

# Use of immobilized *Ganoderma lucidum* living cells for uptake of Ni(II) from wastewater

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## Abstract

A lignolytic white-rot fungus, *Ganoderma lucidum* was used to remove Ni(II) from a single and multiple metal system. The removal of Ni(II) found to occur in two stages: a rapid surface adsorption, within the 60min, and a slow intracellular diffusion till the equilibrium achieved. In first few minutes of contact a sharp decrease in solution pH was noted which was found parallel to the fast metal uptake, probably because of the protons released by the immobilized *Ganoderma lucidum* living cells. At sorption equilibrium solution pH also reached at an equilibrium level. Ni(II) remediation capacity of immobilized *Ganoderma lucidum* biomass increased as the initial metal concentrations ( $C_i$ ) increased up to 800 mg/L, independent of initial pH ( $pH_i$ ) and generally the metal with higher  $C_i$  had a higher uptake capacity. The results also show that some portion of the metal ions sorbed by immobilized *Ganoderma lucidum* biomass was readily released to solution with a decrease in pH. At equilibrium, the maximum total Ni(II) uptake of immobilized *Ganoderma lucidum* biomass was 504.92 mg/g and was reached at  $pH_i$  5 and initial Ni(II) concentration of 800mg/L. Due to presence of larger number of competing ions in wastewater sorption equilibrium was reached in short time. Sulphuric acid (0.1 M) was found to be the better desorbing agent in comparison to other reagents

**Key words:** Heavy metal, white rot fungus, bioremediation, wastewater, desorption.

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## 1. Introduction

Toxic substances generated from agricultural, industrial and domestic activities are responsible for contamination of natural water resources. Growth, survival, reproduction, development and behavior of the organisms are severely affected due to heavy metal contaminants present in aqueous streams. 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 potent toxic metal but has several industrial applications. 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 chemical oxidation or reduction, chemical precipitation, ion exchange filtration, reverse osmosis, electrochemical treatment, membrane technologies and evaporation recovery. When Ni(II) is in the range of 1-100 mgL<sup>-1</sup> these processes may be ineffective or extremely expensive [6-7]. Biosorption is promising alternative to conventional methods [8]. Although freely suspended biomass extensively employed in the past but cell immobilization can enhance its mechanical strength, stability, reusability [9].

In the present study, live white rot fungi was used for uptake of Ni(II) from waste water. 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

### 2.1. Collection and preparation of biomass

White rot fungus biomass was collected from metal contaminated area in a forest near Lahore, Pakistan. Collected fungal cells were multiplied in Biotechnology Laboratory, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan. For immobilization studies, live fungal cells (dry weight = 1g) were suspended in 2% sodium alginate solution to form a homogeneous mixture. Beads were formed by introducing this solution drop wise into the CaCl<sub>2</sub> (0.1M) solution. The biosorbent beads were preserved in 50mM CaCl<sub>2</sub> solution till further use.

### 2.2. Preparation of Ni (II) stock solutions

Stock Ni(II) solution (1000 mg/L) was prepared by dissolving 4.48 g of NiSO<sub>4</sub>·6H<sub>2</sub>O in 100mL of deionized distilled water (DDW) and diluting quantitatively to

1000mL using DDW. Stock solution was diluted to produce Ni(II) solutions of different concentrations.

### 2.3. Batch biosorption studies

In all sets of experiments fixed volume of Ni (II) solutions (100 mL of 100 mg/L) was taken in each 250 mL conical flasks. Weighed amount of live immobilized white rot fungi biomass was added to each conical flask. The pH values of solution were adjusted to the required pH value using 0.1N NaOH and 0.1N HCl. Conical flasks were over sealed with thin film to avoid contamination from foreign particles. After 24 hours of experimental period, samples were filtered to separate liquid and solid phases using Whatman ashless filter paper (No. 40) and stored in plastic sample bottles at 4°C till AAS analysis. The concentrations of Ni (II) were determined using Perkin Elmer AAnalyst 300 Atomic Absorption Spectrophotometer.

### 2.4. Collection of industrial effluents

The industrial wastewater samples were collected from Industrial Zone located in Faisalabad City, Pakistan. Triplicate samples were collected in polyethylene bottles and transported in refrigerator to research laboratory.

### 2.5. Desorption studies

The desorption of Ni(II) from immobilized beads of fungal biomass was carried out using acid desorbent.

### 2.6. Determination of uptake capacity and % removal

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

$$\% \text{ sorption} = (C_i - C_e) 100 / C_i \quad (1)$$

$$q_e = (C_i - C_e) V / 1000 w \quad (2) \quad \text{Where}$$

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

### 2.7. Statistical analysis

All data represent the mean of three independent readings. Results are presented as Mean  $\pm$  Standard deviation.

## 3. Results and discussion

### 3.1. Effect of solution pH

Ionization of functional groups present on cell wall of biomass is strongly related to solution pH [10-12]. Growth rate of microorganisms is also pH dependent phenomenon. Effect of pH on uptake of nickel by immobilized *Ganoderma lucidum* biomass is presented in Fig. 1. Uptake of Ni(II) by immobilized *Ganoderma lucidum* biomass increased with increase in pH from 1 to 5. A further increase in pH beyond 5 resulted in precipitation of Ni(II). Metal uptake was low at lower pH as hydrogen ions effectively compete with metal ions to bind the sorption site [13-16].

### 3.2. Effect of biosorbent dose

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) is presented in Fig. 2. The maximum uptake capacity of Ni(II) was achieved with a

biomass concentration of 0.025 g/L. Metal biosorption capacity was found to decrease with increases the biomass concentration raises the maximum biosorption capacity drops, indicates poor biomass utilization (lower efficiency) [16]. Uptake capacity of biomass increased with an increase in biosorbent dosage. This is because of the availability of more binding sites for complexation of metal ion.

### 3.3. Effect of temperature

Effect of temperature on Ni(II) uptake capacity of immobilized *Ganoderma lucidum* biomass is shown in Fig. 3. Temperature significantly affects metal uptake capacity of biosorbent [15]. The metal uptake capacity of Ni(II) sharply decreases with increase in temperature. According to adsorption theory, adsorption decreases with increase in temperature and molecules adsorbed earlier on surface tend to desorb from the surface at elevated temperature [15, 17-20].

### 3.4. Effect of contact time

Effect of contact time on Ni(II) biosorption is presented in Fig. 4. The sharp increase was observed in first 30 min, and equilibrium was attained after 120 min [19]. In first 30 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 likely resulted from intracellular binding [5,21-23].

### 3.5. Effect of initial metal concentrations

Initial metal concentration provides important driving force to overcome all mass transfer resistance of the metal between the aqueous solution and solid phase [17-18]. The influence of initial concentration on Ni(II) uptake capacity of immobilized *Ganoderma lucidum* biomass was studied at pH 5 (Fig. 5). Initial Ni(II) concentrations strongly affected the uptake capacity of immobilized *Ganoderma lucidum* biomass [24-25].

### 3.6. Wastewater studies

At optimized conditions, Ni(II) removal from industrial wastewater was studied. Sorption equilibrium reached much faster in case of industrial wastewater in comparison to synthetic wastewater using same biosorbent (Fig. 6). This may be due to the presence of large number of co-metal ions in the industrial effluents [7].

### 3.7. Desorption

Immobilized *Ganoderma lucidum* biomass could be reused several times to decrease material cost after desorption of Ni(II) from its surface. The regeneration of the biosorbent is one of the key factors in assessing their potential for commercial application. Ni(II) ions adsorbed onto immobilized *Ganoderma lucidum* biomass were eluted using 0.1M of HCl, H<sub>2</sub>SO<sub>4</sub>, CH<sub>3</sub>COOH and EDTA. The effectiveness of desorbing agents was in following order: H<sub>2</sub>SO<sub>4</sub> > HCl > EDTA > Acetic acid. EDTA removes metals by chelation whereas acid eluent decrease pH to facilitate removal of adsorbed metal ions from biosorbent surface [26-27].

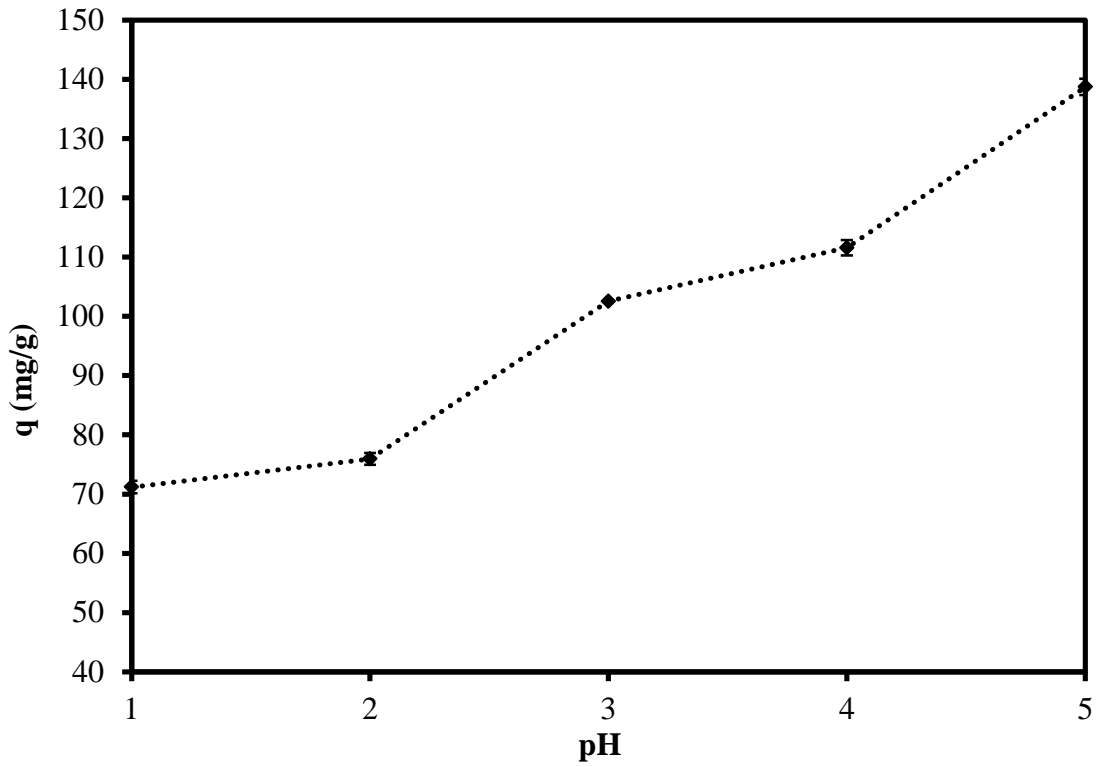


Fig. 1. Effect of pH on uptake of Ni(II) by immobilized *Ganoderma lucidum* living cells

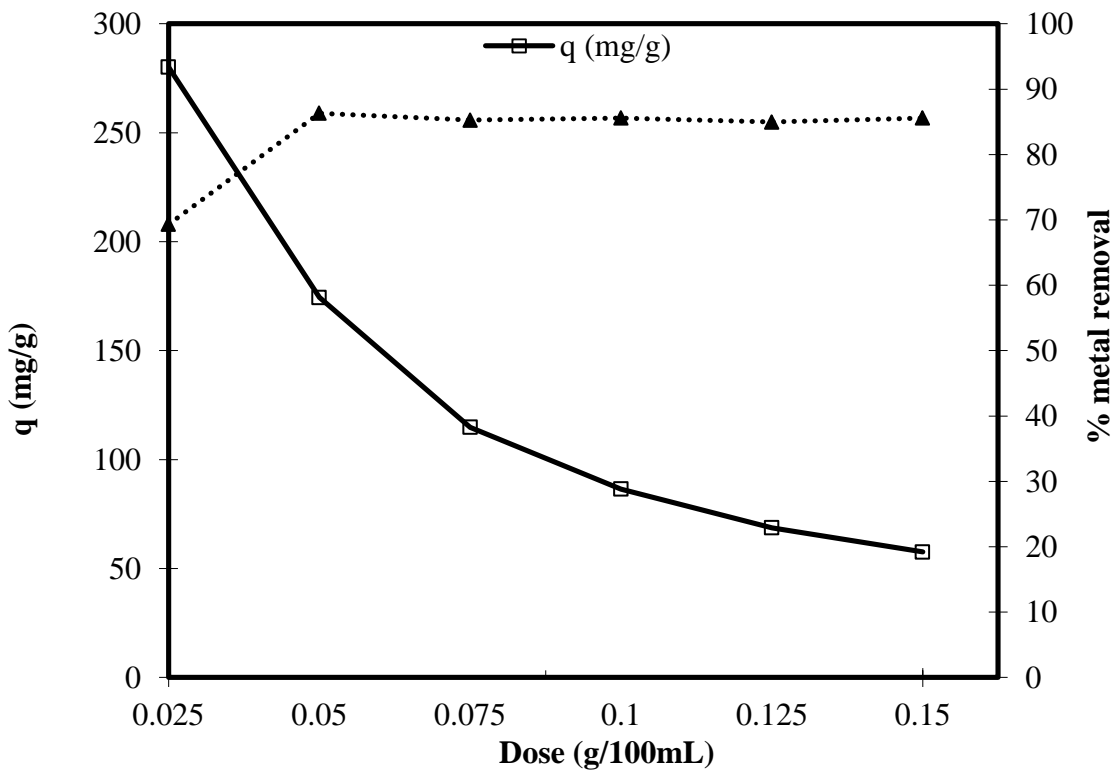
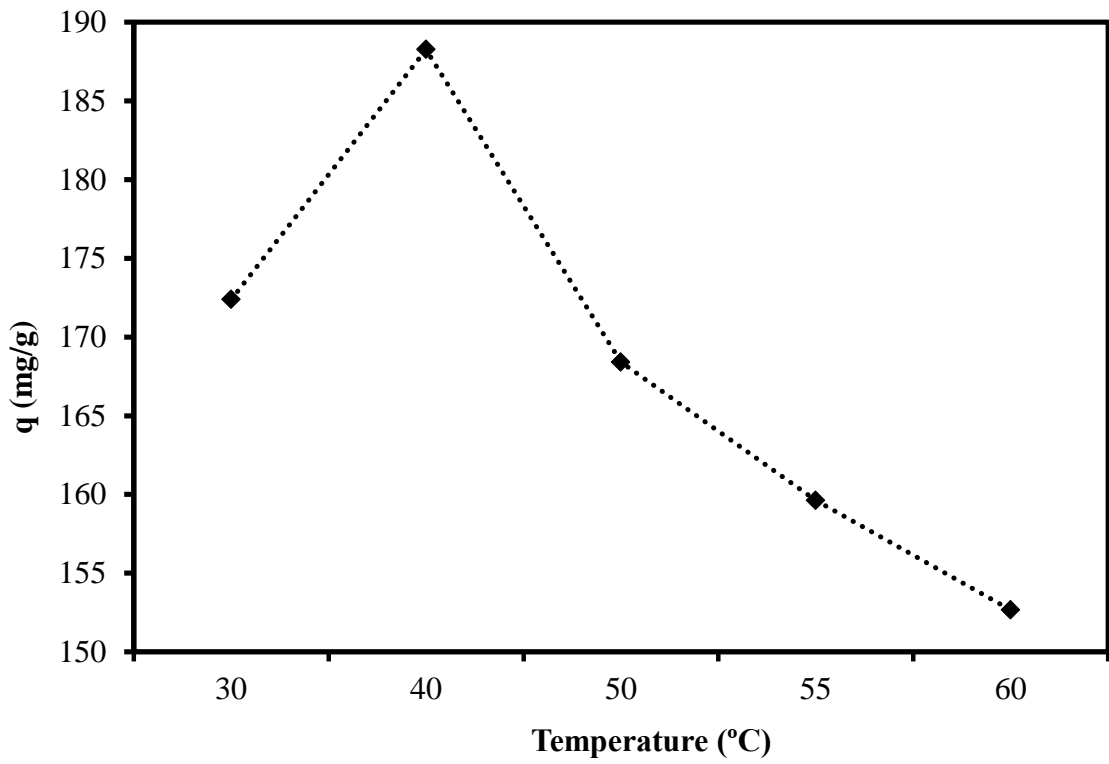
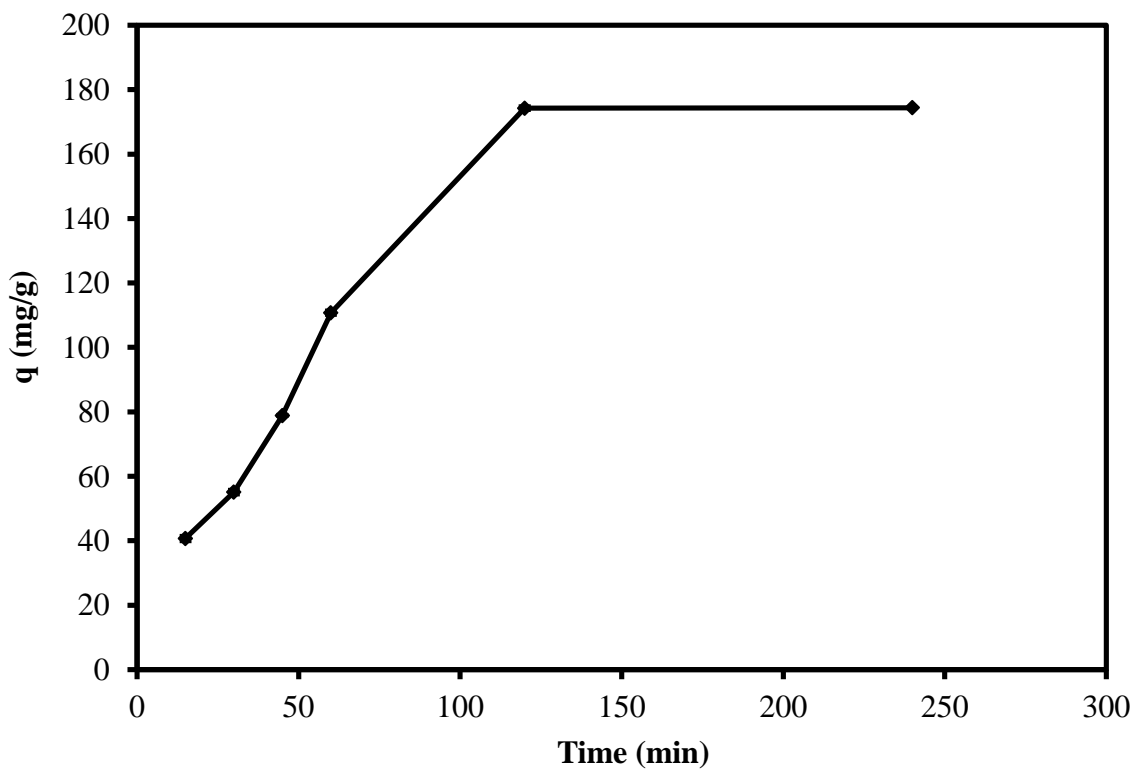


Fig. 2. Effect of biosorbent dose on uptake of Ni(II) by immobilized *Ganoderma lucidum* living cells



**Fig. 3.** Effect of temperature on uptake of Ni(II) by immobilized *Ganoderma lucidum* living cells



**Fig. 4.** Effect of contact time on uptake of Ni(II) by immobilized *Ganoderma lucidum* living cells

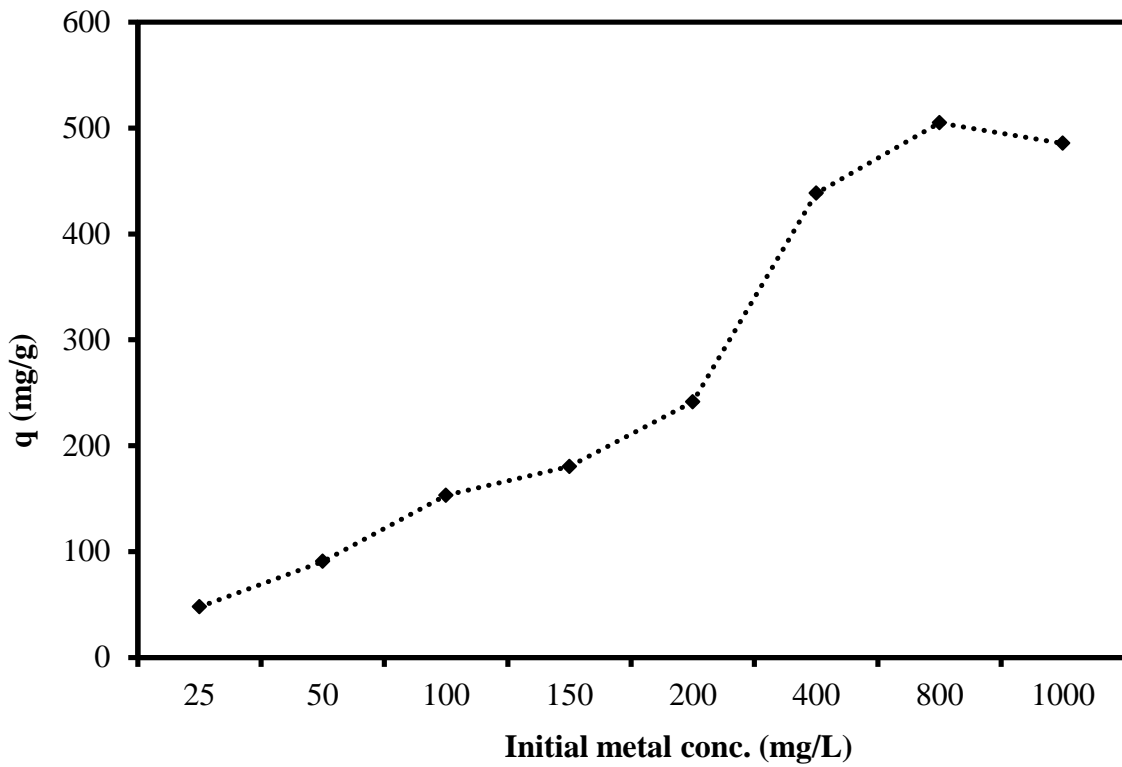


Fig. 5. Effect of initial metal concentration on uptake of Ni(II) by immobilized *Ganoderma lucidum* living cells

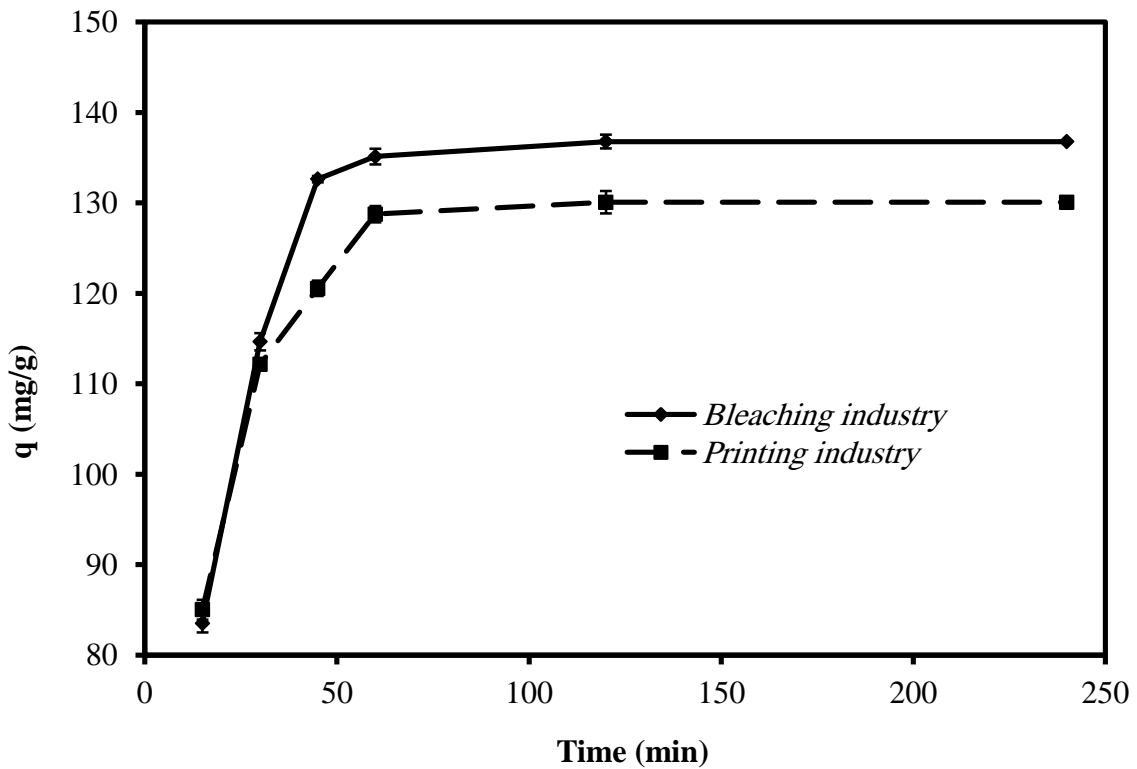


Fig. 6. Removal of Ni(II) from industrial wastewater using immobilized *Ganoderma lucidum* living cells

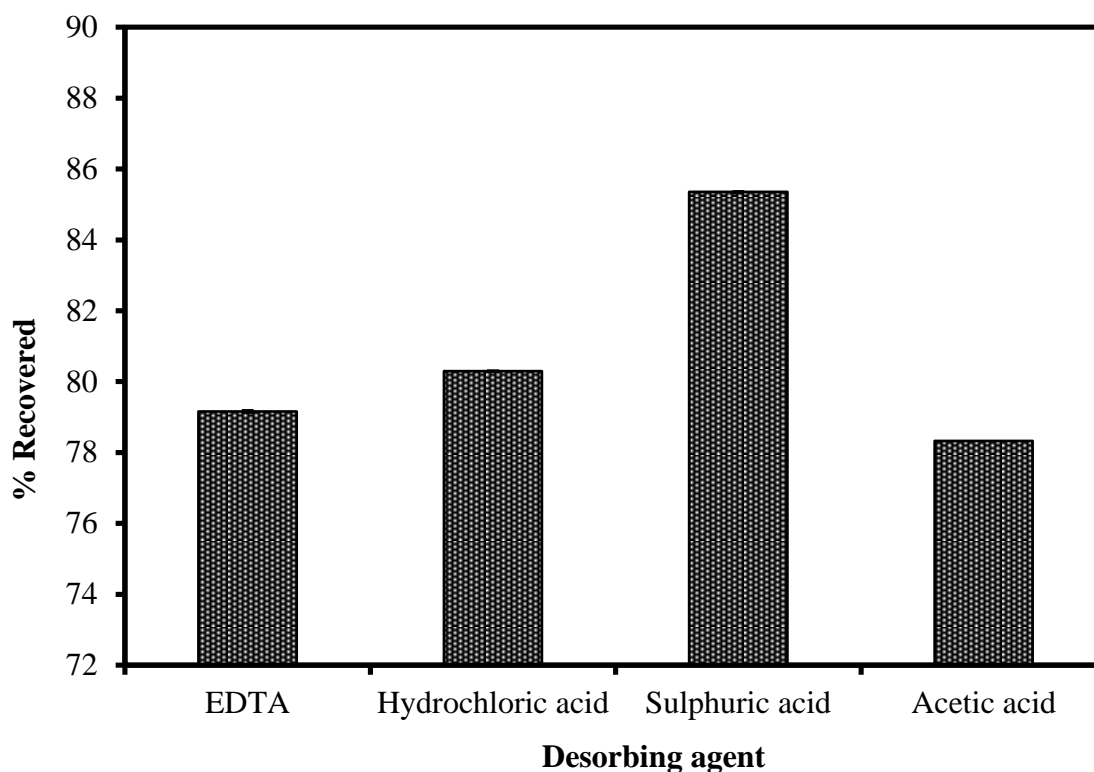


Fig. 7. Desorption of Ni(II) from immobilized *Ganoderma lucidum* living cells using various eluents

#### 4. Conclusions

Following conclusions can be withdrawn from the results of present study:

- Immobilization of biomass could be effectively used to improve metal uptake
- The optimized pH, biosorbent dose, contact time and initial metal concentration were 5, 0.05g/100mL, 240 min and 200mg/L, respectively.
- H<sub>2</sub>SO<sub>4</sub> was found to be most effective eluent for desorption studies.

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