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Bioremediation of nickel from wastewater using immobilized *Phanerochaete chyrsosporium* biomass

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Abstract

Phanerochaete chyrsosporium is a white rot fungus with high heavy metal accumulating ability. In this regard, the present study was planned to assess the Ni (II) remediation potential of live immobilized *Phanerochaete chyrsosporium* from aqueous solutions as well as from real hazardous effluents. The effects of pH, dose, initial metal concentration, time, temperature etc. on bioremediation potential of *Phanerochaete chyrsosporium* were investigated in a well stirred batch system. Langmuir and pseudo second order kinetic model fitted well to adsorption data of Ni(II). For regeneration of metal capacity of *Phanerochaete chyrsosporium* sulphuric acid (0.1 M) was found to be the best desorbing agent.

Key words: Nickel; *Phanerochaete chyrsosporium*, White rot fungus; Bioremediation; Wastewater; Desorption

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1. Introduction

Natural water resources are being contaminated due to toxic substances generated from agricultural, industrial and domestic activites. Heavy metal contaminants present in aqueous streams are severely affecting growth, survival, reproduction, development and behavior of the organisms. Heavy metals are non-degradable and must be reduced to acceptable limits before discharging into environment to avoid threats to living organisms [1-3]. Ni (II) is a nasty toxic heavy metal and a known carcinogen. It appears stuck onto DNA, stuck on to translocator protein and is often present in blood at high levels. Symptoms of nickel toxicity include dizziness, skin rash (called nickel dermatitis), nausea, chest pain, diarrhea, vomiting, headache, weakness and coughing. Contact with nickel vapor can lead to swelling of the brain and liver; degeneration of the liver; throat and nose; irritation to the eyes, and various types of cancer. Like other heavy metals Ni(II) can cause several diseases as well as can reach top of food chain via bioaccumulation [4-5]. Conventional methods for removing Ni(II) from waste water include ion exchange filtration, chemical precipitation, chemical oxidation or reduction, reverse osmosis, electrochemical treatment, membrane technologies and evaporation recovery. At low Ni(II) concentration in the range of 1-100 mgL^{-1} these processes may be ineffective or extremely expensive [6-7]. Bioremediation using natural biomaterials is promising alternative to conventional methods [8]. Immoblized biomass is favorable over freely suspended biomass due to its increased stability, mechanical strength, reusability [9]. Fungal remediation refers to the use of fungi to remediate organic soil contaminants, primarily hydrocarbons and heavy metals. A major limitation of white-rot fungus is its sensitivity to biological process operations. It has been observed that the fungus does not grow well in suspended cell systems. In the present study, white rot fungus was used in immobilized form to improve its growth and metal uptake ability. *Phanerochaete chrysosporium* is the model white rot fungus because of its specialized ability to degrade the abundant aromatic polymer.

Due to *Phanerochaete chrysoporium* specialized degradation abilities, research on its heavy metal uptake potential is needed to optimized to understand the mechanism in order to enhance the bioremediation of a diverse range of pollutants. The effect of different parameters such as pH, dose, initial metal concentration, time, temperature etc was optimized in the present study.

2. Materials and methods

Phanerochaete chrysosporium biomass was collected from metal contaminated area in a forest (Changa Manga) near Lahore, Pakistan. Collected *Phanerochaete chrysosporium* cells were multiplied in Biotechnology Laboratory, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan. One gram of *Phanerochaete chrysosporium* cells was suspended in 2% sodium alginate solution to form a homogeneous mixture. Beads were formed by introducing this solution drop wise into the $CaCl_2$ (0.1M) solution which were preserved in 50mM $CaCl_2$ solution till further use. To a litre of deionized distilled water (DDW) 4.48 g of $NiSO_4 \cdot 6H_2O$ were dissolve to prepare stock Ni(II) solution (1000 mg/L). Ni(II) solutions of varied concentration were prepared by diluting stock solution. In all sets of experiments 100 mL of nickel solution (100 mg Ni(II) per liter) (except concentration study) was taken in triplicate in 250 mL conical flasks. Weighed amount of immobilized *Phanerochaete chrysosporium* was added to each conical flask. Soultion pH was adjusted using 0.1N NaOH and 0.1N HCl. Afterwards, conical flasks were over sealed with thin aluminum film to avoid contamination. After completion experiment (24 h) samples were filtered using Whatman ashless filter paper (No. 40) to separate liquid and solid phases. Carefully filtered samples were stored in plastic sample bottles at 4˚C till AAS analysis. The concentrations of Ni(II) in liquid phase was determined using Perkin Elmer AAnalyst 300 Atomic Absorption Spectrophotometer. The industrial wastewater samples were collected from Industrial Zone located in Faisalabad City, Pakistan. Triplicate samples were collected in polyethylene bottles and transported in a refrigerator to research laboratory. Desorption of Ni(II) from immobilized *Phanerochaete chrysosporium* biomass was study using various acids and EDTA as desorbing agent at 0.1N concentration.

The Ni(II) % sorption and uptake capacity were calculated as follows:

$$\% \text{ sorption} = (Ci - Ce)\, 100 / Ci \quad (1)$$

$$qe = (Ci - Ce)\, V / 1000\, w \quad (2)$$

- Where V is the volume of the solution in mL and W is the mass of the sorbent in g.

All experiments were triplicated and all data represent the mean of three independent readings. Results are presented as Mean ± Standard deviation.

3. Results and Discussion

Percentage of ionization of functional groups present on cell wall of biomass is dependent on solution pH [10-12]. Solution pH also strongly affect growth rate of microorganisms. Effect of pH on uptake of nickel by immobilized *Phanerochaete chyrsosporium* biomass is presented in Fig. 1. Ni(II) uptake by immobilized *Phanerochaete chyrsosporium* biomass increased with increase in pH from 1 to 5. An increase in pH beyond 5 resulted in nickel precipitation. At lower pH values Ni(II) uptake was low as hydrogen ions effectively compete with metal ions to bind the sorption site [13-16]. Biosorbent dose is a crucial parameter to determine the sorbent-sorbate equilibrium of the system [6, 12]. Effect of biosorbent dose on uptake of Ni(II) by immobilized *Phanerochaete chyrsosporium* biomass is shown in Fig. 2. Ni(II) uptake was maximum at a biomass dose of 0.025 g/L. Ni(II) uptake capacity of immobilized *Phanerochaete chyrsosporium* biomass was found to decrease with increase in the biomass concentration due to poor biomass utilization (lower efficiency) [17]. Percentage removal of Ni(II) by immobilized *Phanerochaete chyrsosporium* biomass increased with increase in biomass dose. This is because of the availability of more binding sites for complexation of metal ion on increasing amount of immobilized *Phanerochaete chyrsosporium* biomass.

Effect of temperature on Ni(II) uptake capacity of immobilized *Phanerochaete chyrsosporium* biomass is shown in Fig. 3. Temperature significantly affected Ni(II) uptake capacity of immobilized *Phanerochaete chyrsosporium* biomass. On increasing temperature Ni(II) uptake capacity of immobilized *Phanerochaete chyrsosporium* biomass sharply decreases. According to adsorption theory, adsorption decreases with increase in temperature as molecules adsorbed earlier on surface tend to desorb from the surface at elevated temperature [17-19]. Effect of contact time on Ni(II) biosorption by immobilized *Phanerochaete chyrsosporium* biomass is presented in Fig. 4. The sharp increase was observed in first 60 min, and equilibrium was attained after 120 min [20]. In first 60 minutes biosorption was sharp probably due to decrease in pH of solution because of proton released by the biosorbent. The rapid initial sorption was likely due to extra cellular binding and slow sorption phase observed after first 60 minutes until equilibrium likely resulted from intracellular binding [21-23]. Contact time experiments clearly showed that Ni(II) adsorption following 2^{nd} order kinetics.

Initial metal concentration provides important driving force to overcome all mass transfer resistance of the metal between the aqueous solution and solid phase [18,19]. The influence of initial concentration on Ni(II) uptake capacity of immobilized *Phanerochaete chyrsosporium* biomass was studied at pH 5 by varying concentration from 25 to 1000 mg/L (Fig. 5). Ni(II) uptake by immobilized *Phanerochaete chyrsosporium* biomass increased by increasing concentration from 25 to 800 mg/L. A further increase in concentration resulted in saturation of biomass with metal ions which resulted in decrease in metal uptake capacity [5, 24,25]. Langmuir adsorption isotherm fitted well to concentration data of Ni(II). After optimization of experimental variables, Ni(II) removal from industrial wastewater was studied and results are presented in Fig. 6. Sorption equilibrium reached much faster in case of industrial wastewater in comparison to synthetic wastewater. Saturation of biomass with metal ions in less time was due to presence of large number of competing ions in the industrial effluents [7]. Desorption studies were carried out to determine the feasibility of reuse of immobilized *Phanerochaete chyrsosporium* biomass for uptake Ni (II) from wastewater. Reusability of biosorbent promises its low cost for commercial utilization. Ni(II) ions adsorbed onto immobilized *Phanerochaete chyrsosporium* biomass were eluted using 0.1M of HCl, H_2SO_4, CH_3COOH and EDTA. The effectiveness of desorbing agents was in following order: H_2SO_4 > HCl > Acetic acid > EDTA. Acid eluents which removes metal from biomass surface by lowering pH was found more effective in comparison to metal chelating agent EDTA [26,27].

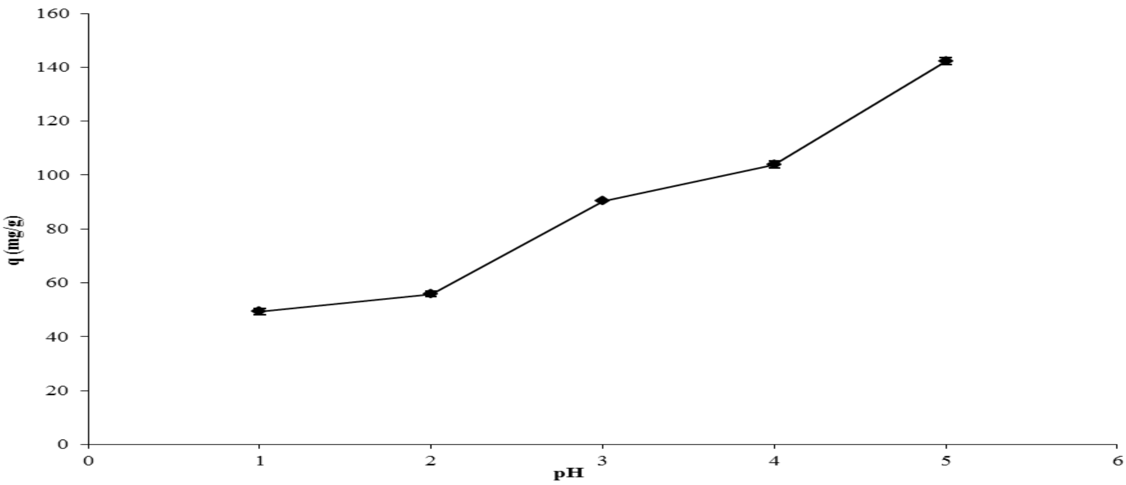

Fig. 1. Effect of pH on uptake of Ni(II) by immobilized *Phanerochaete chyrsosporium* biomass

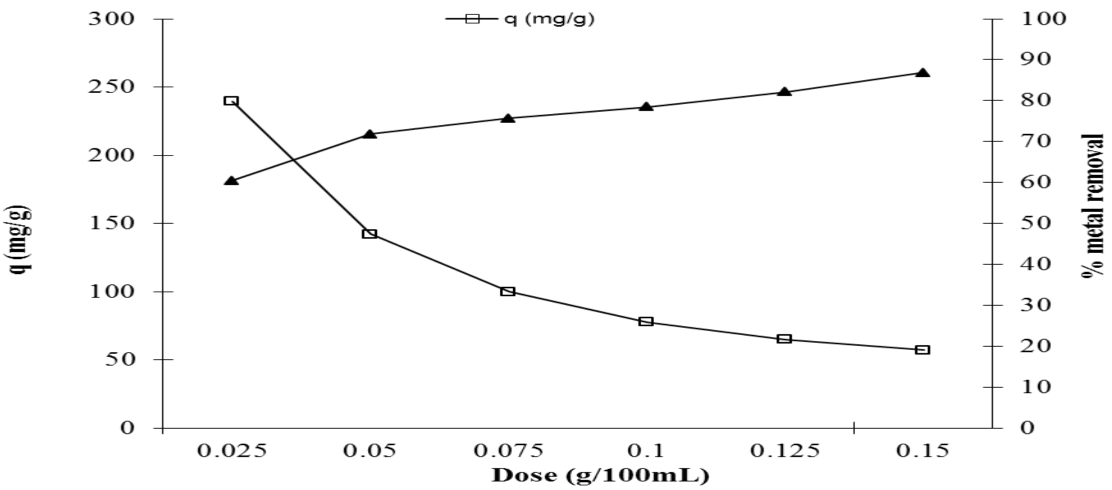

Fig. 2. Effect of biosorbent dose on uptake of Ni(II) by immobilized *Phanerochaete chyrsosporium* biomass

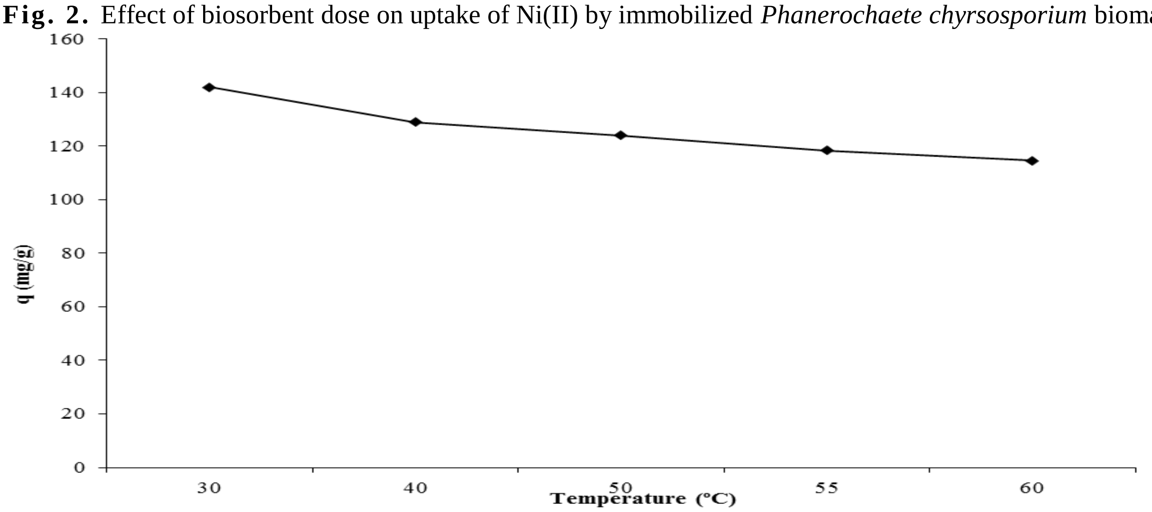

Fig. 3. Effect of temperature on uptake of Ni(II) by immobilized *Phanerochaete chyrsosporium* biomass

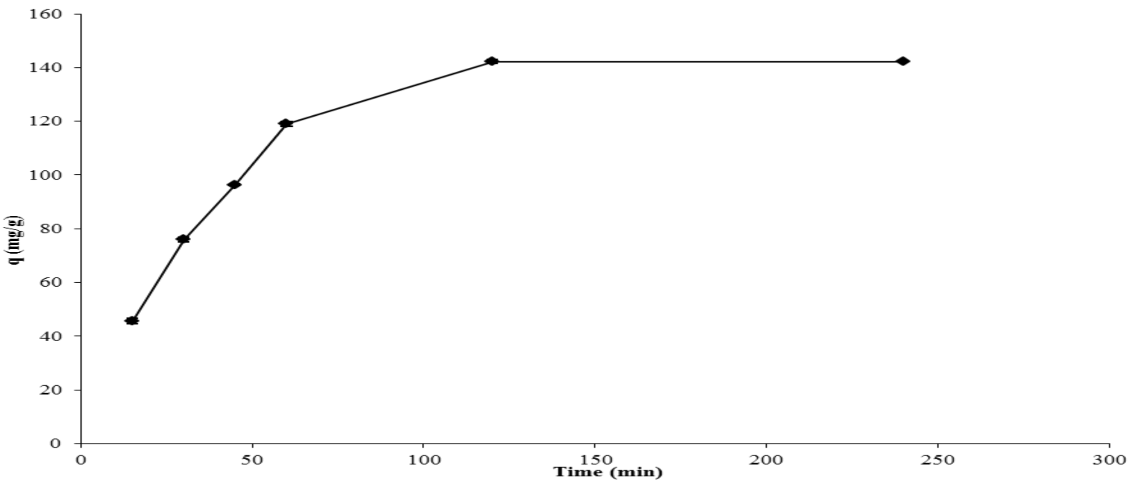

Fig. 4. Effect of contact time on uptake of Ni(II) by immobilized *Phanerochaete chyrsosporium* biomass

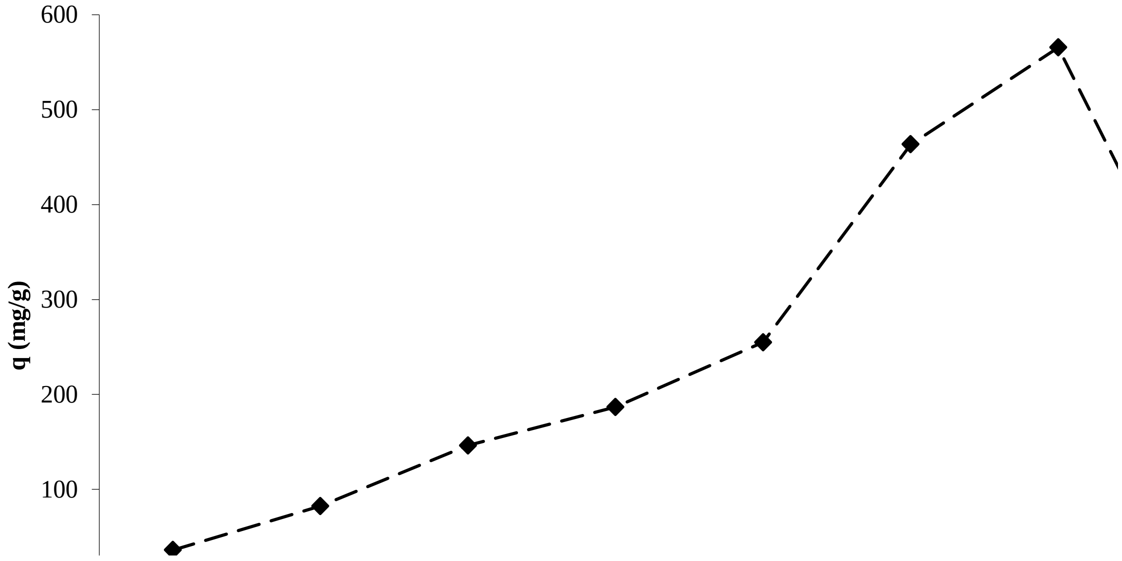

Fig. 5. Effect of initial metal concentration on uptake of Ni(II) by immobilized *Phanerochaete chyrsosporium* biomass

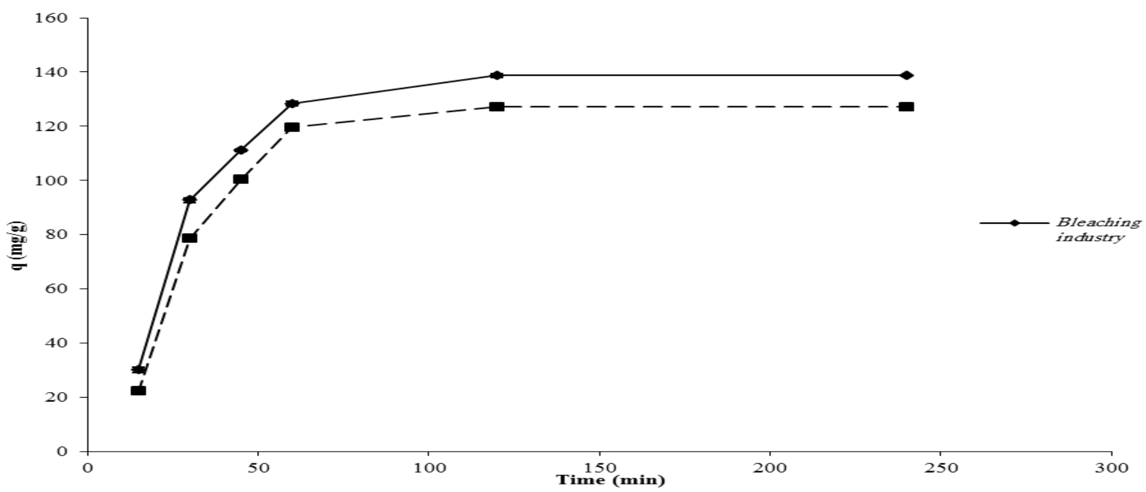

Fig. 6. Removal of Ni(II) from industrial wastewater using immobilized *Phanerochaete chyrsosporium* biomass

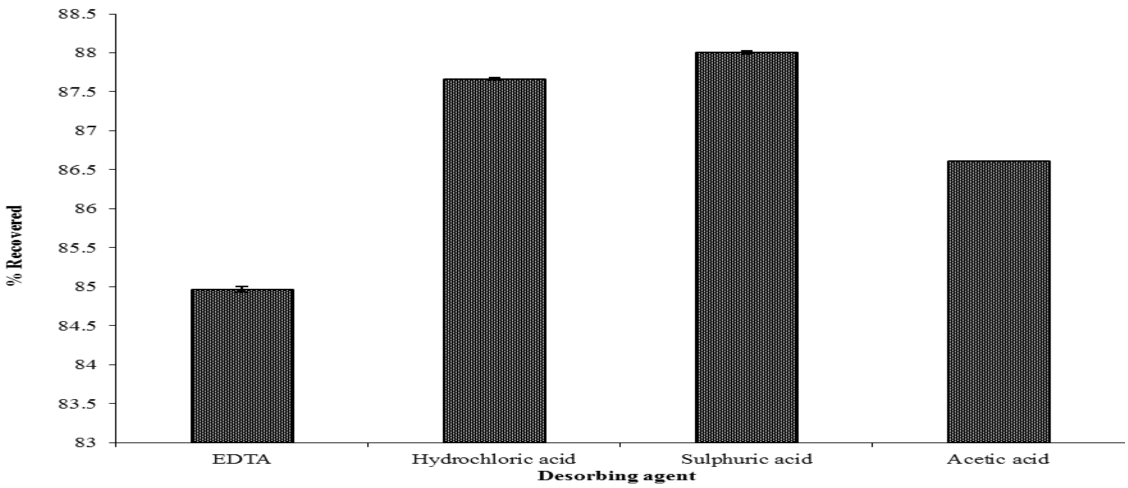

Fig. 7. Desorption of Ni(II) from immobilized *Phanerochaete chyrsosporium* biomass using various eluents

1. Conclusions

The present study was completed by following conclusions:

- Immoblization or granulation of fungal biomass could be effectively used to improve metal uptake capacity.
- *Phanerochaete chyrsosporium* biomass can be easily produced to meat commercial requirements.
- Acid eluents were found more effective than EDTA (chealting agent) for desorption of Ni (II) from *Phanerochaete chyrsosporium* biomass.

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