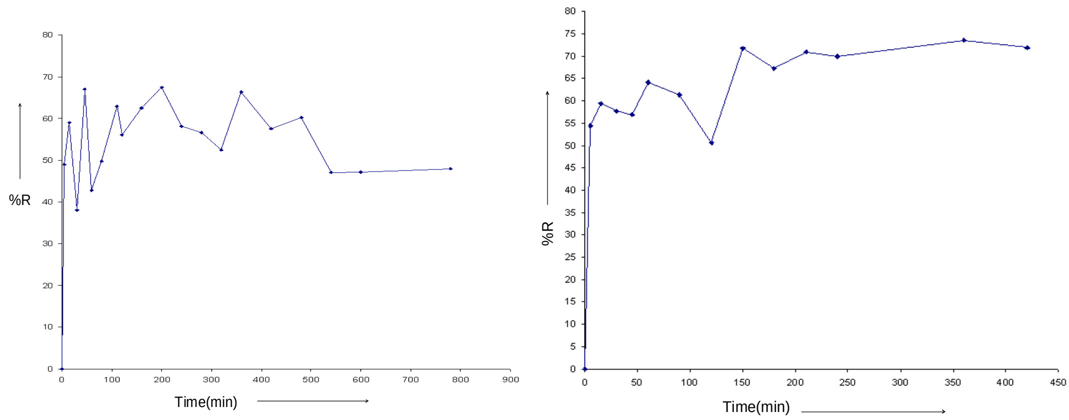

Figure 2: Effect of Sorption time on Nickel and lead.

Effect of concentration: The concentration of metal ions was varied and percentage removal of the metals ions was plotted in the graph below. For each test tube the optimal pH and equilibrium time are maintained .The concentration was varied as 10, 20, 30, 40 and 50 mg/ml. The lead was observed to have maximum adsorption of 50mg/ml for equilibrium time of 5hours and for nickel biosorption was maximum at 75mg/ml for time of 8hours. The biosorption of metals ions to the surface of the cell increase gradually till they reached optimal concentration later it was decreased gradually. The samples of metal solutions prior to and after equilibrium of adsorption were taken. After metal concentration analysis, the final concentration was subtracted from the initial concentration in order to find the metal to be sorbed. The metal ions contents in all the samples were determined by flame atomic absorption spectrometry. The trend observed in the graph might be explained by the ability of active sites to fully absorb the metal ions at lower concentrations, whereas higher concentrations caused saturation of absorption sites.

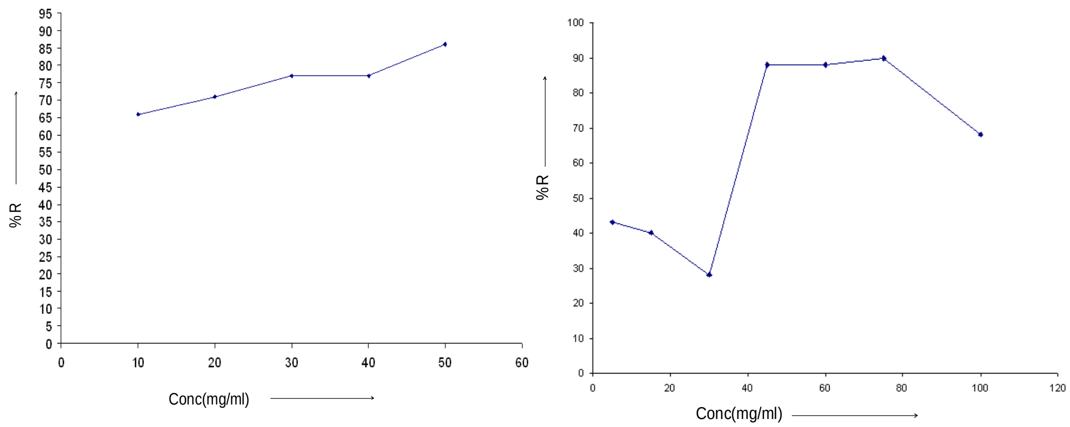

Figure 3: Effect of concentration for lead and Nickel.

Effect of adsorbent dose: The adsorbent dose of biomass was varied and percentage removal of the metals ions was plotted in the graph. The adsorbent dose of pretreated biomass of Aspergillus Niger was added and percentage removal of the metals ions was observed. For each test tube the optimal pH 6 for lead, pH 7 for Nickel and equilibrium time of 5hours for lead and 8hours for Nickel are maintained .The adsorbent dose was varied to 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 gm.

The lead was observed to have maximum adsorption of 0.1gm for equilibrium time of 5hrs.and for nickel biosorption was maximum at 0.2 gm for time 8hrs. The biosorption of metals ions to the surface of the cell increase gradually till they reached optimal concentration later it was decreased gradually. The percentage removal of metals ions of lead and Nickel was observed to be maximum 93% at 0.1gm and 67% at 0.2gm respectively.

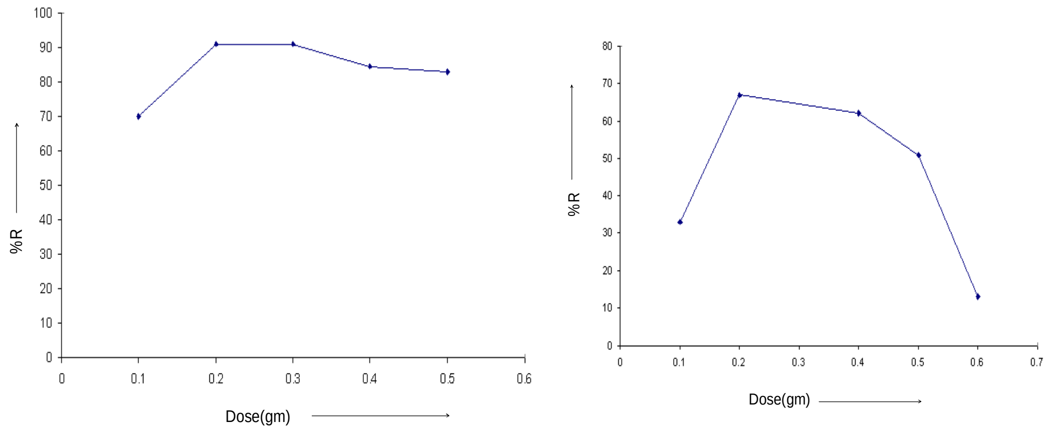

Figure 4: Effect of adsorbent dose for lead and Nickel.

4. Binary Metal System (BMS)

In the BMS, one metal ions is used as the main metal, the initial concentration of which was varied to determine the maximum amount absorbed in the presence of fixed concentration of co-ions. Comparing the intercationic effect in BMS, the biosorption of Lead and Nickel, the data clearly indicates that adsorbents are competing for same binding sites.

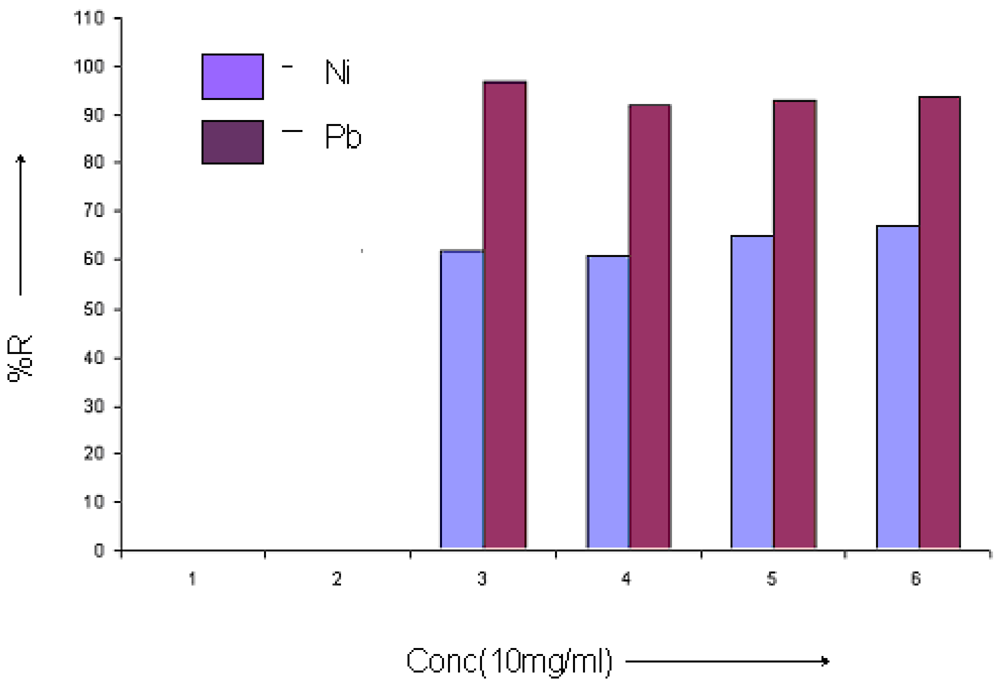

Figure 5: Effect of concentration on percentage of metal removal of Lead in presence of co-ion Nickel (25 mgl^{-1})

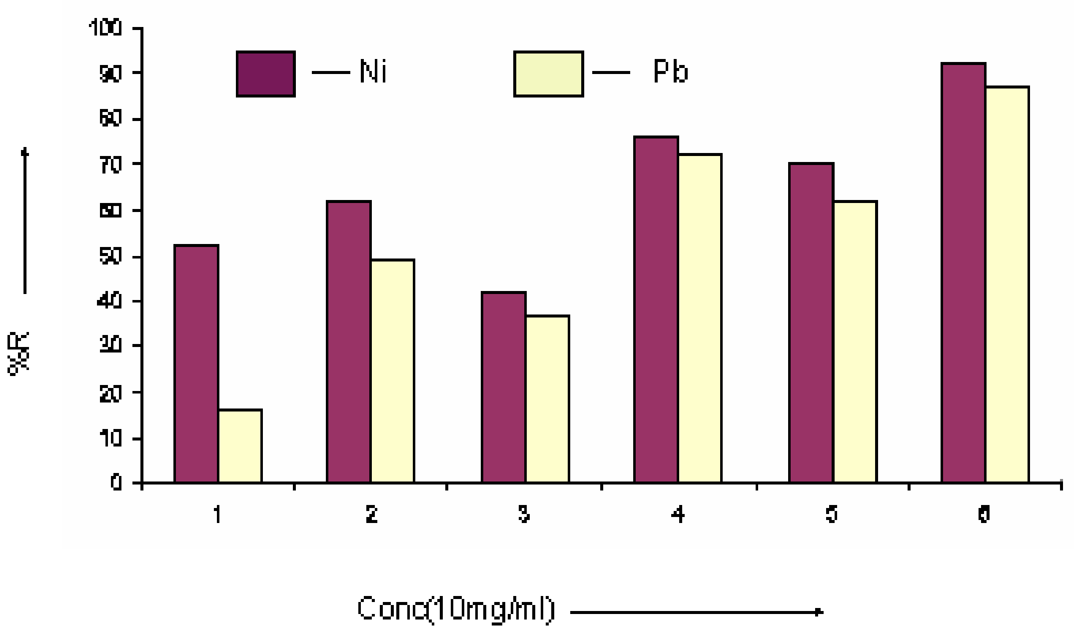

Figure 5: Effect of concentration on percentage of metal removal of Nickel in presence of co-ion Lead (25 mgl^{-1})

In 100 ml volume of BMS solutions with various concentrations (10, 20, 30, 40, 50 and 60 mgl^{-1}) of main metal and 25 mgl^{-1} can affect metal concentration or co-ion in the same solution in conical flasks of 100 - 200 ml, working volume was thoroughly mixed with biosorbent dose of 0.1 g at 30 ± 1°C and 150 rpm shaking speed for optimal equilibrium time of respective single metal removal. The pH range was adjusted 6 by using 0.1 M HNO3 and 0.1 M HCl solutions. The flasks were kept on rotating shaker with constant shaking. At the end of experiment, the solution was separated from the biomass by filtration through filter paper.

5. Conclusion

From the results obtained and discussions made above, the following conclusions are made:

The pretreated biomass of *Aspergillus Niger* is found as a potential source for the biosorption process for the removal of heavy metals like lead and Nickel. The different parameters, which effect the biosorption process were studied and effect of the parameter on the percentage removal are plotted in the graph. Increasing adsorption capacity with pH under single system is related to the influence of functional groups present on cell surface. Heavy metal removal was inhibited, possibly as a result of a positive charge density on metal binding sites due to a high concentration of protons in solution. With an increase pH, the negative charge density on the cell surface increase due to deprotonation of the metal binding sited and thus increases biosorption. For Lead and Nickel optimal pH is observed to be 7 and 6 respectively. For single metal systems, the higher percentage adsorptions at low metal concentrations imply that at low concentrations metals are adsorbed by specific adsorption sites, while at higher concentrations, the specific sites are saturated and exchange sites filled resulting in lower effective adsorption. The equilibration time for maximum biosorption at optimal pH for both Lead and Nickel was observed to be 5hours and 8hours respectively. Smaller sorbent mass get saturated faster with respect to the adsorbed metal because of Surface area effect and Solution : Solid ratio. Hence decrease in adsorption capacity with increasing adsorbent dose. In the binary metal system, physico-chemical characteristics of respective metals (i.e. ionic potential, ionic radius, ionic stability limit) seems to be exert some additional influence on the competitive preferential adsorption on metals on the sorbent pretreated *Aspergillus niger* biomass. In presence of co-ion lead, the percentage removal of Nickel is observed to be 92% which is greater than the Single metal system removal. In presence of Nickel, maximum biosorption and removal was observed to be 97%.

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