

Removal of Ni (II) and Cu (II) from their Solutions and Waste Water by Nonliving Biomass of *Pseudomonas oleovorans*

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Abstract

The nonliving biomass of *Pseudomonas oleovorans* was used for the removal of Nickel and Copper from their single and binary solutions. The optimum conditions such as pH, initial metal ion concentration, contact time and biomass dose were determined experimentally. q_{max} and 'b' values for Ni(II) were 95.6 mg/g and 0.010 and for Cu(II) 137.5 mg/g and 0.008 respectively. The removal of the metals was studied in binary metal systems also. The developed method was applied for the removal of the metals from the wastewater samples. The investigations on the speciation of the metals revealed that both Ni and Cu exist as hydrated Ni (II) and Cu (II) ions and precipitates of Ni(OH)₂ and Cu(OH)₂ at different pH ranges.

Keywords: Pretreatment; pH; Time; Langmuir isotherm; Binary; Stability.

Introduction

The removal of toxic metal contaminants from wastewater is one of the most important environmental concerns being faced by many countries these days. Although this issue has been addressed for many years, effective treatment options are limited. Chemical precipitation, ion exchange, reverse osmosis, and solvent extraction are the most commonly used procedures for removing metal ions from dilute solutions [1]. However these methods are not appreciably successful at low metal concentrations often encountered in wastewater. Biosorption, the process of passive cation binding by dead or living biomass, represents a potentially cost-effective way of eliminating toxic heavy metals from industrial wastewater. The abilities of bacterial masses to remove metal ions in solutions have been studied by different workers [2-4] because of their high surface to volume ratio and high content of potentially active chemisorption sites. As single toxic metallic species rarely exists in natural and waste waters, any approach that attempts to remove heavy metals from multi-component systems using bacteria will be of great use. The present study was undertaken to probe the potential of nonliving biomass of *Pseudomonas oleovorans* (*P.oleovorans*) to remove nickel and copper by biosorption process from single metal ion and binary metal ion systems and from the waste water. *P.oleovorans* is a gram-negative bacterial species whose cell wall contains peptidoglycan, lipopolysaccharide and protein [5] which make available donor atoms to form bonds with metals. It was also aimed to investigations on the speciation of the metals in the solution at different pH ranges were made on the basis of biosorption process.

Materials and Methods

Growth of bacterial biomass

Culture of *Pseudomonas oleovorans* (MTCC-617) was procured from microbial type culture collection and gene bank, Institute of Microbial Technology (CSIR) Chandigarh, India. Revival of culture was done by growing the species on prescribed growth medium (Composition-nutrient agar). The medium was prepared, autoclaved and allowed to cool. Loop full of the bacterial culture was taken and streaked on the petriplates containing prescribed medium in laminar air flow chamber. The inoculated plates of *P.oleovorans* were incubated

at 25°C for 24 hours. Biomass of the species was separated from the medium by centrifugation and was dried in hot air oven at 60°C.

Pretreatment of nonliving biomass of *P.oleovorans*

10 g of the harvested biomass of the bacterial species was treated with 100 mL of 0.1 N NaOH for 1 hour. The pretreated biomass was centrifuged and the residue of the biomass was taken in a 100 mL beaker and was washed for 3-4 times with double distilled water and again centrifuged. The centrifuged biomass was dried at 60°C for 24 hours in hot air oven.

Solutions

The aqueous solutions of metal ions were prepared by using AnalR grade chemicals in double distilled water. Stock solutions of nickel and copper ions each of 1000 ppm concentration were prepared by taking nickel chloride and copper sulphate respectively. From these stock solutions, the solutions of desired concentrations were prepared by diluting with double distilled water. Desired pH values (measured by systronics digital pH meter) of different systems were adjusted by adding 0.1 N NaOH and 0.1 N HCl. Binary metal ion solutions of nickel and copper were prepared by taking metal ion concentrations in 1:1 ratio. Study on the speciation of metals was made by changing the pH of the metal ion solutions in contact with the biomass during biosorption experiments. The waste water samples were collected from two sites; from Tawa River at a distance of three kilometers from coal mines, Pathakhara, Sarni, District Betul discharging effluents in the river and from the outlet of upper Lake of Bhopal, India.

Apparatus

Metal ion concentrations were determined using Perkin-Elmer

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Instruments, Shelton, USA, Model A analyst 100 with air – acetylene flame (AAS). The wave lengths used for nickel and copper were 232.0 nm and 324.8 nm respectively.

Biosorption experiments

To study the effect of pH (2.0-10.0), biomass dose (40-240 mg in 100 mL solution), contact time (20-120 min) and initial metal ion concentration (25-200 mg/L), the biosorption experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL solution of the metal ion of varying concentrations and different amounts of nonliving biomass of *P.oleovorans*. In each experiment, one of the conditions was changed while other conditions were kept constant. The flasks were agitated on an incubator shaker (Innova 4230, New Brunswick Scientific, USA) at a constant speed of 150 rpm for different time periods. The temperature during experiments was 30°C. It is to be added here that in case of temperature contrasting results have been obtained regarding its effect on biosorption by different biomasses. However, the temperature does not seem to influence the biosorption process in the range of 20°C-35°C [6]. Also, biosorption process is usually not operated at high temperatures because it will increase the operational cost [7].

After each interaction of metal ion with the biomass at a predetermined time period, the matrix was centrifuged and the amount of metal in the supernatant was determined by AAS technique. Before measurement, the supernatants of each metal were appropriately diluted with double distilled water to ensure that the metal concentration in the sample was linearly dependent on the absorbance. Biosorption experiments were conducted in duplicate and average values were used in the analysis. The biosorbent amount was calculated as follows:

$$q = V (C_i - C_f) / m$$

Where q is the metal uptake (mg metal per gram biosorbent), V the liquid sample volume (mL), C_i the initial concentration of the metal in the solution (mg/L), C_f the final (equilibrium) concentration of the metal in the solution (mg/L) and m is the amount of the added dried biosorbent (mg).

The equilibrium of the biosorption process is often described by fitting the experiment points with models such as Langmuir and Freundlich isotherm models [2]. In the present study only Langmuir model [8] which works for monolayer adsorption has been used.

The Langmuir equation can be given as

$$q = \frac{b C_f q_{\max}}{1 + b C_f}$$

On rearranging the above equation, we get

$$\frac{1}{q} = \frac{1}{b q_{\max}} + \frac{1}{C_f q_{\max}}$$

Where q is the amount of metal sorbed (mg/g), C_f is the final (equilibrium) concentration of the metal in solution (mg/L) and q_{\max} is the maximum amount of metal ion which can be taken up by the biosorbent (mg/g), 'b' is the Langmuir constant. The linear Langmuir plot was obtained by plotting $1/q$ vs $1/C_f$ on y and x -axis respectively. The values of q_{\max} and 'b' were calculated from the y-axis intercept and slope of the linear plot respectively. The value obtained for the q_{\max}

signifies the maximum absorption capacity (mg/g) and 'b' is a constant related to the affinity between the metal and the biomass.

Biosorption experiments on the binary system of nickel and copper

To study the binary system of copper and nickel, 100 mL mixture solution of the metal (concentration of each metal ion 100 mg/L) were treated with nonliving biomass of *P.oleovorans* at optimum conditions in respect of pH, contact time, biomass dose. Other steps followed were same as for single metal systems.

Application of the developed method for the determination of metals in wastewater samples

Samples of wastewater from two sites were collected in plastic container from 15 cm depth below the surface of wastewater in triplicate and mixed to get composite sample. The samples were preserved in refrigerator by adding 1.5 mL of concentrated HNO_3 to 1 L of waste water samples. The samples were digested with HNO_3 as per procedure recommended by APHA, AWWA, WFCF [9]. The digested water was filtered and the concentration of metal ion was determined by AAS. After that the developed method was applied to wastewater samples following optimum conditions and the analyses of results were made.

Metal speciation studies

The biosorption of the metals by the biomass depends on their chemical speciation which, in turn, depends on the change in pH range of the solutions. Thus, biosorption capacity can be used for determining the speciation of metals. The biosorption experiments carried out to investigate the effect of pH on biosorption capacity were used for studying the speciation of the metals in solutions.

Results and Discussion

Effect of pH

The pH of the solution has a very significant effect on metal ion solubility and surface charge of the biomass [10] which, in turn, influences the biosorption process. The nature of the curves (Figure 1) of Ni (II) and Cu(II) biosorption by *P.oleovorans* shows that in case of Ni(II), pH rises sharply from 2.0 to 5.0 and then becomes nearly constant from 5.0 to 7.0 After that, there is only slight increase in the biosorption. In case of Cu (II), the biosorption increases sharply from pH 2.0 to 4.0, then becomes nearly constant from pH 4.0 to 6.0 and after that there is only slight increase from pH 6.0 to 10.0. The less amount of biosorption at lower pH values is due to the fact that the cell wall of the bacterial biomass is closely associated with H_3O^+ and access of metal ions to cell wall would be obstructed as a result of repulsion. The metal uptake increases from pH 2.0-5.0 in case of Ni(II) and 2.0-4.0 in case of Cu(II). This is due to the fact that the more ligands with negative charge are exposed with the subsequent increase in attraction sites to positively charged metal ions [11]. After some pH range the sorption becomes higher due to the precipitation of hydroxide of metals which depend on the solubility products of their hydroxides (solubility products of Ni (OH)₂ and Cu(OH)₂ are 2×10^{-15} and 5×10^{-20} respectively) [12]. Therefore, at the same concentration of each metal ion the pH required for precipitation will fall in the order Ni > Cu which is in consonance with the results. It can be concluded that the percentage removal of Ni (II) and Cu (II) at high pH values is mainly due to simultaneous biosorption and precipitation [13].

Effect of biomass dose

Biomass dose of the species when varied from 40mg-240mg in 100mL metal ion solution of 100mg/L concentration, it was found that there was an increase in biosorption with the increase of biomass but at higher biomass dose the increase was marginal (Figure 2). The increase in percentage removal is due to the increase in surface area of the biomass and the availability of the adsorption sites [14]. The reason for the constancy of the removal after some point lies in the fact that the screen effect takes place amongst the dead cells which block the active sites of the cells by an increase of biomass [15]. On the basis of the above study, 0.1g of biomass in 100 mL solution of metal ions was chosen for further studies.

Effect of contact time

The effect of contact time on the biosorption of Ni (II) and Cu (II) is shown in Figure 3. It is observed that the biosorption increased with the time in the beginning but after 80 minutes contact time it remained constant for both the metal ions. It is known that the role of metal uptake is influenced by the factors affecting mass transfer from the bulk solution to binding sites [16]. In the present case, the experimental conditions allowed normal mixing of the metal ions and the biomass in the system which partially suppressed some kinetic factors leading to attainment of the equilibrium at a short time (80 min). However, to ensure the complete constancy of time 90 minutes time period was used for further studies.

Effect of initial metal Ion concentration

When 0.1g of biomass of *P.oleovorans* was treated with 100 mL solution of both the metals in different concentrations (25 mg -200 mg/L), percentage removal of each metal regularly decreased with the increase in metal concentration (Table 1). This is due to the fact that at lower concentration the metal ions are fully absorbed at the active sites and later only a few sites are available for absorption. For further studies 100 mg/L solution of both the metal ions was used with the intent of showing the usefulness of the metal for removal of metal ions from dilute solutions where classical methods fail.

For the quantification of the capacity of the biomass of *P.oleovorans* to remove the title metals from the aqueous solutions, the data of change in the amount of biosorption with initial concentration were used for calculating q (specific metal uptake in mg/g) values. The Langmuir [8] isotherms were drawn by plotting $1/q$ vs $1/C_r$. It was found that the

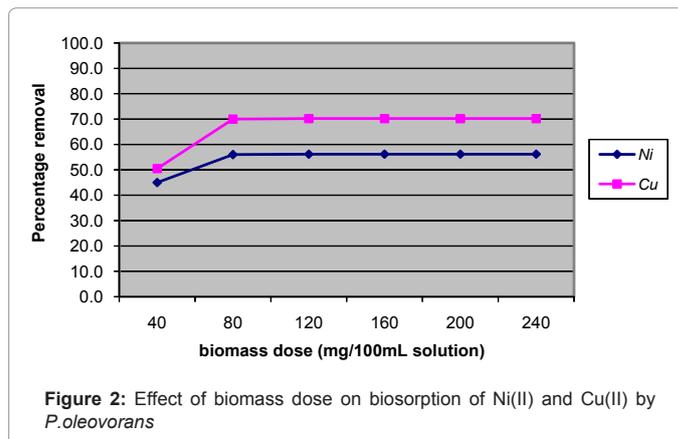


Figure 2: Effect of biomass dose on biosorption of Ni(II) and Cu(II) by *P.oleovorans*

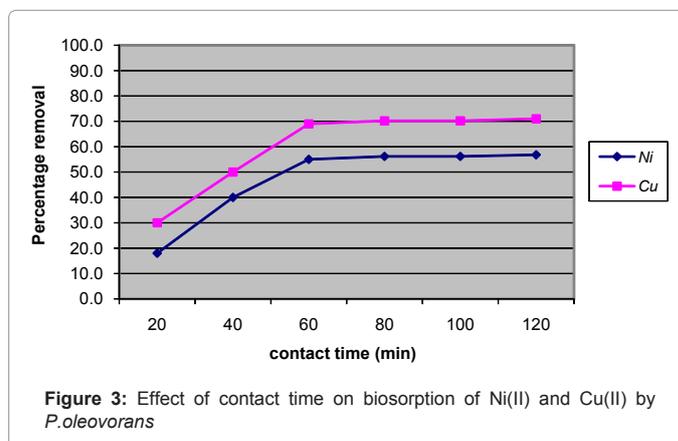


Figure 3: Effect of contact time on biosorption of Ni(II) and Cu(II) by *P.oleovorans*

Ni (II)		Cu (II)	
Initial concentration (ppm)	Percent removal	Initial concentration ppm	Percent removal
25	84.0	25	90.0
50	72.0	50	85.4
75	66.8	75	78.0
100	56.2	100	70.2
125	51.2	125	66.0
150	48.3	150	61.0
175	44.0	175	58.1
200	42.0	200	51.0

pH 6.0, temp 60°C, Contact time 90 minutes, biomass dose 0.1 g in 100 mL metal ion solution

Table 1: Effect of Initial concentrations of Ni (II) and Cu (II) on their Biosorption by *P.oleovorans*.

graphs were linear at low concentration range but deviated at high concentration it may be attributed to the use of Lineweaver-Burk [17] equation which is strongly biased towards fitting data in the low concentration range or to sites (such as $-\text{COO}^-$, OH^- , $-\text{NH}_2$) at surface not having equal affinity for adsorbate which is requirement for validity of Langmuir model [8]. q_{max} and 'b' values were calculated from the data related low concentration range (q_{max} and 'b' values for Ni are 95.6 mg/g and 0.010 and for Cu 137.5 mg/g and 0.008 respectively). The q_{max} value in case of Cu (II) is more than Ni (II) which shows that Cu (II) has more affinity for the biomass than Ni (II). This is in consonance with the Irving – Williams series [18] developed for the stability constants for the formation of complexes. The 'b' value for Cu (II) is lesser than

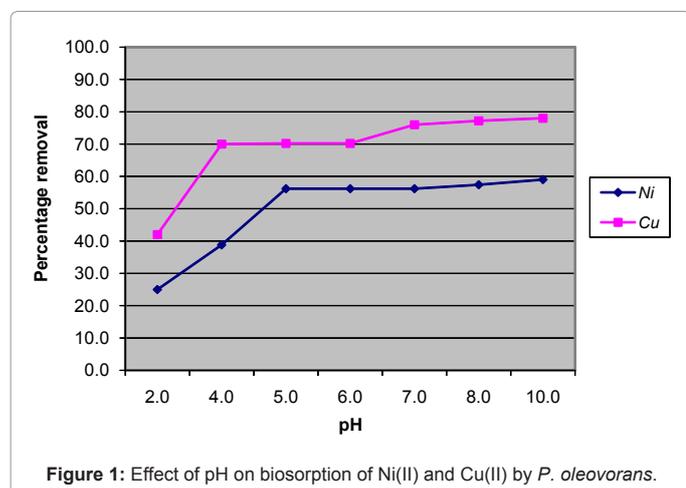


Figure 1: Effect of pH on biosorption of Ni(II) and Cu(II) by *P.oleovorans*.

that of Ni. This is expected as lesser value of 'b' shows more affinity of the metal for biomass resulting high q_{max} value of Cu (II) [19].

Biosorption of binary mixture of (Ni + Cu) by nonliving biomass of *P.oleovorans*

The industrial effluents generally include more than one metal. The presence of multi metals in the solutions affects the removal of a particular metal by absorption due to competitive interactions with the biomass of bacterial species. When binary solutions of Ni (II) and Cu (II) taken in 1 : 1 ratio (optimum concentration of each metal, 100 mg/L) were treated with 0.1 g of biomass of *P.oleovorans* under same optimum conditions as used in single metal systems, the percentage removal was 38.2 % for Ni (II) and 54.8 % for Cu (II) which in both the cases is lesser than the single metal ion systems [56.2 % for Ni (II) and 70.2 % for Cu (II)].

This decrease in percentage removal is due to increased competition between the same charged metals (+2) for binding sites of the biomass.

Application of the developed method for the removal of metals from wastewater

The proposed method was applied for the removal of Ni(II) and Cu (II) metals present in the wastewater samples collected from the Tawa River, Sarni, District Betul, and outlet of Upper Lake, Bhopal (India). The concentration of Ni and Cu in the sample determined following the procedure developed by APHA, AWWA, WPCF [9] were Ni 8.4ppm and Cu 4.64 ppm in Tawa river and Ni, NIL, Cu 1.6 ppm on the outlet of Upper Lake. The method could remove 62.0 % Ni and 65.0 % Cu from Tawa River and 63.6 % Cu from the outlet of Upper Lake (nickel being NIL in the upper lake)

Speciation analysis of Ni (II) and Cu (II) by the biosorption onto the biomass of *P.oleovorans*

The data of percent removal with the pH change were exploited for the speciation analysis of Ni and Cu metals in solution. From the Figure 1 it is clear that there is sharp increase in biosorption from pH 2.0 to 5.0 in case of Ni and from 2.0 to 4.0 in case of copper. After that pH range remains constant 5.0 to 7.0 for Ni and 4.0 to 6.0 for Cu. After that in both the cases again there is increase which is due to biosorption and precipitation of both the metals. From these data it can be concluded that in the beginning only hydrated metal ions are present and at higher pH values (7.0 for Ni and 6.0 for Cu), the hydroxide formation starts which increases the sorption. Therefore above pH 7.0 in case of Ni and 6.0 in case of Cu, hydroxides are also present.

Conclusion

The present study clearly indicates that at nearly normal optimum conditions, the nonliving biomass of *Pseudomonas oleovorans* can be used for the removal of Ni and Cu from their aqueous solution and waste water samples. The method can work satisfactorily for dilute solutions also. The optimum pH, biomass dose, contact time and initial metal ion concentration are 6.0, 0.1 g in 100 mL, 90 min, 100 mg/L respectively. Nickel and copper are found as their hydrated ions and hydroxides at different pH ranges.

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