STUDIES ON BIOSORPTION OF NICKEL USING IMMOBILIZED FUNGUS, RHIZOMUCOR TAURICUS

K. Kishore Kumar,*a M. Krishna Prasad,b B. Sarada,c and Ch. V. R. Murthy a

Rhizomucor tauricus, an industrial fungus, was immobilized in sodium alginate and used as adsorbent for the removal of nickel from aqueous solutions. The biosorption capacity of Ni(II) was found to be 394 mg/g of immobilized biomass. It was observed that an increase in pH from 3 to 6 increased the percent adsorption, and an increase in liquid-to-solid ratio from 2 to 10 increased the metal uptake. The percent adsorption was increased when increasing the initial metal concentration from 25 to 100 mg/L. The equilibrium biosorption data was evaluated by Langmuir, Freundlich, and Langmuir-Freundlich (L-R) isotherm models, and was best described by the Langmuir and Freundlich isotherms. FTIR analysis revealed that –NH (bending), C–H (stretching), C=O (stretching), and –OH functional groups were mainly responsible for Ni(II) biosorption. Thus, this study demonstrated that the immobilized Rhizomucor tauricus biomass could be used as an adsorbent for the treatment of Ni(II) from aqueous solution.

Keywords: Rhizomucor tauricus; Fungus; Immobilization; Isotherms; FTIR

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INTRODUCTION

Water pollution by heavy metals is very toxic in nature and eventually accumulates throughout the food chain, causing serious threat to mankind. These toxic metals can cause accumulation poisoning, cancer, brain damage, and renal malfunctioning when found above the tolerance limit. Pollution by nickel is of considerable concern because the metal has found widespread use in electroplating, metal finishing, etc. The presence of Ni(II) ions above critical levels may cause various types of acute and chronic disorder in human health, such as severe damage of lungs and kidney, gastrointestinal distress (e.g. nausea, vomiting, and diarrhea), pulmonary fibrosis, renal edema, and skin dermatitis. The toxic limit of Ni(II) as insoluble compounds of Ni is 1 mgm$^{-3}$, soluble compounds of Ni 0.1 mgm$^{-3}$, nickel carbonyl of 0.05 to 0.12 mgm$^{-3}$, and nickel sulphide of 1 mgm$^{-3}$ (Lothongkum et al. 2009; Pahlavanzadeh et al. 2010).

Biosorption refers to metal sequestration by biomass through adsorption, ion-exchange, coordination, complexation, etc. Application of fungal biomass to remove heavy metals from industrial wastewater is economically valuable, technically sound, socially acceptable, and is attractive for industry. Many fungal species, such as Aspergillus niger (Kapoor et al. 1999; Srivastava and Thakur, 2005; Park et al. 2005),

Kishore et al. (2012). “Ni(II) biosorption by R. tauricus,” BioResources 7(4), 5059-5073. 5059
Saccharomyces cerevisiae (Lin et al. 2005), Mucor hiemalis (Tewari et al. 2005), Botrytis cinerea (Akar and Tunali 2005), Neurospora crassa (Tunali et al. 2005), Lentinus sajor-caju (Bayramoglu et al. 2005), and Phanerochaete chrysosporium (Iqbal and Asma 2007) have been extensively studied for heavy metal biosorption.

Natural polymers such as alginate, chitosan, chitin, and cellulose derivatives have been used as the matrix for the immobilization of microbial cells via an entrapment technique. A major advantage of alginate gel entrapment is that immobilized cells do not suffer extreme physical-chemical condition changes during the immobilization process. Permeability, null toxicity, and transparency of the formed matrix imply a very gentle environment for immobilized cells (Smidsrød and Skjåk-Braek 1990; Kumar et al. 2006).

Immobilized fungal cells have been found to be far more stable during experimentation in the batch system than the free fungal cells. Several researchers reported immobilized fungal biomass for biosorption of heavy metal ions, using calcium alginate, such as Trametes versicolor (Bayramoglu et al. 2003), Rhizopus arrhizus (Liao et al. 2004), Tetraselmis chuii (Moreno-Garrido et al. 2005), and Rhizomucor tauricus (Kumar et al. 2009).

Some of the workers used dead fungal biomass, eliminating the need for nutrient use. Fungal biomass varieties can also be used as biosorbents, such as Botrytis cinerea (Akar and Tunali 2005), Mucor hiemalis (Tewari et al. 2005), and S. cerevisiae (Lin et al. 2005; Murthy et al. 2007). The -COOH, -NH₂, -OH, and -SH groups on microbial cell walls provide the binding sites for interaction of metal ions (Kuyucak and Volesky 1998). Rhizomucor tauricus was chosen as biosorbent material because it is a waste industrial fungus that removes Ni(II) ions from aqueous solution.

**EXPERIMENTAL**

**Materials**

**Immobilization of Rhizomucor tauricus**

The fungus Rhizomucor tauricus MTCC 1976 was purchased from MTCC, Chandigarh. R. tauricus was produced by standard culture technique using potato dextrose broth and incubated for 72 hours at 30 °C. The biomass was filtered through a vacuum filtration unit to separate the unused nutrient broth. It was rinsed twice, filtered through the vacuum filtration unit, and resuspended in distilled water. Sodium alginate (4%) solution was prepared in hot distilled water at room temperature.

Pre-determined weighed biomass (wet weight) in alginate solution was mixed thoroughly with a magnetic stirrer. The uniform mixture of fungus and sodium alginate solution (2%) was pumped through the peristaltic pump into the 0.5 M calcium chloride dihydrate solution. The beads were stored at 4 °C overnight for curing with 0.25 M calcium chloride dihydrate solution. Beads (4 mm) were washed twice with distilled water to avoid the excess calcium chloride dihydrate; these beads were used for equilibrium studies.
Methods

Biosorption experiments

The primary stock solution was prepared with approximately 2000 ppm of Ni(II) in solution. From the stock solutions, experimental test solutions were prepared by diluting the primary stock solution with de-mineralized water. Samples of 100 mL of nickel solution of known concentration were taken in 250 mL conical flasks, and 25, 12.5, and 10 mL of immobilized beads were added at a constant agitation speed (160 rpm) using an orbital shaker (Kemi make).

The volume of immobilized biomass was taken by replacement method at a known wet weight of biomass. The flasks were placed in an orbital shaker for a definite amount of time. The required pH was adjusted with 0.1 N hydrochloric acid and 0.1 N NaOH.

Analytical procedure

The concentrations of unadsorbed nickel ions in the sample supernatant liquid were determined using an atomic absorption spectrophotometer (Perkin Elmer model AA200) with an air acetylene flame at the wave number 232 nm. \( C_A \) and \( C_S \) were then calculated and tabulated for subsequent analysis of the data. The metal uptake (\( C_S \)) was calculated using the general definition,

\[
C_S = \frac{V(C_T - C_A)}{M}
\]  

where \( C_S \) is the metal uptake of Ni(II) g\(^{-1}\) biomass, \( V \) is the volume of metal-containing solution in contact with the biosorbent in mL, \( C_T \) and \( C_A \) are the initial and equilibrium (residual) concentration of metal in the solution mg L\(^{-1}\), respectively, and \( M \) is the amount of added biosorbent on wet basis in g.

The metal % of removal by \( R. \) tauricus was determined by Equation 2 as follows,

\[
R(\%) = \frac{C_T - C_A}{C_T} \times 100
\]  

where \( R \) is the percentage of Ni(II) adsorbed by biomass, \( C_T \) is the initial concentration of metal ions in mg L\(^{-1}\), and \( C_A \) is the concentration of metal ions at time \( t \) in mg L\(^{-1}\).

FTIR analysis

The powdered biomass before adsorption and after adsorption was air dried, and the moisture was removed completely at 60\(^\circ\)C with a humidity control oven. The powder was analyzed by Fourier transform infrared spectrophotometer by potassium bromide (KBr) pellet method in the wave number range of 400.00 cm\(^{-1}\) to 4000.00 cm\(^{-1}\) (Perkin-Elmer make serial No. 72425).
RESULTS AND DISCUSSION

Effect of Equilibrium Time

The time required to reach equilibrium metal concentration was first estimated (Fig. 1) and found to be 3½ hours. All the experiments were conducted for 4 hours to ensure the attainment of equilibrium. The effect of different process variables, such as weight of biomass, metal concentration, pH effect, and L/S ratio on equilibrium adsorption was studied.

![Figure 1](image1.png)

**Fig. 1.** Time vs. concentration of Ni\(^{2+}\) on *Rhizomucor tauricus* beads

Effect of pH

Figure 2 reveals that metal uptake increased with increasing pH within the range 3<pH<6. This behavior is probably due to preferential adsorption of hydrogen ion at low pH when compared to metal ion. The low level of Ni(II) uptake at lower pH values could be attributed to the increased concentration of hydrogen (H\(^+\)) and hydronium (H\(_3\)O\(^+\)) ions competing for Ni(II) binding sites on the biomass.

![Figure 2](image2.png)

**Fig. 2.** Variation of metal uptake with pH at different Initial metal load
The increase in Ni(II) biosorption at higher pH values (5 to 6) may be explained by the ionization of functional groups on the cell surface that serve as the binding sites related to the isoelectric point of the cells (Aksu et al. 2002). Heavy metal ions such as Ni(II) have a strong affinity for proteins of the cell wall. At pH values above the isoelectric point, there is a net negative charge on the cell surface, and the ionic state of ligands such as carboxyl, phosphoryl, sulphydryl, hydroxyl, and amino groups will be such so as to promote reaction with the metal cations. The same results were shown in the case of fungus A. niger (Kapoor and Viraraghavan 1998).

**Effect of Initial Metal Concentration**

The adsorption of Ni(II) by immobilized *R. tauricus* biomass was studied at different Ni(II) ion concentrations ranging from 25 to 100 mg L$^{-1}$. The percent adsorption decreased with increasing initial Ni(II) ion concentration. This reduction may be due to in unit biosorption of metal ion with the enhanced metal concentration. The findings indicate that the biosorbent is effective in dilute metal solutions. These results may be explained by the fact that the ratio of sorptive surface of fungal biomass to Ni(II) availability is high, hence Ni(II) may interact with the biosorbent and then is removed. Figure 3 shows the variation of percent adsorption with total metal concentration. It clearly indicated that the percent adsorption decreases with an increase in total metal concentration. The same results were reported on immobilized biomass of *Rhizopus arrhizus* for chromium (VI) biosorption (Prakasham et al. 1999).

**Effect of Liquid/Solid Ratio**

The L/S ratio of immobilized biomass loading was studied with Ni(II) from 2 to 10, while other variables were kept constant. The volume of biomass beads significantly influenced the extent of Ni(II) biosorption; increase in the biomass quantity decreased the metal uptake. Variation of metal concentration in the immobilized beads with aqueous metal concentration at different pH values is shown plotted in Fig. 4 at 30 °C.
It can be observed that an increase in the L/S ratio to 10 increased the metal uptake. This trend could be explained as a consequence of partial aggregation of biomass at higher biomass bead volume, which results in the decrease in effective surface area for the biosorption (Karthikeyan et al. 2007). Therefore the optimum L/S ratio was 10, and all the experiments were conducted (Fig. 5) with L/S ratio of 10.

**Effect of Temperature**

The temperature of the process also influenced the adsorption capacity. The temperature range, 10 to 50 °C (10, 30, and 50 °C), was studied with the L/S ratio of 10, while other variables were kept constant. While an increasing trend of adsorption was observed with increasing temperature from 10 ± 1 °C to 30 ± 1 °C, the percent adsorption
of Ni(II) on immobilized *R. tauricus* biomass did not change substantially when temperatures rose higher than 30 ± 1 °C.

**Fig. 6.** Effect of temperature on the adsorption of Ni(II) at pH 6

The percentage of adsorption was little changed between the temperatures of 30 ± 1 °C and 50 ± 1 °C. Increases in percent adsorption with temperature may be attributed to either an increase in the number of active surface sites available for adsorption on the adsorbent or a decrease in the thickness of the boundary layer surrounding the adsorbent with temperature, so that the mass transfer resistance of adsorbate in the boundary layer decreases. The same results were drawn in the case of *P. chrysosporium* (Iqbal and Edyvean 2004) and *R. oligosporus* (Duygu et al. 2008); that is the increase in metal uptake at increased temperature is due to either higher affinity of sites for the metal or an increase in binding sites on relevant biomass.

**Thermodynamic Parameters**

Gibbs Free Energy $\Delta G^0$ is the basic criterion for deciding whether the chemical process does occur/proceed or not. The spontaneity of the reaction can also be judged by the sign and magnitude of $\Delta G^0$. To design any chemical process system, one should have the knowledge of changes that are expected to occur during a chemical reaction. The rate and extent of changes are more informative in the design of process equipment.

In view of the above, analysis has been carried out on the effect of thermodynamic parameters on the biosorption of Ni(II) on *R. tauricus*. The thermodynamic parameters such as changes in standard free energy change $\Delta G^0$, enthalpy $\Delta H^0$, and entropy $\Delta S^0$ for any given adsorption process could be determined from the following equations:

$$\Delta G^0 = -RT\ln K_c$$

(3)

where $\Delta G^0$ is the free energy change, expressed as J/mol. $K_c$ is the apparent equilibrium constant for the process. $K_c$ can be derived from
where $C_S/C_a$ can be defined as the 'adsorption affinity'.

The enthalpy changes ($\Delta H^0$) and entropy changes ($\Delta S^0$) for the adsorption process for Ni(II) using immobilized Rhizomucor tauricus for all the initial metal concentrations in the aqueous solutions were obtained from the plot of log $(C_S/C_a)$ versus $1/T$ (Fig. 7), and the calculated thermodynamic data are compiled in Table 1.

![Graph](image)

**Fig. 7.** Vant Hoff plot for adsorption of Ni (II) on *R. tauricus* at various initial metal concentrations

<table>
<thead>
<tr>
<th>$C_0$ (mg/L)</th>
<th>$\Delta H^0$ (KJ/mol)</th>
<th>$\Delta S^0$ (J/mol*K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>-9.43188</td>
<td>-17.0027</td>
</tr>
<tr>
<td>45</td>
<td>-8.72152</td>
<td>-17.7303</td>
</tr>
<tr>
<td>90.2</td>
<td>-8.15668</td>
<td>-17.0027</td>
</tr>
</tbody>
</table>

The enthalpy and entropy changes were calculated as -9.431 KJ mol$^{-1}$ and -17.002 J mol$^{-1}$ K$^{-1}$, respectively, for *R. tauricus* at various initial Ni (II) concentrations. The free energy values increased positively with an increase in temperature for the adsorption of Ni(II) with biosorbents. The spontaneity of the biosorption process decreases with increasing temperature. The negative $\Delta H^0$ values indicated an exothermic nature of the...
adsorption. The negative values of $\Delta S^0$ suggested a decrease in the randomness at solid/solution interface during the adsorption of Ni(II) ions onto immobilized *Rhizomucor tauricus* (Ahmet and Mustafa 2008). Another thermodynamic parameter, standard free energy change $\Delta G^0$, was also calculated at various temperatures of the process. In all cases, large negative values of $\Delta G^0$ for biosorbent indicate the spontaneity of the biosorption process at a given temperature (Table 2).

**Table 2. Gibbs Free Energy Change for Ni (II) Biosorption on *R. tauricus***

<table>
<thead>
<tr>
<th>Temp. (°K)</th>
<th>$\Delta G^0$ (KJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>283</td>
<td>-4.62</td>
</tr>
<tr>
<td>293</td>
<td>-4.450</td>
</tr>
<tr>
<td>303</td>
<td>-4.280</td>
</tr>
</tbody>
</table>

**Equilibrium Isotherms**

*Freundlich isotherm*

The Freundlich adsorption isotherm was proposed by Boedecker and was later modified by Freundlich (1926). The Freundlich adsorption equation can be written as:

$$C_S = K_f C_A^{n_f}$$

Taking the logarithm on both sides, one obtains

$$ln q_e = ln K_f + \frac{1}{n_f} ln C_e$$

where $q_e$ is equilibrium adsorption capacity (mg g$^{-1}$), $C_e$ is the equilibrium concentration of the adsorbate in solution, and $K_f$ and $n_f$ are constants related to the adsorption process such as adsorption capacity and intensity, respectively. The plots in Fig. 8 give the Freundlich isotherm for biosorption of Ni(II) onto immobilized *R. tauricus*. The values of $n_f$ and $K_f$ are found to be 0.737 and 11.26, respectively, at pH 6, and values at pH 5 and 3 were also calculated and shown in Table 3. The values of the constants were obtained by analyzing the data graphically. Figure 8 shows that the data were well fitted to the Freundlich equation. The constant $n$ is found to be independent of pH, while the constant $K_f$ varies with pH. This is also suggestive that the metal ion under study could well be separated from its aqueous solution with high adsorption capacity.

*Langmuir isotherm*

The most widely used isotherm equation for modeling equilibrium is the Langmuir equation, which is valid for monolayer sorption onto a surface with a finite
number of identical sites. A basic assumption of the Langmuir theory is that sorption takes place at specific homogeneous sites within the adsorbent.

This model can be written in non-linear form (Langmuir 1918), and it is represented by the equation,

\[
\frac{C_e}{q_e} = \frac{1}{q_m b} + \frac{C_e}{q_m} \tag{8}
\]

where \(q_m\) is the maximum amount of the metal ion per unit weight of adsorbent to form a complete monolayer on the surface bound at high \(C_A\) (mg/g), and \(b\) is a constant that accounts for the affinity of the binding sites (L/mg). \(q_m\) represents the limiting adsorption capacity when the surface is fully covered with metal ions and helps in the evaluation of adsorption performance, particularly in cases where the sorbent did not reach its full saturation during contact. From the plots between \((C_e/q_e)\) and \(C_e\), the slope \((1/q_m)\) and the intercept \((1/b)\) can be calculated.

![Fig. 8. Freundlich plot for Ni\(^{2+}\) on Rhizomucor tauricus beads at different pH values](image)

The Langmuir adsorption constants evaluated from the isotherms at different temperatures with correlation coefficients are presented in Table 3. From the Langmuir isotherm for cadmium, the biosorption affinity constants \(b\) and maximum capacity \((q_m)\) to form a complete monolayer on to the surface of the \(R.\ tauricus\) biomass at 25 °C were estimated as 0.00780 (L mg\(^{-1}\)) and 622.20 mg g\(^{-1}\), respectively, with \(R^2\ 0.993\) indicating the present sorption data could be best represented by the Langmuir model. The higher adsorption capacity, \(q_m\ (\gg 1)\) indicated a strong electrostatic force of attraction.

**Langmuir-Freundlich isotherm**

The Langmuir-Freundlich isotherm equation is characterized by the heterogeneity factor, \(n\), and employed to describe heterogeneous systems. The isotherm can be expressed as Equation 9,

\[
C_s = \frac{K_{LF} C_A^n}{1 + a_{LF} C_A^n} \tag{9}
\]
where $K_{LF}$ is the Langmuir-Freundlich constant (Lmg$^{-1}$), $a_{LF}$ is the affinity coefficient (Lmg$^{-1}$), and $n$ is the heterogeneity coefficient. At low concentrations it effectively reduces to the Freundlich isotherm and thus does not obey Henry’s law.

### Table 3. The Constants Obtained From the Isotherm Models at Different Initial pH Values

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Isotherm</th>
<th>Parameter</th>
<th>pH 3</th>
<th>pH 5</th>
<th>pH 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Experimental</td>
<td>$q_{\text{max}}$ (mg g$^{-1}$)</td>
<td></td>
<td></td>
<td>394</td>
</tr>
<tr>
<td>2</td>
<td>Freundlich</td>
<td>$N$</td>
<td>0.813</td>
<td>0.744</td>
<td>0.737</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$K_f$ (mg g$^{-1}$)</td>
<td>3.130</td>
<td>8.577</td>
<td>11.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R^2$</td>
<td>0.960</td>
<td>0.970</td>
<td>0.998</td>
</tr>
<tr>
<td>3</td>
<td>Langmuir</td>
<td>$q_{\text{max}}$ (mg g$^{-1}$)</td>
<td>493.66</td>
<td>622.20</td>
<td>730.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$b$ (mg$^{-1}$)</td>
<td>0.0036</td>
<td>0.00629</td>
<td>0.00780</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R^2$</td>
<td>0.955</td>
<td>0.9670</td>
<td>0.993</td>
</tr>
<tr>
<td>4</td>
<td>Langmuir-Freundlich</td>
<td>$a_{LF}$</td>
<td>-2.6874</td>
<td>-79.365</td>
<td>-476.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$K_{LF}$</td>
<td>-0.8615</td>
<td>-0.2206</td>
<td>-0.0351</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$n$</td>
<td>0.028</td>
<td>0.278</td>
<td>0.543</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R^2$</td>
<td>0.978</td>
<td>0.987</td>
<td>0.999</td>
</tr>
</tbody>
</table>

At high sorbate concentrations, it predicts a monolayer sorption capacity characteristic of the Langmuir isotherm. $K_{LF}$, the Langmuir-Freundlich constant (Lmg$^{-1}$), $a_{LF}$, the affinity coefficient (Lmg$^{-1}$), and $n$, the heterogeneity coefficient, at pH 6 was -0.0351, -476.19, and 0.543, respectively with an $R^2$ value 0.999. Nonlinear regression analysis was carried out in Sigma Plot 8.0 in order to determine the $K_f$ and $n$ values for various pH, which were then tabulated in Table 3. The adsorption capacity of Ni(II) on *R. tauricus* is 394 mg g$^{-1}$ which is higher than the immobilized hyphal cells of the fungus *Polyporus squamosus* (Wuyep et al. 2007) which is 131.092 mg g$^{-1}$ entrapped in the same matrix.

### FTIR Spectroscopic Analysis

The FTIR spectroscopic analysis of the biomass, dead powder of *R. tauricus* before adsorption of heavy metal ions (Fig. 9), indicated a broad adsorption band at 3423.28 cm$^{-1}$, representing $–$NH stretching (Singh et al. 2010; Venkata Subbaiah et al. 2010); 2925.21 cm$^{-1}$ and 2854.16 cm$^{-1}$ represented $–$CH$_2$ stretching. The absorption band at 1742.68 cm$^{-1}$ could be attributed to a C=O group of carboxylic acid (Lalhruaitluanga et al. 2010), and the absorption band at 1417.14 cm$^{-1}$ represents a carboxylate group. Further, the band at 1180.53 cm$^{-1}$ indicates an $–$OH group in sugars, and the bands at 1076.53 cm$^{-1}$ and 1031.88 cm$^{-1}$ represent amide C-N stretching and $–$P=O stretching, respectively.
In all the FTIR spectroscopic analysis of the biomass, dead powder of *R. tauricus* after adsorption of heavy metal ions (Fig. 9) exhibited shifts of peaks at 3423.23 cm\(^{-1}\), 1742.68 cm\(^{-1}\), and 1180.53 cm\(^{-1}\), indicating that the amide –NH bending, CH stretching, carboxylic acid, and hydroxylic groups are the main functional groups involved in the complexation of metal ions.

![FTIR Spectra](image)

**Fig. 9.** FTIR of *Rhizomucor tauricus* a) before treatment, b) after treatment with Ni(II)

**CONCLUSIONS**

1. It was observed that the increase in pH and biomass concentration increases the metal adsorption.
2. The biosorption capacity of Ni(II) was found to be 394 mg/g of immobilized biomass.
3. The equilibrium data were well fitted to Freundlich and Langmuir type adsorption isotherms. The adsorption data were represented by adsorption distribution coefficient (ratio of metal concentration in solid immobilized beads to that in aqueous solution) as a function of aqueous metal concentration, pH, and biomass concentration in immobilized beads.
4. Free energy change (\(\Delta G\)) with negative sign reflects the feasibility and spontaneous nature of the process. The negative enthalpy values indicate an exothermic nature and negative entropy value point toward a decrease in randomness at solid liquid interface.
5. In the biosorption process, amide –NH (bending), CH (stretching), carboxylic acid, and hydroxylic groups were involved.
6. *Rhizomucor tauricus* may be used as efficient adsorbent to remove nickel (II) from effluents.
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