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BIOSORPTION AND DESORPTION OF ZINC AND NICKEL FROM WASTEWATER BY USING DEAD FUNGAL BIOMASS OF ASPERGILLUS FLAVUS

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Abstract—Effluents containing heavy metals can be remediated with the help of dead microorganisms by the process known as biosorption. In this study the dead biomass [1]of fungus Aspergillus flavus was used for the biosorption of heavy metals i.e., Zinc and Nickel. The capacity of biosorption by the dead biomass of Aspergillus flavus was evaluated at room temperature with different parameters which are; pH, contact time, biomass concentration and metal ion concentration. The biosorption capacity for Zn was found to be 47.36% at room temperature, at pH 6.5, with biomass concentration of 2g/L having contact time of 50 min and solution concentration of 2ppm. Biosorption capacity for Ni was found to be 61.60% at room temperature, at pH 5, with biomass concentration of 2g/L having contact time of 60 min and solution concentration of 2ppm. . In this study, desorption of the heavy metals by 0.1M HCl was found to be effective. Fungal biomass was recovered for reuse.

Index Terms — Biosorption, Aspergillus flavus, heavy metals, Zinc, Nickel.

I. INTRODUCTION

In present times because of increasing population, unplanned industrialisation environmental pollution has become a major problem. Prime reason for water and soil pollution by heavy metals is due to the discharge untreated effluent from various industries involving heavy metals. Removal of heavy metals is important because they are toxic to the ecosystem. The World Health Organization has recommended the maximum acceptable concentration of Nickel and Zinc in drinking water as 0.07 and 0.05 mg/L respectively [1]. The most toxic heavy metal pollutants are zinc and nickel which are released into the environment from various industries like electroplating, metal plating ,paint and powder, batteries, alloy, brass manufacturing, mining, processing crudes, etc. [2-9]. The concentration level of these two heavy metals varies widely in the environment [10]. Zinc and nickel are essential micronutrients in humans [11]. They are important for all living beings. When zinc is present in excess amount it is toxic to living organisms. In humans, it can cause anaemia, zinc fumes have corrosive effect on skin and cause metal fume fever, damages pancreas, lungs, nerve membrane, causes nausea and vomiting and nephritis [4, 7, 12, 13, 14]. The free zinc ion in solution is highly toxic to plants and aquatic life [8] Nickel in excess amount, causes decrease in body weight, heart and liver damage, skin irritation, dermatitis, gastro-intestinal distress[4, 7, 12, 11].

Various traditional methods including chemical precipitation, lime coagulation, ion exchange, reverse osmosis, solvent extraction, electrochemical treatment, evaporation, ultra filtration, membrane processing have been devised for the removal of heavy metals from industrial wastewater [10,13, 15, 16, 17, 18, 19]. These physico-chemical methods have several disadvantages like production of toxic sludge, high concentration of toxic reagents, energy requirements and generation of secondary pollutants [10, 18, 19]. Alternative methods have been devised which led to the development of new eco-friendly biological technologies for the removal of heavy metals. Biological approach is more applicable as it is cost effective, easy, simple, highly efficient, and eco- friendly [7, 10, 19].

Microorganisms play a vital role in removal of heavy metals, since they are used as adsorbents for their removal. Removal of heavy metals or their recovery is done using living cells or dead cells. For biosorption studies, use of dead biomass is preferred over living cells. The dead biomass has several advantages viz. non requirement of additional nutrients, reuse of biosorbents, no toxicity limits for heavy metals, regeneration of biosorbent, recovery of biosorbed metals by easy desorption methods and displays high affinity for removal of metal ions from aqueous solutions[7, 10,12, 19]

Dead fungal cells sequester heavy metal ions on cell surface by adsorption. Heavy metal ion uptake by surface adsorption is irrespective of the surface functional groups such as carboxyl, amide, thiol, phosphate, and hydroxide [20].Thus the application of dead biomass is very effective method in the treatment of heavy metal removal. In the present study, the experiment was carried out for detecting the maximum sorption capacity of the dead biomass for Zinc and Nickel from aqueous solution. We determined the effect at room temperature of concentration of metal ion, pH, biomass concentration and contact time on the efficiency of biosorption.

II. MATERIAL AND METHODS

A. Isolation and Biomass Preparation.

The culture of *Aspergillus flavus* (identified by Agharkar Reaearch Institute, Pune) was isolated from soil and maintained on GPYA (Glucose peptone yeast extract agar) plate, at room temperature. To obtain fungal biomass, *Aspergillus flavus* was inoculated in GPYB and was kept on rotator shaker at 170 rpm with constant stirring till the

required biomass growth was obtained. The biomass was autoclaved for 15 min, at 15lbs and 121^0 C. Then the biomass was recovered by filtering and it was dried at 37^0 C for 24 hrs. The biomass was crushed to powder in mortal and pestle and then it was used for sorption studies.

B. Preparation of Reagents.

All chemicals used in this study were of analytical grade and solutions were prepared using de-ionized and distilled water. Aqueous solution of Zn and Ni (100 mmol/L) was prepared by dissolving metal salts ($ZnSO_4$ and $NiSO_4$) in distilled water for the determination of lambda max, by using UV/Vis spectrophotometer. Lambda max for Zn and Ni was found to be 200 nm. Stock solutions of 10ppm of Zn and Ni were prepared and different concentrations were obtained by diluting this stock solution.

C. Kinetic experiment.

Kinetic experiment were carried out using parameters that include biomass concentration and initial metal ion concentration.

- To study the effects of biomass concentration, experiment was carried out using different biomass concentration ranging, 1 to 4g/L.
- To study the effects of metal ion concentration experiment was carried out using different concentration where initial was 1ppm ranging to 4ppm for Zn and Ni solution.

D. Effect of pH on Zn and Ni adsorption.

To investigate the effect of pH, a series of experiment with 2ppm in 50ml of Zn and Ni solution was conducted. The pH was adjusted from 4.0 to 6.5 for Zn and 4.0 to 6.0 for Ni by0.1M HCl and 0.1M NaOH.

E. Adsorption equilibrium.

For equilibrium studies, the concentrations of Zn solution was 47.36% and Ni was 61.60% and biosorbent dosage was 2g/L. The pH was adjusted to 6.5 for Zn and 5 for Ni using 0.1M HCl or 0.1M NaOH hourly throughout the experiment. The mixtures were agitated on a rotary shaker (agitation rate, 170 rpm) for 50 min at room temperature. Then the biosorbent was filtered through a Whattman filter paper no.1. The amount of Zn and Ni uptake by *Aspergillus flavus* (Biosorption efficiency) in each flask was determined using the following mass balance equation (Eq. 1):

$$\% \text{ of Biosorption} = \frac{\text{Initial Absorbance} - \text{Final Absorbance}}{\text{Initial absorbance}} \times 100 \quad (1)$$

F. Effect of Cations and Anions on biosorption.

The effect of light metal ions and anions ligands were determined by using 0.1g of biomass and 50ml of Zn and Ni solution containing Cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) and Anions (NO_3^-, Cl^-, and EDTA – Ethylene diamine tetraacetic acid) respectively. Blank samples without light metals and anionic ligands were used as controls.

G. Desorption.

For desorption studies, 0.1g of biomass was used in 50ml of Zn and Ni solution. After absorption experiment, biomass was filtered and washed with distilled water for 3 times, for the removal of Zn and Ni present on the surface. Then it was transferred to 50ml desorbent solution (0.1 M HCL). The mixture was shaken at the interval of every 10 min, then the filtrates were used to determine the concentration of Zn and Ni after desorption.

H. IR Spectrum of Aspergillus flavus.

The IR spectrum of the dead dried biomass (80^0C for 8 hours) as native and loaded with Zn and Ni were analyzed in the 400-4000cm^{-1} area using a FT-IR 4100 spectrometer (Jasco, Japan). Amount of 5mg dead biomass native and loaded with Zn and Ni were used for the analysis.

III. Results and Discussion

A. Effect of concentration.

1) Effect of Biomass concentration.

It has been found that increase in biomass concentration decreases the biosorption efficiency [12].This occurs due to decrease in surface area on biosorbent for binding of adsorbent [21]. In this Biomass Concentration study we have found that 2g/L biosorbent shows maximum biosorption for 1L of Zn and Ni solution respectively. Thus 2g/L biomass concentration is found to be effective {Fig. 1(a), (b)}

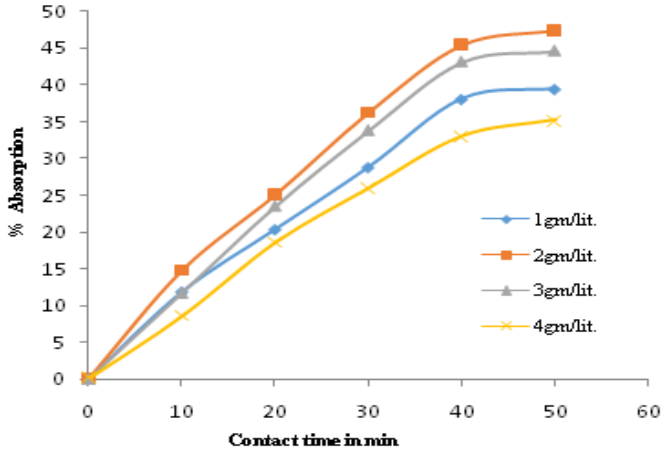

Figure 1(a). Effect of biomass concentration on biosorption of Zn by *Aspergillus flavus*

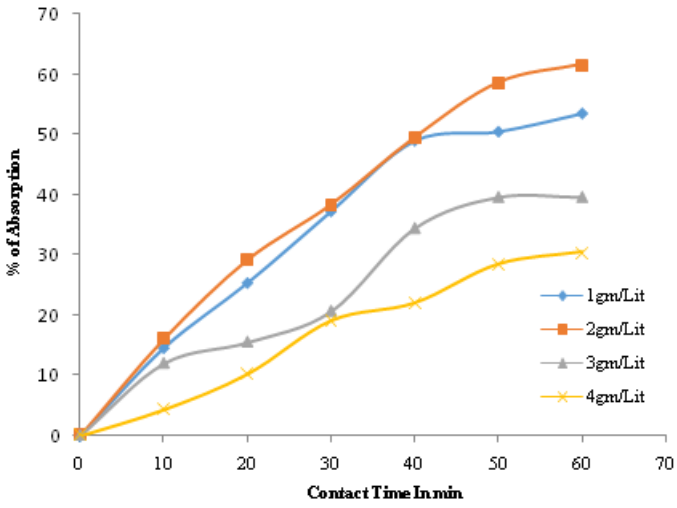

Figure 1(b). Effect of biomass concentration on biosorption of Ni by *Aspergillus flavus*.

2) Effect of initial concentration of metal ions

The initial concentration of metal ions in the solution plays an important role as a driving for us to overcome the mass transfer resistant between aqueous and solid phase. Thus

biosorption increase with an increase as in initial concentration till it reaches an optimum but after a certain point there is decrease in biosorption due competition between metal ions [22].The effect of initial concentration of solution on biosorption was studied. It has been found that *Aspergillus flavus* shows maximum biosorption for both Zn and Ni at concentration of 2ppm, within 50 min. {Fig. 2(a), (b)}. Percent biosorption was found to be 47.36% for Zn, 52.36% for Ni.

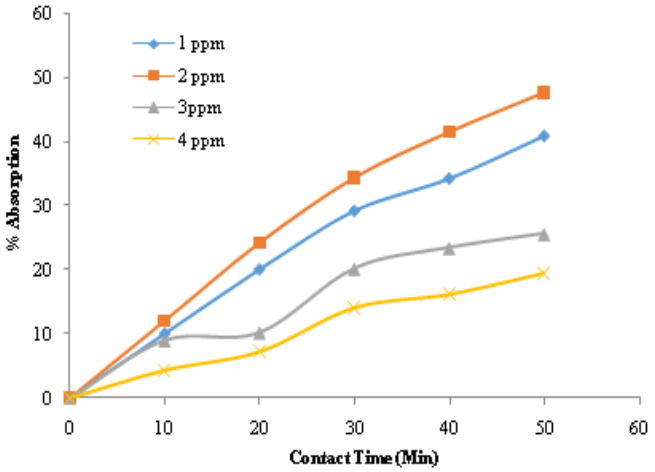

Figure 2(a). Biosorption of Zn by *Aspergillus flavus*

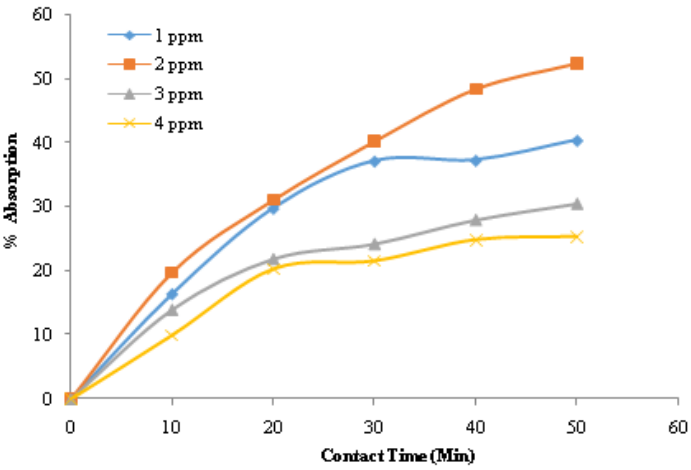

Figure 2 (b). Biosorption of Ni by *Aspergillus flavus.*

B. Effect of pH of solution.

The fungus contains many ionisable group (e.g. carboxyl groups)on the cell wall which are responsible for biosorption. There is very less biosorption at lower pH such as 2.0 and subsequent increase in pH increase the rate of biosorption and reaches an optimum at 6.5 pH for Zn and pH 5 for Ni and biosorption decreases after increasing pH from the optimum pH. The biosorption capacities of biosorption depend upon various functional groups and at a low pH [21, 22].

Many studies have shown that pH is an important factor affecting biosorption of heavy metals [14]. It is well known that pH affects the protonation of the functional groups on the biomass as well as the metal chemistry [21, 22]. The effect of pH on Zn and Ni adsorption was studied and the results are shown in Fig. 3(a), (b). While removal percentage was zero without biosorbent. Maximum biosorption was recorded at pH 6.5, for Zn and 5 for Ni respectively. At pH 6.5 Zn was adsorbed 47.36% within 40 min, while at pH 5 Ni adsorbed 61.60 %within 50 min.

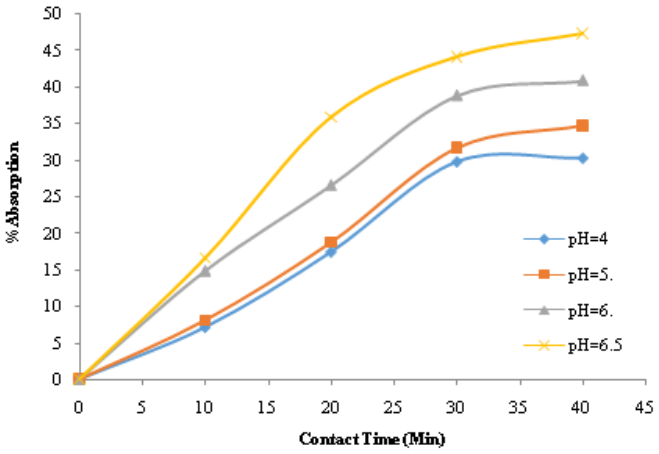

Figure 3(a). Effect of pH on biosorption of Zn by *Aspergillus flavus.*

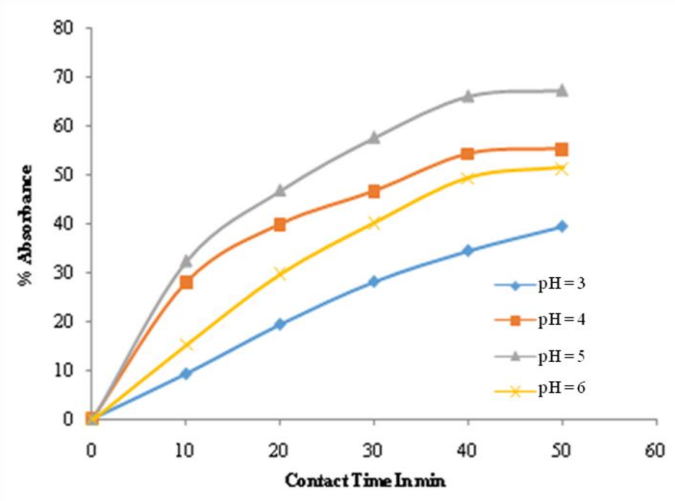

Figure 3(b). Effect of pH on biosorption of Ni by *Aspergillus flavus.*

C. Desorption and recycling of biosorbent (dead biomass).

After determining the biosorption profile, the reuse and recycling possibility of biosorbent was investigated. In this study, desorption of Zinc and Nickel was carried out using 0.1M HCl solution. Fig. 4 shows that 0.1M HCl was effective desorbent for Zn and Ni.

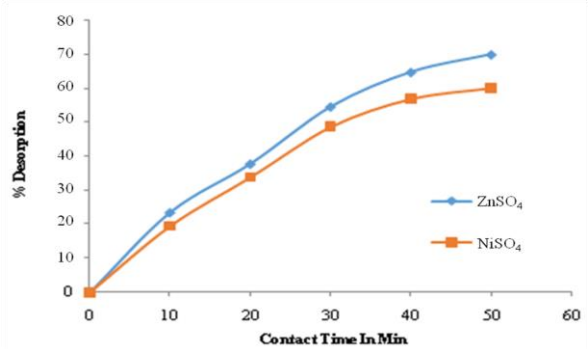

Figure 4. Desorption of Zn and Ni from *Aspergillus flavus.*

D. Effect of cations and anions.

The effects of cations and anions on adsorption of Zn and Ni were shown in Fig. 5(a) (b). Industrial effluents often contains ions such as Na^+, K^+, $Mg2^+$ and $Ca2^+$, which may interfere with the uptake of heavy metal ions by biomass[23].

The effect of cations on adsorption was studied and the result was shown in Fig. 5(a).

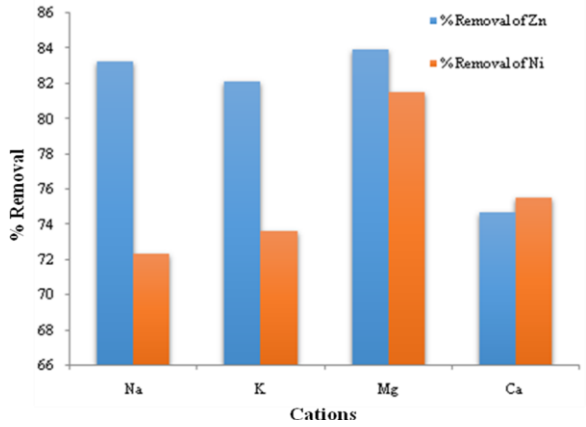

Figure 5(a) The effect of cations on Zn And Ni adsorption.

The three types of anions was investigated, including sodium salts of chloride, nitrate, and EDTA. The EDTA affected the adsorption remarkably {Fig. 5(b)}. This is because Zn and Ni can combine with EDTA strongly.

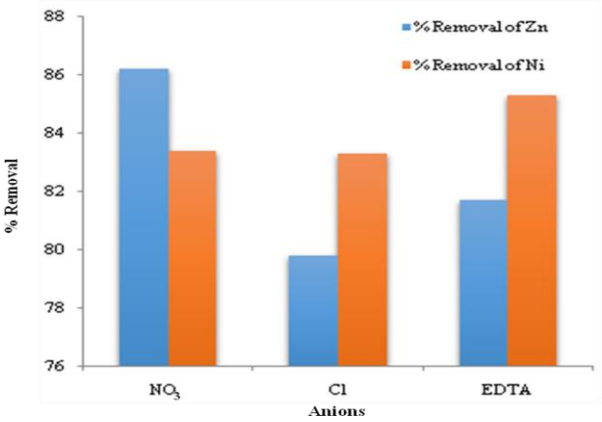

Figure 5(b). The effect of anions on Zn And Ni adsorption.

E. IR spectrum of Aspergillus flavus

FTIR (Fourier Transform Infra-red Spectroscopy) was performed, the obtained values were found to be similar with standard values of FTIR for functional group. Values were confirmed by plotted graphs.

The absorbance spectrum of *Aspergillus flavus* with that loaded with Zn is shown in Fig. 6(a). Some intense characteristic bands were obtained from the functional groups presented in proteins and polysaccharides. The intense peak at 3360.36 cm^{-1} was caused by the hydroxy stretching of carboxylic groups and also stretching of amido group. The strong peaks at 1633.41 cm^{-1} and 1279.54 cm^{-1} were assigned to C=O and sulphate, respectively. The peaks at 1279.54 cm^{-1} and 1062.26 cm^{-1} were due to C–O existence. Some bands in fingerprint region could be attributed to the phosphate groups. After adsorbing Zn, the peaks at 3360.36 cm^{-1}, 1633.41 cm^{-1} and 1279.54 cm^{-1} reduced to 3350.36 cm^{-1}, 1630.40 cm^{-1} and 1270.54 cm−1, respectively, which suggested amido, hydroxy, C=O and C–O could combine intensively with Zn [24].

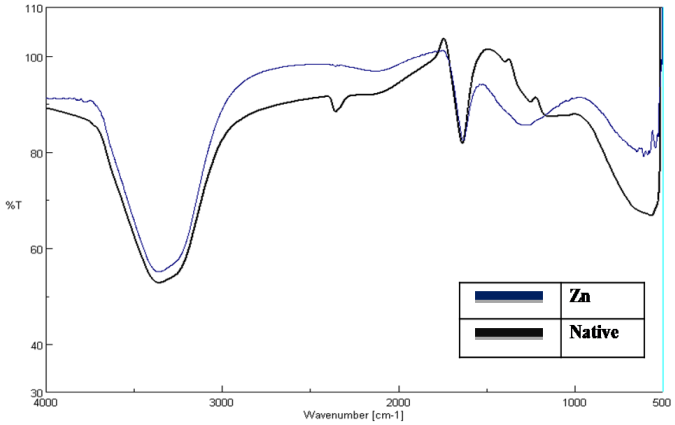

Figure 6(a). IR spectrum of *Aspergillus flavus* (top: fungal biomass loaded with Zn; bottom: native fungal biomass). There was overlap between only biomass (native) and biomass + Zn.

The absorbance spectrum of *Aspergillus flavus* with that loaded with Ni is shown in Fig. 6(b). Some intense characteristic bands were obtained from the functional groups presented in proteins and polysaccharides. The intense peak at 3355.53 cm^{-1} was caused by the hydroxy stretching of carboxylic groups and also stretching of amido. The strong peaks at 1629.55 cm^{-1} and 1400.07 cm^{-1} were assigned to C=O and sulphate, respectively. The peaks at 1320.04 and 1182.15 cm^{-1} were due to C–O existence. Some bands in fingerprint region could be attributed to the phosphate groups. After adsorbing Ni, the peaks at 3355.53 cm^{-1}, 1629.55 cm^{-1}, and 1400.07 cm^{-1} reduced to 3350.31 cm^{-1}, 1628.41 cm^{-1} and 1382.59 cm^{-1}, respectively, which suggested amido, hydroxy, C=O and C–O could combine intensively with Ni [24].

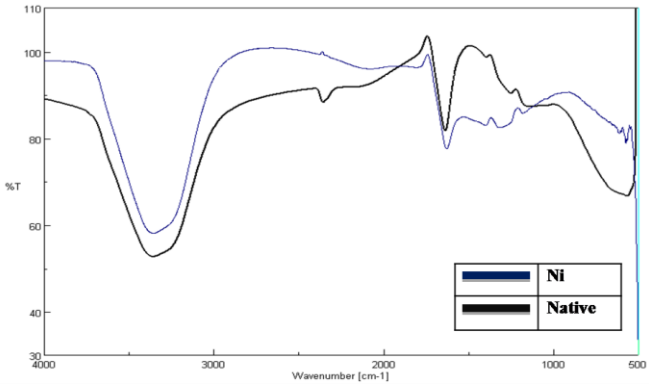

Figure 6(b). IR spectrum of *Aspergillus flavus* (top: fungal biomass loaded with Ni; bottom: native fungal biomass). There was overlap between only biomass (native) and biomass + Ni.

IV. Conclusion

This study indicated that the dead fungus *Aspergillus flavus* can be efficient biosorbent material for the removal of Zn and Ni. The maximum biosorption capacityfor Zn was found to be 47.36% at room temperature, at pH 6.5, with biomass concentration of 2g/L having contact time of 50 minutes and solution concentration of 2ppm.The maximum biosorption capacity for Ni was found to be 61.60% at room temperature, at pH 5, with biomass concentration 2g/L having contact time of 60 minutes and solution concentration of 2ppm. No effect was found by cations and anions except EDTA on the uptake of Zn and Ni. It was found that EDTA can recover 81% Zn

and 85% Ni. Desorption experiment proved that 0.1M HCl was found to be an efficient desorbent for the recovery of Zn and Ni.

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