Full Length Research Paper

Bioremediation of Ni(II) and Cu(II) from wastewater by the nonliving biomass of *Brevundimonas vesicularis*

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The nonliving biomass of *Brevundimonas vesicularis* was used for the biosorption of nickel and copper from their single and binary solutions after its pretreatment with 0.1 N NaOH. The optimum conditions, such as pH, initial metal ion concentration, contact time and biomass dose were determined experimentally. From the linear plot of Langmuir isotherm model, maximum absorption capacities (q_{max}) of the metal ion in mg/g of the biomass and 'b' (Langmuir constant) values were determined. The values of q_{max} and 'b' for Ni(II) and Cu(II) are 91.6 mg/g, 0.013 and 129.4 mg/g, 0.009, respectively. The removal of the metals was also studied in binary metal systems. The developed method was applied for the removal of the metals from the wastewater samples.

Key words: Pretreatment, pH, time, binary, stability, complexation.

INTRODUCTION

The removal of toxic metal contaminants from wastewaters 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 (Rich and Cherry, 1987). However, these procedures have signifi-cant disadvantages, such as incomplete metal removal, high reagent or energy requirements and generation of toxic sludge or other waste products that require disposal. These disadvantages are particularly apparent at low metal concentrations often encountered in waste-waters. 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 wastewaters. The ability of bacterial masses to remove metal ions in solutions was demonstrated in several studies by different workers (Gadd, 1998; Kang et al., 2005; Ozdemir et al., 2009; Chatterjee and Chandra,

2010; Pandiyan and Mahendradas, 2011) due to their high and fast sorption capacity. *Brevundimonas vesicularis* is a Gram negative bacterial species which has potential chemisorption sites, such as peptidoglycan, lipopolysaccharide and protein in its cell wall (Salton and Kim, 1996). Heavy metals known for their toxicity and non-biodegradability can lead to different ecological and health problems (Gadd, 1992). The most common adverse effect of nickel in humans is an allergy. People suffer from impaired lung functioning and chronic bronchitis if nickel is absorbed through food or water (Cempel and Nikel, 2006). Copper in high doses causes anemia, liver and kidney damages and stomach and intestinal irritation. Patients suffering from Wilson's disease are at greater risk for health effects from exposure to copper (ATSDR, 2002). Nickel and copper form highly stable complexes upon interaction with the species containing oxygen and nitrogen donor compounds. Therefore, the harmful effects of such heavy metals might be remediated by removing them from water bodies after their interaction with the nonliving biomasses, such as Gram negative bacteria which contain oxygen and nitrogen donor compounds. The present study was undertaken to probe the potential of the nonliving biomass of *B. vesicularis* to remove nickel

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and copper by biosorption process from single and binary metal ion systems and from the wastewater samples.

MATERIALS AND METHODS

Growth of bacterial biomass

Culture of *B. vesicularis* (MTCC-1933) was obtained from microbial type culture collection and gene bank, Institute of Microbial Technology (CSIR) Chandigarh, India. Revival of culture was carried out by growing the species on prescribed growth medium (Composition: rabbit blood agar) obtained from MTCC Chandigarh, India. The inoculated plates of *B. vesicularis* were incubated at 25°C for 48 h. 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 *B. vesicularis*

Harvested biomass (10 g) of the bacterial species was treated with 100 ml of 0.1 N NaOH for 1 h. The pretreated biomass was centrifuged and the residue of the biomass was taken in a 100 ml beaker and was washed for 3 to 4 times with double distilled water and again centrifuged. The centrifuged biomass was dried at 60°C for 24 h in hot air oven.

Solutions

Stock solutions of nickel and copper ions at 1000 mg/L concentration were prepared by dissolving nickel chloride and copper sulphate (AnalaR Grade chemicals, Delhi, India), respectively in double distilled water. From these stock solutions, the solutions of desired concentrations were prepared by diluting with double distilled water. Binary metal ion solutions of nickel and copper were prepared by dissolving metal ion concentrations in 1:1 ratio. The wastewater samples were collected from two sites: Tawa River at a distance of 3 km from coal mines, Pathakhara, Sarni, district Betul, discharging effluents in the river and from the outlet of Upper Lake of Bhopal, India. 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. Desired pH values (measured by systronics digital pH meter, Delhi, India) of different systems were adjusted by adding 0.1 N NaOH and 0.1 N HCl.

Apparatus

Metal concentrations were determined using Perkin-Elmer Instruments, Model A analyst 100 with air-acetylene flame (AAS) Shelton, USA. The wave lengths used for nickel and copper were 232.0 and 324.8 nm, respectively.

Biosorption experiments

To study the effect of pH (2.0 to 10.0), biomass dose (40 to 240 mg in 100 ml solution), contact time (20 to 120 min) and initial metal ion concentration (25 to 200 mg/L), the biosorption experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml solution of the metal ion at interval concentrations and different amounts of nonliving biomass of *B. vesicularis*. In each experiment, one of the conditions was changed, while other conditions were kept constant. The flasks were agitated using an incubator shaker (Innova 4230, New Brunswick Scientific, USA) at a constant speed of 150 rpm for different incubation times. The temperature during experiments was

30°C. It is added here that the variation in temperature within 20 to 35°C range does not influence the biosorption process (Aksu, 1992). On the other hand, biosorption process is usually not operated at high temperatures because it will increase the operational cost (Wang, 2002).

For each treatment, the matrix was centrifuged and the amount of metal in the supernatant was determined by AAS technique. Prior to 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:

$$q = V (C_i - C_f)/m \qquad (1)$$

where q is the metal uptake (mg metal per gram biosorbent), V is the liquid sample volume (ml), C_i is the initial concentration of the metal in the solution (mg/L), C_f is 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 (Gadd et al., 1988). In the present study, only Langmuir (1918) model suitable for monolayer adsorption has been used. The Langmuir equation can be given as:

$$q = \frac{bC_f\,q_{max}}{1 + bC_f} \qquad (2)$$

On rearranging Equation 2, we get:

$$\frac{1}{q} = \frac{1}{b.q_{max}} \cdot \frac{1}{C_f} + \frac{1}{q_{max}} \qquad (3)$$

where q is the amount of metal sorbed (mg/g), C_f is the final (equilibrium) concentration of the metal in solution (mg/L), q_{max} is the maximum amount of metal ion which can be taken up by the biosorbent (mg/g) and 'b' is the Langmuir constant. The linear Langmuir plot was obtained by plotting $1/q$ versus $1/C_f$ on y and x axes, 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} represents 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 Ni(II) and Cu(II)

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 *B. vesicularis* at optimum conditions in respect to pH, contact time and biomass dose. The metal mixture concentrations were determined similarly to single metal systems.

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

Wastewater samples from the two sites were collected in plastic containers at 15 cm depth to the surface, keeping in view the
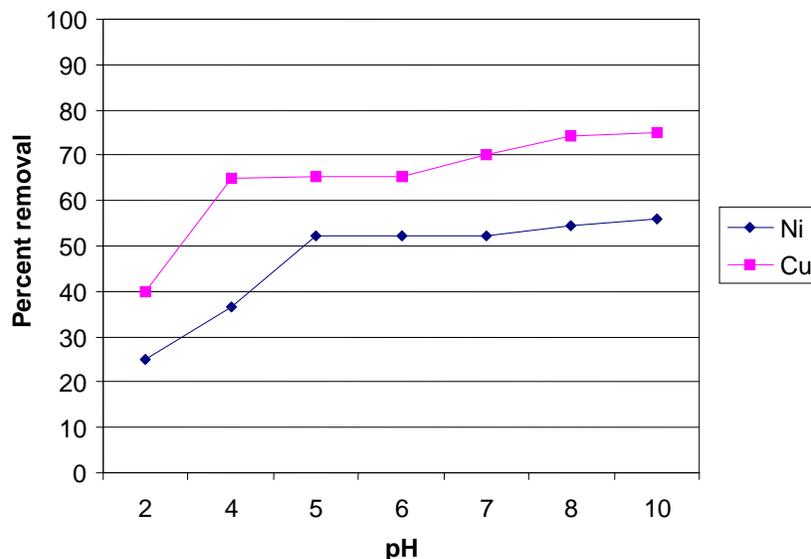
Figure 1. Effect of pH on biosorption of Ni(II) and Cu(II) by *B. vesicularis*.

representativeness, practical viability and safety considerations. Samples taken in triplicate were mixed to get composite sample. The samples were preserved in refrigerator by adding 1.5 ml of concentrated HNO_3 to 1 L of wastewater samples. The samples were digested with HNO_3 following APHA, AWWA and WFCF (1995) recommendations and were filtered. Following sample preparation, the concentrations of metals were determined by AAS. After that the developed method was applied to wastewater samples at optimum conditions and the results were analysed as previously explained.

RESULTS AND DISCUSSION

Effect of pH

In the present study, the percent biosorption was determined at different pH values and the curves were drawn by plotting percent biosorption against pH for Ni(II) and Cu(II) systems (Figure 1). The pH of the solution has a very significant effect on metal ion solubility and surface charge of the biomass (Guibal et al., 1994) which in turn influences the biosorption process. The present study also showed that in the case of Ni(II), biosorption increased sharply from pH 2.0 to 5.0 and then became stable from 5.0 to 7.0. Above the pH value of 7, a slight increase in the biosorption was observed. On the other hand, the biosorption of Cu(II) increased sharply from pH 2.0 to 4.0, then became nearly constant from pH 4.0 to 6.0. Finally, there was a slight increase from pH 6.0 to 10.0. Low biosorption observed at lower pH values can be explained by the fact that the cell wall of the bacterial biomass is closely associated with H_3O^+ and the access of metal ions to cell wall would be obstructed as a result of repulsion (Kapoor et al., 1999). The metal uptake increased from pH 2.0 to 5.0 in case of Ni(II) and 2.0 to

4.0 in case of Cu(II). This could be explained by the fact that the more ligands with negative charge are exposed with the subsequent increase in attraction sites to positively charged metal ions (Selatnia et al., 2004). At high pH labels, the sorption resulted 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} mol^3/L^3, respectively) (Agasyan and Tsyurupa, 1976). Therefore, at the same concentration of each metal ion, the pH required for precipitation will fall in the order Ni > Cu which is in agreement 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 (Al-Quodah, 2006)

Effect of biomass dose

The data on biosorption were recorded by varying biomass dose of the species from 40 to 240 mg in 100 ml metal ion solution of 100 mg/L concentration. The curves were drawn by plotting percent biosorption against biomass dose (Figure 2). The curves indicated an increase in biosorption with the increase of biomass, but at higher biomass dose, the increase was not significant. The increase in percentage removal is due to the increase in surface area of the biomass and the availability of the adsorption sites (Burno et al., 2008). 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 (Hammain et al., 2007). Based on Figure 2 results, 100 mg of biomass in 100 ml solution of metal ions was chosen for further studies.
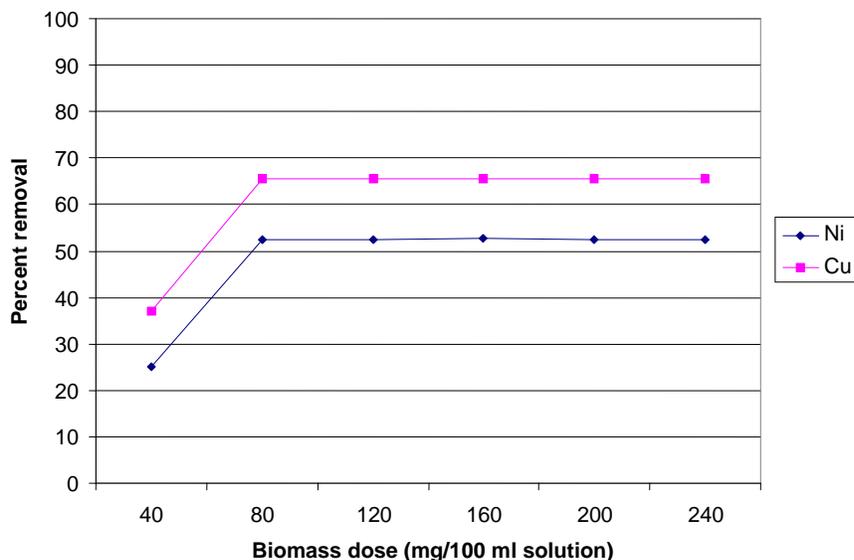
Figure 2. Effect of biomass dose on biosorption of Ni(II) and Cu(II) by *B. vesicularis*.
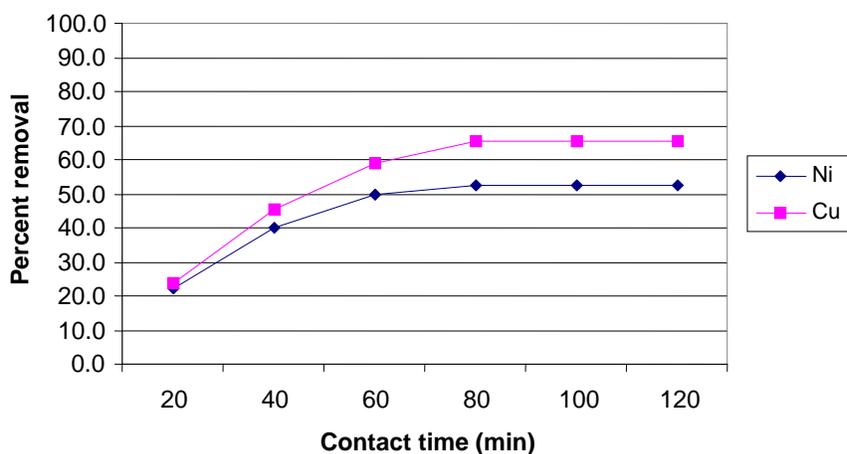

Figure 3. Effect of contact time on biosorption of Ni(II) and Cu(II) by *B. vesicularis*.

Effect of contact time

The effect of contact time on the biosorption of Ni(II) and Cu(II) is as shown in Figure 3. The results exhibited that the biosorption increased in time-dependent manner up to 80 min contact time prior to stable values for both metal ions. Weber (1985) demonstrated that the role of metal uptake is influenced by the factors affecting mass transfer from the bulk solution to binding sites. In the present study, the experimental conditions allowed normal mixing of the metal ions and the biomass in the system which partially suppressed some kinetic factors leading the equilibrium in short time (80 min). However, to ensure the complete constancy of time, 90 min time period was used for further studies.

Effect of initial metal ion concentration

When 0.1 g of biomass of *B. vesicularis* was treated with 100 ml solution of both metals in different concentrations (25 to 200 mg/L), percentage removal of each metal regularly decreased with increase in metal concentration (Table 1). This trend might be explained by the ability of active sites to fully absorb the metal ions at lower concentrations, whereas higher concentrations caused saturation of absorption sites. For further studies, 100 mg/L solution of both metal ions was used with the intent of showing the usefulness of the biomass for removal of metal ions from dilute solutions, where classical methods failed.

For the quantification of the capacity of the biomass of

Table 1. Effect of initial concentrations of Ni(II) and Cu(II) on their biosorption by *B. vesicularis*.

Ni(II)			Cu(II)		
Initial concentration (mg/L)	Final concentration (mg/L)	Percent removal	Initial concentration (mg/L)	Final concentration (mg/L)	Percent removal
25	4.5	82.0	25	2.7	89.2
50	14.0	72.0	50	9.0	82.4
75	30.0	60.0	75	20.3	73.0
100	47.6	52.4	100	34.6	65.4
125	66.0	47.2	125	43.5	65.2
150	85.2	43.2	150	58.5	61.0
175	105.0	40.0	175	73.5	58.0
200	122.0	39.0	200	91.6	54.2

pH = 6.0, contact time = 90 min, biomass = 100 mg in 100 ml solution.

B. vesicularis to remove Ni(II) and Cu(II) from the aqueous solutions, variation of biosorption amount in comparison with initial concentration were used for calculating q (specific metal uptake in mg/g) values. The Langmuir (1918) isotherms were drawn by plotting $1/q$ versus $1/C_f$ and q_{max} (maximum absorption capacity) and 'b' values were calculated. The deviations from Langmuir model were observed at higher concentration ranges. The latter results may be attributed to the use of Lineweaver–Burk equation which is strongly biased towards fitting data at low concentration range (Lineweaver and Burk, 1934) or due to extra cellular precipitation (Ahalya et al., 2003) which could take place both in the solution and on the cell surface (Ercole et al., 1994). In the present case, the q_{max} and 'b' values were calculated from the linear plots observed in lower concentration range. The calculated q_{max} and 'b' values for Ni are 91.6 mg/g and 0.013 and for Cu are 129.4 mg/g and 0.009, respectively. The q_{max} value in the case of Cu(II) is higher than Ni(II) which shows that Cu(II) has more affinity for the biomass than Ni(II). The latter result is in consonance with the Irving and Williams (1953) series developed for the stability constants for the formation of complexes. The 'b' value for Cu(II) is lower than that of Ni(II). This was expected as lower value of 'b' shows more affinity of the metal for biomass resulting high q_{max} value of Cu(II) (Jalal et al., 2002).

Biosorption of binary mixture of Ni(II) and Cu(II) by the nonliving biomass of *B. vesicularis*

The industrial effluents generally include a mixture of metals. 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) prepared in 1 : 1 ratio (optimum concentration of each metal, 100 mg/L) were treated with 0.1 g of biomass of *B. vesicularis* under same optimum conditions as used

in single metal systems, the percentage removal is 37.0% for Ni(II) and 51.4% for Cu(II) (Table 2) which is lower in both cases than the single metal ion systems [52.4% for Ni(II) and 65.4% for Cu(II)]. This decrease in percentage removal is due to a higher competition between the same charged metals (+2) for binding sites of the biomass.

Removal of Ni(II) and Cu(II) from wastewater samples

The suggested 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 the outlet of Upper Lake, Bhopal (India). The concentration of Ni and Cu in the samples was Ni (8.4 ppm) and Cu (4.64 ppm) in Tawa River and Ni (NIL) and Cu (1.6 ppm) on the outlet of Upper Lake. This method could remove 60.4% Ni and 63.5% Cu from Tawa River and 59.6% Cu from the outlet of Upper Lake (nickel being NIL in the Upper Lake).

Conclusion

The present study clearly showed that at nearly normal optimum conditions (pH and temperature near to generally found for water systems and environment respectively), the nonliving biomass of *B. vesicularis* could be used for the removal of Ni and Cu from their aqueous solution and wastewater samples. The method might be equally useful with satisfactory results for dilute solutions. The optimum pH, biomass dose, contact time and initial metal ion concentration were 6.0, 0.1 g in 100 ml, 90 min and 100 mg/L, respectively. Nickel and copper were found as their hydrated ions and hydroxides at different pH ranges. The biosorption potential of *B. vesicularis* can be further enhanced by immobilizing it with sodium alginate, polysulphone, polyacrylamide, etc. However, immobilizing the biomass usually increases process costs.

Table 2. Percent removal of Ni(II) and Cu(II) in single and binary systems by *B. vesicularis*.

Metal	Percent removal (single system)	Percent removal (binary system)
Ni	52.4	37.0
Cu	65.4	51.4

pH = 6.0, contact time = 90 min, biomass = 100 mg in 100 ml solution.

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