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Biosorption of Lead(II), Nickel(II), and Copper(II) on *Rhizopus arrhizus* from Binary and Ternary Metal Mixtures

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Biosorption of Lead(II), Nickel(II), and Copper(II) on *Rhizopus arrhizus* from Binary and Ternary Metal Mixtures

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ABSTRACT

The biosorption of three divalent metal ions [Pb(II), Ni(II), and Cu(II)] frequently encountered together in industrial waste waters and in binary and ternary systems was studied using *Rhizopus arrhizus*, a filamentous fungus, in batch stirred reactors. The multimetal biosorption data were evaluated in terms of equilibrium isotherms and adsorption yields. The single-metal equilibrium data were analyzed using the Freundlich adsorption model. The individual Freundlich adsorption constants were determined and used to compare biosorptive capacity of the microorganism for different metal ions. The effects of various combinations of the metal ions on the biosorption capacity of *R. arrhizus* are discussed and the actions of the synergistic or antagonistic metal ion combinations were determined. For the two-metal adsorption equilibrium, competitive adsorption isotherms have been also developed. The six-parameter empirical Freundlich model, restricted to bicomponent systems, was used successfully to characterize simultaneous biosorption of Pb(II), Ni(II), and Cu(II) ions by *R. arrhizus* from two-metal systems. The biosorption capacity of Pb(II) in the two binary and one ternary systems, in agreement with the single-metal data, was greater than that of the Ni(II) and Cu(II) ions. The relative capacities were Pb(II) $>$ Ni(II) $>$ Cu(II) at pH 5.0 in single, binary, and ternary systems.

Key Words. Waste water treatment; Heavy metal ion; Biosorption; *Rhizopus arrhizus*; Multimetal mixtures; Empirical Freundlich adsorption model

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INTRODUCTION

Multimetal systems are often encountered in industrial operations. Three widely used metals that often appear in metal-bearing industrial effluents are lead, nickel, and copper. These ions are frequently encountered in a mixture in industrial waste waters during mining, metal cleaning, plating, electroplating, metal processing, dyeing, porcelain enameling, and $CuSO_4$ manufacture. In plating rinse, Ni(II), Cu(II), and Pb(II) concentrations can approach 2–900, 20–120, and 2–140 mg L^{-1}, respectively. Waste waters from paint and ink formulation, porcelain enameling, and $CuSO_4$ manufacture industries contain concentrations ranging over 0–40 mg L^{-1} Ni(II), 0–100 mg L^{-1} Cu(II), and 86 mg L^{-1} Pb(II); 0.25–67 mg L^{-1} Ni(II), 0–12 mg L^{-1} Cu(II), and 2.9 mg L^{-1} Pb(II); average 22 mg L^{-1} Ni(II) and 221–433 mg L^{-1} Cu(II), respectively (1). The most reasonable policy to diminish the escape of heavy metals from industrial sources is the adoption of low waste-generating technologies coupled with effective effluent purification processes.

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 waste waters. Although much research has been carried out on the uptake of heavy metal ions by biomaterials, the accepted approach has been restricted to employing single metal ions with one microorganism at a time (2–8). Because single toxic metallic species rarely exist in natural and waste waters, any approach that attempts to removal heavy metals from multimetal systems using biosorbents would be more realistic. Equilibria and capacity relationships for monocomponent systems are well established and quantitatively expressed by various types of adsorption isotherms (9–11). To describe the competitive adsorption equilibrium, several isotherms ranging from simple models related to the individual isotherm parameters (such as the competitive Langmuir model, the three-parameter Redlich-Peterson model, and the ideal adsorption solution theory [IAS]), to more complex models related to the individual isotherm parameters and to correction factors, (such as the modified competitive Langmuir model, the multicomponent Freundlich isotherm, and the empirical bicomponent Freundlich isotherm) have been proposed (12–15).

Two-metal biosorption studies are particularly important for assessing the degree of interference with a biosorption process of common metal ions in waste waters. The biosorption behavior of a two-metal system involving cadmium and iron on *Sargassum fluitans*, a brown marine alga, was evaluated (16). Despite the higher affinity of *Sargassum* biomass for Cd than for Fe, the uptake of Cd decreases in the presence of Fe. The Cd presence also affects the uptake of Fe very strongly. Two-metal biosorption studies lead to the assessment of the behavior of more complex (ternary) systems. Equilibrium metal uptake performance of *Ascophyllum nodosum* seaweed biomass was studied using aqueous solutions containing copper, cadmium, and zinc ions in binary and ternary mixtures (17). Results from the binary and ternary data show that the metal selectivity of the biomass is of the order Cu $>$ Cd $>$ Zn. The behavior of selective biosorption of *Pseudomonas aeruginosa*, a bacterium, with solutions containing lead, copper, and cadmium was investigated (18). The biomass has the highest saturation capacity for copper in the single-metal system, followed by lead and cadmium, whereas in three-metal adsorption the maximal capacity of the metals decreases in the order of Pb $>$ Cu $>$ Cd. The ability of the fungal biomass *R. arrhizus* to bind Cr(VI) and Fe(III) simultaneously in solution was also investigated (19). Although Cr(VI) ions, in agreement with the single-metal data, are adsorbed selectively from the binary metal mixtures, Fe(III) ions compete strongly with Cr(VI) ions to bind to active sites on the fungus. In all of the studies mentioned above, the multimetal adsorption equilibrium was described by three models, two of which originate from single-component Langmuir isotherm, and the third which uses the single-component Langmuir model as the starting structure and is established empirically.

The Freundlich expression is an empirical equation based on heterogeneous energetic distribution of active sites on the surface of adsorbent; thus, interaction between adsorbed metals is reversible. The Freundlich equation for single-component systems is commonly presented as (20)

$$q_{\mathrm{eq}} = a^0 C_{\mathrm{eq}}^{b^0} \tag{1}$$

This nonlinear equilibrium equation is linearized to determine the individual Freundlich constants from the slope and intercept, which are equal to b^0 and $\ln a^0$ at $C_{\mathrm{eq}} = 1.0$, respectively.

$$\ln q_{\mathrm{eq}} = b^0 \ln C_{\mathrm{eq}} + \ln a^0 \tag{2}$$

Magnitude of a^0 and b^0 shows easy separation of metal ions from waste water and favorable adsorption. The intercept of the linearized Freundlich equation a^0 is an indication of the adsorption capacity of the adsorbent; the slope b^0 indicates the effect of concentration on the adsorption capacity and represents the adsorption intensity.

For multimetal systems, models should be modified in order to take into account all metals and cover experimental data over a wide range of solution concentrations. The empirical extension of the Freundlich model, restricted to binary mixtures, was proposed by Fritz and Schluender (21) and has been shown to fit successfully bisolute mixtures of organics onto activated carbon that could not be described by the IAS-Freundlich expression (22):

$$q_{1,\mathrm{eq}} = \frac{a_1^0 \cdot C_{1,\mathrm{eq}}^{b_1^0 + b_{11}}}{C_{1,\mathrm{eq}}^{b_{11}} + a_{12} \cdot C_{2,\mathrm{eq}}^{b_{12}}} \tag{3a}$$

$$q_{2,\mathrm{eq}} = \frac{a_2^0 \cdot C_{2,\mathrm{eq}}^{b_2^0 + b_{22}}}{C_{2,\mathrm{eq}}^{b_{22}} + a_{21} \cdot C_{1,\mathrm{eq}}^{b_{21}}} \tag{3b}$$

In this isotherm, a_i^0 and b_i^0 are derived from the corresponding individual Freundlich isotherm equations. The six new parameters are the competition coefficients and can be derived experimentally in bisolute adsorption tests. The Freundlich model physically provides a more realistic description of metal adsorption by organic matter because it accounts for different binding sites.

In the present study, competitive biosorption of Pb(II), Ni(II), and Cu(II) ions on *R. arrhizus* from binary and ternary mixtures is described. The metal binding capacity of *R. arrhizus* is shown to be a function of pH, metal combination, the number of metals competing for binding sites in the fungal cells, and levels of metal concentration. The applicability of Freundlich-type adsorption models in explaining the mechanism of single and simultaneous biosorption of Ni(II), Pb(II), and Cu(II) from binary mixtures by *R. arrhizus* is tested.

EXPERIMENTAL SECTION

Microorganism and Preparation of the Microorganism for Biosorption

R. arrhizus, a filamentous fungus, was obtained from the U.S. Department of Agriculture Culture Collection (NRRL 2286). Batch experiments were conducted with 100 mL cultures in 250 mL Erlenmeyer flasks. The flasks were agitated on a shaker for 96 h at 150 rpm and at a temperature of 30°C. The growth medium contained malt extract (17.0 g L^{-1}) and soya peptone (5.4 g L^{-1}). The pH was adjusted to 5.4–5.6 with H_2SO_4.

After the growth period, *R. arrhizus* was washed twice with distilled water, inactivated using 1% formaldehyde, and then dried in an oven at 60°C for 24 h. Some methods of killing cells may actually improve the biosorption properties of the biomass. Better killing methods, such as immersion in formaldehyde, would cross-link the cells, thereby simultaneously immobilizing them. That inactivated biomass of *R. arrhizus,* killed with formaldehyde solution, can endure more chemically aggressive environments such as multimetal mixtures has been reported (19, 23, 24). For biosorption studies, 1.0 g of dried cells was suspended in 100 mL of distilled water and homogenized for 20 min in a homogenizer (Ultra-Turrax T 25, Janke and Kunkel, IKA-Labortechnik) at 8000 rpm.

Preparation of Biosorption Media Containing Single Metal Ions

Ni(II), Pb(II), and Cu(II) solutions were prepared by diluting 1.0 g L^{-1} of stock solutions of nickel(II), lead(II), and copper(II), obtained by dissolving

$Ni(NO_3)_2 \cdot 6H_2O$, $Pb(NO_3)_2$ and $CuN_2O_6 \cdot 3H_2O$ in distilled water, respectively. To determine the isotherms, initial metal ion concentrations were varied between 25 and 200 mg L^{-1} while the dry cell weight in each sample was constant at 1.0 g L^{-1}. Before the solutions were mixed with the fungal suspension, pH was adjusted to the required value by adding 1 mol L^{-1} of HNO_3.

Preparation of Binary and Ternary Metal Mixtures

The applicability of the empirical Freundlich model to the biosorption of Pb(II) and Ni(II) ions from the binary metal mixtures by *R. arrhizus* was checked for four different sets of data. The initial concentrations of Pb(II) ions were varied between 25 and 200 mg L^{-1}, whereas the Ni(II) ion concentration in each metal mixture was held constant at 50, 100, 150, or 200 mg L^{-1}. For the determination of biosorption characteristics of Cu(II) and Ni(II) ions in binary metal mixtures, the initial concentrations of Cu(II) ions were varied between 25 and 200 mg L^{-1}, whereas the Ni(II) ion concentrations in each biosorption medium were held constant over the 25–150 mg L^{-1} range. For the determination of biosorption characteristics of Pb(II) and Cu(II) ions in binary metal mixtures, the initial concentrations of Pb(II) ions were varied between 25 and 205 mg L^{-1}, whereas the Cu(II) ion concentrations in each biosorption medium were held constant over the 25–100 mg L^{-1} range. Competitive biosorption experiments of Pb(II)–Ni(II), Cu(II)–Ni(II), and Pb(II)–Cu(II) in binary mixtures were performed at pH 5.0, 4.0, and 5.0, respectively. For the determination of biosorption characteristics of Pb(II), Ni(II), and Cu(II) ions in ternary metal mixtures, the initial concentrations of Pb(II) ions were varied between 25 and 200 mg L^{-1}, whereas the Ni(II) and Cu(II) ion concentrations in each biosorption medium were held constant over the 50–200 mg L^{-1} range. The pH of ternary metal mixtures was adjusted to pH 5.0 with 1 mol L^{-1} HNO_3.

Biosorption Studies

The fungal suspension (20 mL) was mixed with 180 mL of the desired metal solution in an Erlenmeyer flask. The flasks were agitated on a shaker at 25°C for 48 h, which is sufficiently long for adsorption equilibrium. Agitation rate was 150 rpm. Before the fungal suspension and the metal-bearing solution were mixed, a 3-mL sample was taken from the metal-bearing solution. Subsequently, samples were taken at 5-min intervals at the beginning of adsorption and at 25–30-min intervals after samples reached equilibrium. The samples were centrifuged at $6030 \times g$ for 3 min and the supernatant liquid was used for metal analysis.

Measurement of Heavy Metal Ions

The concentrations of unadsorbed Ni(II), Pb(II), and Cu(II) ions in the sample supernatant were determined using an atomic adsorption spectrophotometer (ATI-UNICAM 929 England) with an air–acetylene flame. LIKOM (Japan) Ni and ATI-UNICAM Pb, Cu hollow cathode lamps were used. Ni(II), Pb(II), and Cu(II) were measured at 232.0, 217.0, and 324.8 nm, respectively.

RESULTS AND DISCUSSION

Pb(II), Ni(II), and Cu(II) Biosorption on *R. arrhizus:* Single-Metal Situation

The effect of pH on the biosorption of heavy metal ions by microorganisms was investigated in detail in our earlier papers and Pb(II), Ni(II), and Cu(II) ions were demonstrated to be best adsorbed on the biomass at pH in the range 4.0–5.0 (10, 25–27). At pH values higher than 5.5, Pb(II) and Cu(II) ions precipitated and adsorption studies at these pH values could not be performed. Adsorption isotherms were developed for optimum conditions and adsorption equilibrium data fit the noncompetitive Freundlich model (Fig. 1). Values of a^0 and b^0 presented in Table 1 were obtained by evaluating the noncompetitive Freundlich adsorption isotherms. A higher value of the a^0 parameter for Pb(II) than for Ni(II) and Cu(II) implies that the biosorbent has a higher capacity for Pb(II) than for Ni(II) and Cu(II). The magnitude of a^0 also reflects the affinity and selectivity of the biosorbent for a metal. Comparison of ad-

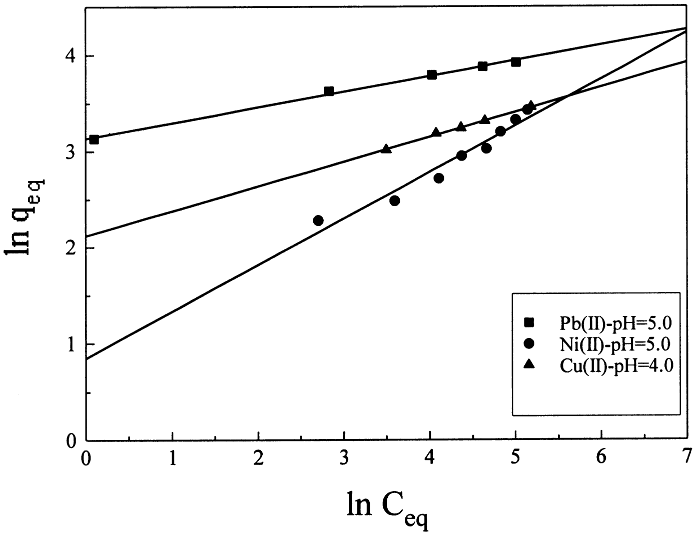

FIG. 1 The noncompetitive Freundlich adsorption isotherms obtained at optimum pH for Pb(II), Cu(II), and Ni(II) biosorption on *R. arrhizus.*

TABLE 1
Comparison of Individual Constants Obtained from Noncompetitive Freundlich Adsorption Isotherms for Metal Ions at Different pH Values

pH	a^0 ($mg^{b^0+1}g^{-1}L^{-b^0}$)	b^0	ε (%)	R
Lead				
4.0	7.34	0.28	2.63	0.995
5.0	22.94	0.16	1.65	0.998
Nickel				
4.0	2.03	0.52	8.22	0.966
5.0	2.34	0.48	7.78	0.974
Copper				
4.0	8.33	0.26	0.57	0.999
5.0	3.80	0.25	0.77	0.997

sorption isotherms for different metal ions under optimum conditions shows that well-adsorbed metal ions have shallow slopes and were located in the upper surface concentration q_{eq} values, i.e., Pb(II) biosorption at pH 5.0. As metal ions become more poorly adsorbed, their isotherms have increased slopes and lower q_{eq} vs. C_{eq} values. The highest a^0 values were found to be 22.94 mg^{b^0+1} $g^{-1}L^{-b^0}$ for Pb(II) ions at pH 5.0 and 8.33 mg^{b^0+1} $g^{-1}L^{-b^0}$ for Cu(II) ions at pH 4.0. *R. arrhizus* had a higher adsorption capacity, a^0, but a lower adsorption intensity for the treatment of waste water containing Pb(II) and Cu(II) ions than for the treatment of waste water containing Ni(II) ions. *R. arrhizus* was much more efficient in removing higher concentrations of Ni(II) ions.

R. arrhizus, which belongs to a large class of phycomycetes (algalike fungi), is known for its strong metal-sorbent properties which are common to members of the entire genus. The heavy metal biosorption capacity of *R. arrhizus* strain used in this study was compared with that of the common fungi, which belong to different classes. Maximum adsorbed metal ion quantities per dry weight of *R. arrhizus* were determined to be 50.42 mg Pb(II) g^{-1} at pH 5.0, 31.71 mg Cu(II) g^{-1} at pH 4.0, and 30.86 mg Ni(II) g^{-1} at pH 5.0. A maximum Cu(II) uptake by *Ganoderma lucidum*, a mushroom (basidiomycetes), was measured as 64.50 mg g^{-1} (28). A copper accumulation of 10.11 mg g^{-1} by *Aspergillus niger* (ascomycetes) was documented (28). A maximum lead accumulation of 116.0 mg g^{-1} and copper accumulation of 8.52 mg g^{-1} by

Penicillium chrysogenum was observed (29). *Fusarium flocciferum* (deuteromycetes), also known as a good biosorbent, reached uptake values of 51.99 mg Ni(II) g^{-1} and 39.97 mg g^{-1} Cu(II) (30). Maximum adsorbed metal ion quantities per dry weight of *Penicillium digitatum* were determined to be 18.65 mg Pb(II) g^{-1}, 14.67 mg Ni(II) g^{-1}, and 14.62 mg Cu(II) g^{-1} (23). Some values of maximum adsorbed metal ion quantities per dry weight of biomass are not comparable with other values found for the same metal. It may depend not only on different biosorption abilities of fungi, but also on operating conditions that are not identical. In fact, works of different authors cannot be compared directly: operating conditions are often different even if they are nominally equal.

Simultaneous Biosorption of Pb(II) and Ni(II) on *R. arrhizus* at pH 5.0 from Binary Metal Mixtures

Because Pb(II) ions were best adsorbed on the biomass at pH 5.0 and *R. arrhizus* had approximately the same adsorption capacity and intensity for Ni(II) ions at both pH 4.0 and 5.0, studies on the simultaneous biosorption of a mixture of Pb(II) and Ni(II) were carried out at pH 5.0. The empirical Freundlich adsorption isotherms for the simultaneous biosorption of Pb(II) ions on *R. arrhizus* in the presence of increasing concentrations of Ni(II) ions are given in Fig. 2. The empirical Freundlich adsorption isotherms for the simultaneous

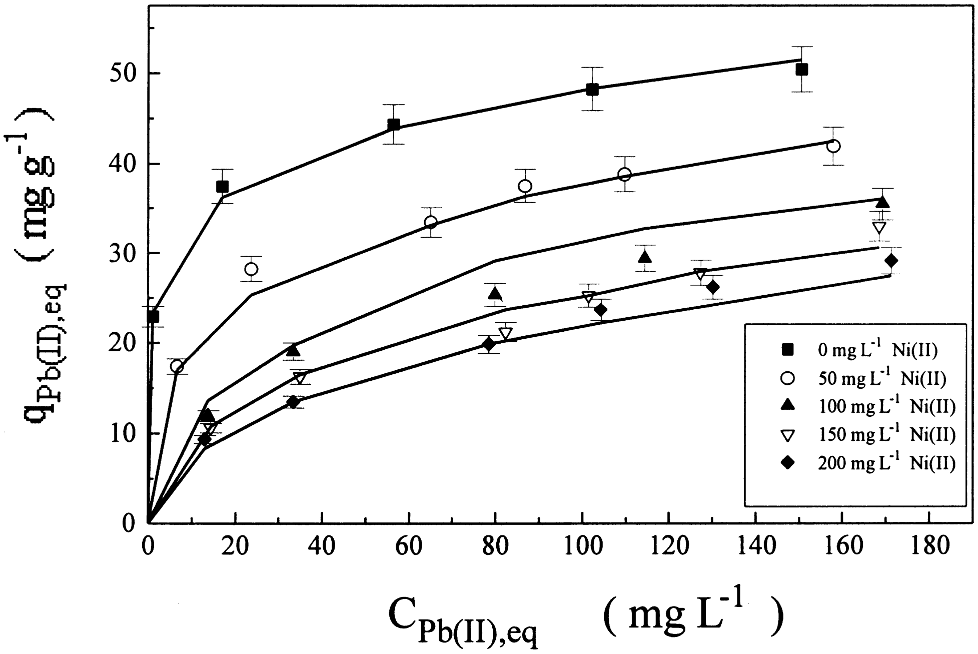

FIG. 2 The empirical Freundlich adsorption isotherms of Pb(II) in the presence of increasing concentrations of Ni(II) at pH 5.0.

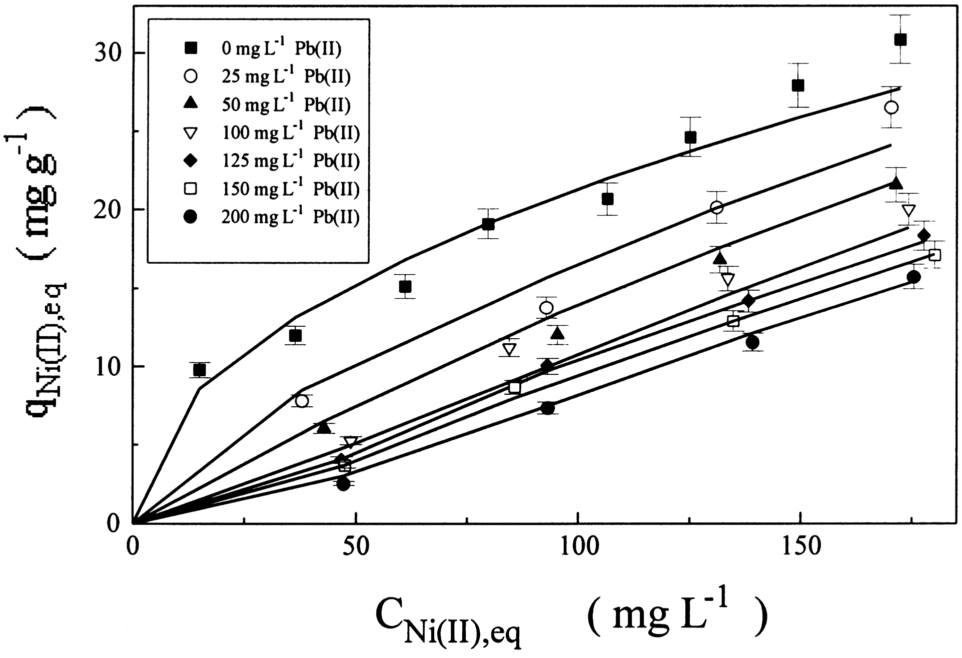

FIG. 3 The empirical Freundlich adsorption isotherms of Ni(II) in the presence of increasing concentrations of Pb(II) at pH 5.0.

biosorption of Ni(II) ions on *R. arrhizus* in the presence of increasing concentrations of Pb(II) ions are also given in Fig. 3. In the figures where competitive metal uptake equilibrium data are shown, the Freundlich model profiles are presented in full lines, whereas the symbols denote experimentally obtained values. The error bars indicated in the figures represent 5% error between the predicted and the experimental values. Competition coefficients for the empirical Freundlich model given by Eqs. (3a) and (3b) were estimated from the competitive adsorption data of Pb(II) and Ni(II) by using an MS Excel 7.0 computer program (Table 2). Average percentage errors between the experimental values and the predicted values using the empirical Freundlich model for the entire data set of Pb(II) and Ni(II) ions were 5.27 and 5.68%, respectively. Correlation coefficients between the predicted and the experimental values for the entire data set ranged between 0.917 and 0.999. The adsorp-

TABLE 2
Competition Coefficients of Empirical Freundlich Model for Biosorption of Pb(II) and Ni(II) Ions on *R. arrhizus* at pH 5.0; 1 Denotes Pb(II) and 2 Denotes Ni(II)

a_1^0	b_1^0	b_{11}	a_{12}	b_{12}	a_2^0	b_2^0	b_{22}	a_{21}	b_{21}
22.94	0.16	0.50	0.04	1.11	2.34	0.48	1.24	14.15	1.11

tion data of Pb(II) and Ni(II) in the binary mixtures exhibited good agreement with the empirical Freundlich isotherm. Pb(II) ions were adsorbed selectively from the binary mixtures of Pb(II) and Ni(II) ions by *R. arrhizus*. The inhibition effect of Pb(II) ions on the biosorption of Ni(II) ions, as is evident from the competition coefficients a_{12} and a_{21}, was greater than that exerted by Ni(II) ions.

Simultaneous Biosorption of Cu(II) and Ni(II) on *R. arrhizus* at pH 4.0 from Binary Metal Mixtures

To obtain selectivity for Cu(II) ions, pH of biosorption media was adjusted to 4.0, the optimum value for the biosorption of Cu(II) ions. Adsorption isotherms of Cu(II) and Ni(II) in the presence of constant concentrations of other metal were computed from the empirical Freundlich isotherm and the comparison between experimental and computed values is shown in Figs. 4 and 5. Competition coefficients for the empirical Freundlich model are summarized in Table 3. Average percentage errors between the experimental values and the predicted values using the empirical Freundlich model for the entire data set of Cu(II) and Ni(II) ions were 8.35 and 7.19%, respectively. Correlation coefficients between the predicted and the experimental values for the entire data set ranged between 0.985 and 0.998. A good agreement for the experimental data with the empirical Freundlich model was obtained for the

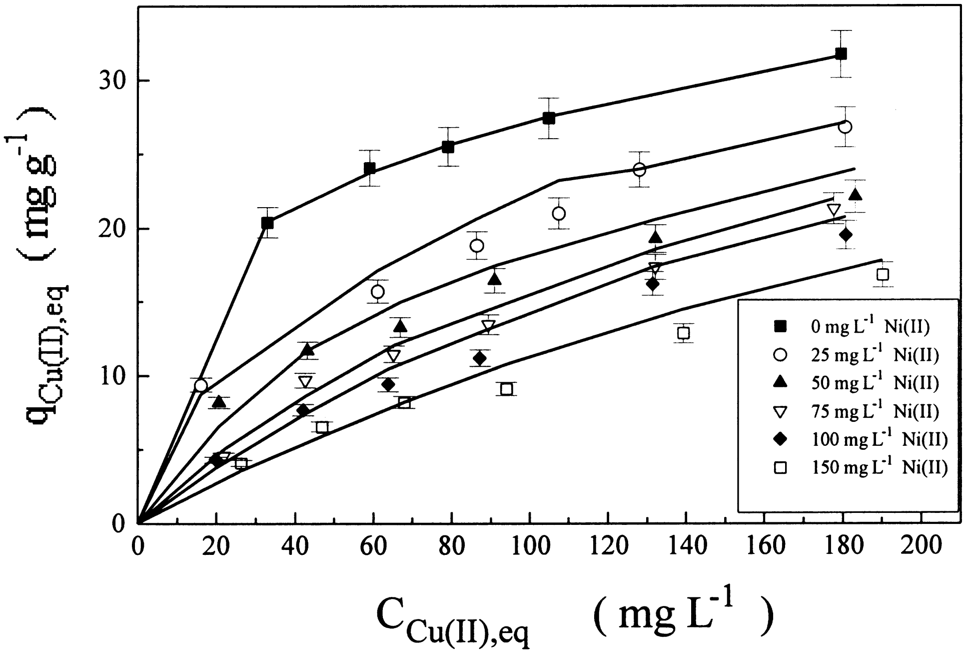

FIG. 4 The empirical Freundlich adsorption isotherms of Cu(II) in the presence of increasing concentrations of Ni(II) at pH 4.0.

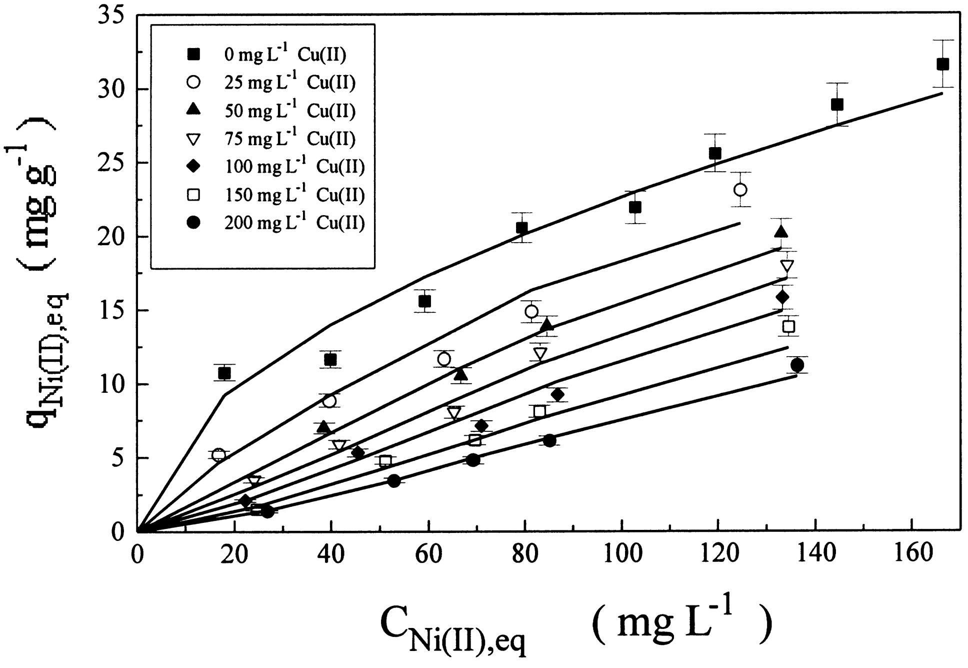

FIG. 5 The empirical Freundlich adsorption isotherms of Ni(II) in the presence of increasing concentrations of Cu(II) at pH 4.0.

bicomponent system Cu(II)–Ni(II). Cu(II) ions were adsorbed selectively from binary mixtures containing Cu(II) and Ni(II) ions at equal concentrations. However, equilibrium uptake of Ni(II) ions increased when Ni(II) concentration and/or ratio of Ni(II) ion concentration was increased with respect to total metal-ion concentration. *R. arrhizus*, as is evident from the b_1^0 and b_2^0 values, showed a higher affinity for Ni(II) than for Cu(II) at higher initial metal ion concentrations.

Simultaneous Biosorption of Pb(II) and Cu(II) on *R. arrhizus* at pH 5.0 from Binary Metal Mixtures

The biosorption of Cu(II) was significantly inhibited by Pb(II), whereas the influence in the reverse situation was milder (Figs. 6 and 7). Competition coefficients estimated from the competitive adsorption data of Pb(II) and Cu(II)

TABLE 3
Competition Coefficients of Empirical Freundlich Model for Biosorption of Cu(II) and Ni(II) Ions on *R. arrhizus* at pH 4.0; 1 Denotes Cu(II) and 2 Denotes Ni(II)

a_1^0	b_1^0	b_{11}	a_{12}	b_{12}	a_2^0	b_2^0	b_{22}	a_{21}	b_{21}
8.33	0.26	0.91	0.71	1.00	2.03	0.52	0.96	0.77	1.03

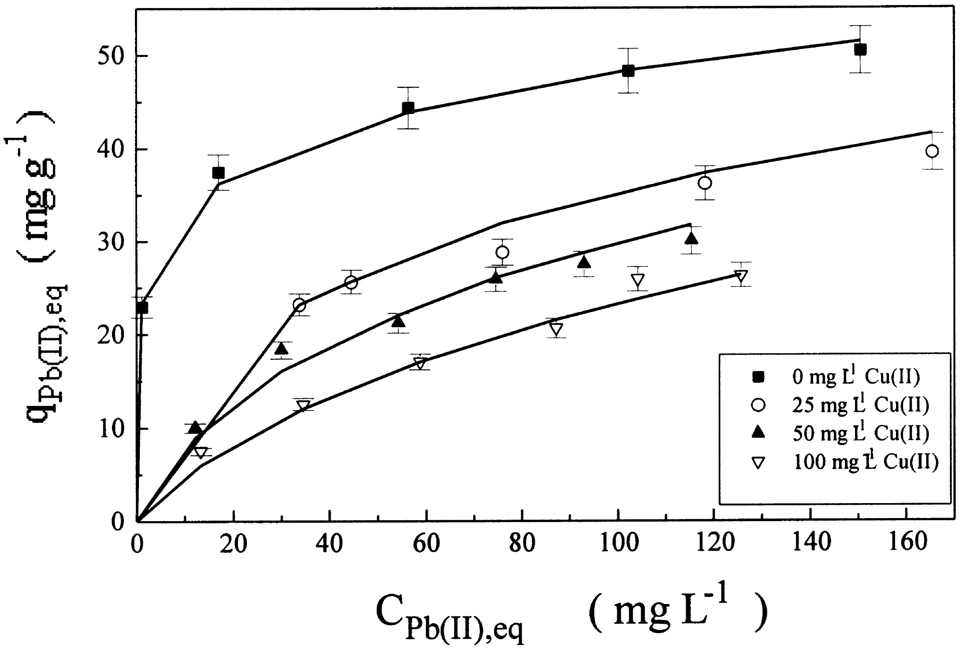

FIG. 6 The empirical Freundlich adsorption isotherms of Pb(II) in the presence of increasing concentrations of Cu(II) at pH 5.0.

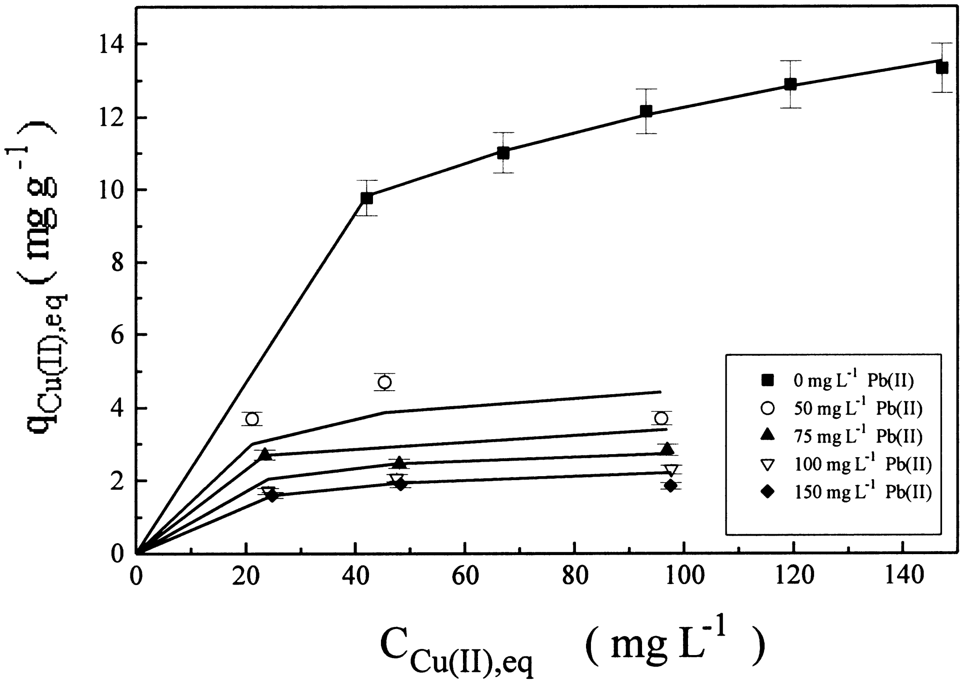

FIG. 7 The empirical Freundlich adsorption isotherms of Cu(II) in the presence of increasing concentrations of Pb(II) at pH 5.0.

TABLE 4
Competition Coefficients of Empirical Freundlich Model for Biosorption of Pb(II) and Cu(II) IOns on *R. arrhizus* at pH 5.0; 1 Denotes Cu(II) and 2 Denotes Pb(II)

a_1^0	b_1^0	b_{11}	a_{12}	b_{12}	a_2^0	b_2^0	b_{22}	a_{21}	b_{21}
3.80	0.25	0.01	0.14	0.73	22.94	0.16	0.76	1.00	0.78

ions are given in Table 4. Average percentage errors between the experimental values and the predicted values using the empirical Freundlich model for the entire data set of Pb(II) and Cu(II) ions were 5.57 and 15.93%, respectively. Correlation coefficients between the experimental values and the predicted values ranged between 0.963 and 0.999. To describe the adsorption isotherms mathematically, some well-known relationships for multimetal adsorption (12–15) were also tested, but there was no satisfactory agreement with the measured adsorption data of Pb(II), Ni(II), and Cu(II) ions from binary mixtures. Competitive models related only to the individual isotherm parameters, such as the competitive Langmuir model and the competitive Redlich–Peterson model, failed for the data. Individual adsorption constants used alone cannot define exactly the multicomponent adsorption behavior of the metal mixtures. Multicomponent systems are characterized by additional features beyond those of single components, such as possible interaction effects between different species in solution, potential interactions on the surface in particular depending on the adsorption mechanism, and competition between the different metal ion species for surface sites depending on the ionic characteristics. For that reason, better accuracy may be achieved by using competitive isotherms related to the individual isotherm parameters and to correction factors such as in the competitive Freundlich model.

Simultaneous Biosorption of Pb(II), Ni(II), and Cu(II) on *R. arrhizus* at pH 5.0 from Ternary Metal Mixtures

Biosorption data of Pb(II), Ni(II), and Cu(II) ions in ternary mixtures are summarized in Table 5. Adsorbed Pb(II) ion quantity per unit weight of dried biomass at equilibrium increased with increasing initial Pb(II) ion concentration, but decreased with increasing concentrations of Ni(II) and Cu(II) ions. A similar biosorption pattern was also observed for Ni(II) ions. The adsorption of Cu(II) ions at pH 5.0, however, was significantly low and relatively constant [3.1–4.9 mg Cu(II) g^{-1}] throughout the entire set of experiments. Increasing the concentration of Cu(II) ions did not correspondingly increase uptake of Cu(II) ions. In other words, irrespective of the initial Cu(II) ion concentration, a fixed number of Cu(II) ions bound to the active sites of the

TABLE 5
Comparison of Pb(II), Ni(II), and Cu(II) Biosorption on *R. arrhizus* at pH 5.0: As Single Metal and in the Presence of Increasing Concentrations of the Metal Ions in Ternary Metal Mixtures

$C_{\mathrm{Pb(II),i}}$ (mg L^{-1})	$C_{\mathrm{Ni(II),i}}$ (mg L^{-1})	$C_{\mathrm{Cu(II),i}}$ (mg L^{-1})	$q_{\mathrm{Pb(II),eq}}$ (mg g^{-1})	$q_{\mathrm{Ni(II),eq}}$ (mg g^{-1})	$q_{\mathrm{Cu(II),eq}}$ (mg g^{-1})	$Y_{\mathrm{Pb(II),eq}}$ (%)	$Y_{\mathrm{Ni(II),eq}}$ (%)	$Y_{\mathrm{Cu(II),eq}}$ (%)	$q_{\mathrm{t,eq}}$ (mg g^{-1})	$Y_{\mathrm{t,eq}}$ (%)
54.46	–	–	37.47	–	–	69	–	–	37.47	69
100.74	–	–	44.35	–	–	44	–	–	44.35	44
150.38	–	–	48.23	–	–	32	–	–	48.23	32
201.03	–	–	50.42	–	–	25	–	–	50.42	25
–	48.27	–	–	11.98	–	–	25	–	11.98	25
–	98.65	–	–	19.10	–	–	19	–	19.10	19
–	149.84	–	–	24.65	–	–	16	–	24.65	16
–	203.10	–	–	30.86	–	–	15	–	30.86	15
–	–	51.84	–	–	9.78	–	–	19	9.78	19
–	–	105.24	–	–	12.15	–	–	12	12.15	12
–	–	132.35	–	–	12.88	–	–	10	12.88	10
–	–	160.45	–	–	13.32	–	–	8	13.32	8
48.53	47.27	53.75	17.98	8.30	4.30	37	18	8	30.58	20
99.54	45.54	53.42	29.91	7.30	4.10	30	16	8	41.31	21
157.76	51.98	51.83	36.60	6.17	3.30	23	12	6	46.07	18
208.63	46.04	49.17	40.89	5.40	3.10	20	12	6	49.39	16
50.90	97.36	99.10	15.63	11.08	4.90	31	11	5	31.61	13
98.67	96.65	101.19	27.21	10.40	3.30	28	11	3	40.91	14
151.71	102.34	99.46	34.29	9.47	4.03	23	9	4	47.79	14
200.50	98.62	100.61	36.86	7.80	4.30	18	8	4	48.96	12
51.32	156.59	152.25	9.60	13.72	4.42	19	9	3	27.74	8
101.06	158.45	150.94	23.00	13.21	4.36	23	8	3	40.57	10
157.76	155.23	154.93	31.00	12.76	4.03	20	8	3	47.79	10
203.78	153.16	155.15	32.82	11.70	3.63	16	8	2	48.15	9

cells owing to the preferential adsorption of Pb(II) and Ni(II) ions at this pH. The capacity of Pb(II) in ternary systems, in agreement with the single- and dual-metals data, was always significantly greater than that of other metal ions.

Because the equilibrium uptake of Pb(II), Ni(II), and Cu(II) was reduced by the presence of increasing concentrations of other metals, compared with the single-metal systems, the combined action of Pb(II), Ni(II), and Cu(II) ions on *R. arrhizus* was generally found to be antagonistic. The most logical reason for the antagonistic action is claimed to be the competition for adsorption sites on cells and/or the screening effect by other metal ions. Increasing concentrations of metal ions that are not adsorbed can mask preferentially adsorbed metal ions. However, the total adsorbed metal ion quantities per unit weight of dried biomass at equilibrium were higher than the adsorbed Ni(II) and Cu(II) ion quantities per unit weight of dried biomass at equilibrium obtained in the biosorption media containing Ni(II) and Cu(II) ions alone at the same concentrations. For example, the total adsorbed metal ion quantity obtained in a mixture containing equal concentrations (150 mg L^{-1}) of the metal ions was determined as 47.79 mg Me(II) g^{-1}, whereas the equilibrium uptake of Pb(II), Ni(II), and Cu(II) obtained in the biosorption media containing Pb(II), Ni(II), and Cu(II) ions alone at a constant concentration of 150 mg L^{-1} was found to be 48.23 mg Pb(II) g^{-1}, 24.65 mg Ni(II) g^{-1}, and 13.32 mg Cu(II) g^{-1}. Although individual adsorption yields were decreased with the increasing initial metal ion concentrations, the total adsorption yields were also generally higher than the individual adsorption yields of Ni(II) and Cu(II) ions in the ternary metal mixtures. In this case, the total interactive effects of Pb(II), Ni(II), and Cu(II) ions on *R. arrhizus* are thought to be synergistic. This can be attributed to the fact that increases in the total metal ion concentration compared with the single metal ion systems result in a large difference in concentration between adsorbent surface and metal solution or a strong driving force and further uptake of the metal ions in the multimetal mixtures. Another possible explanation for the observed synergism is that there exists a variety of binding sites on the biomass that are specific or partially specific for individual metal species. The correlation of the multicomponent isotherms with the adsorption of these metal ions in ternary metal mixtures is currently being studied.

CONCLUSIONS

The equilibrium biosorption of three metal ions [Pb(II), Ni(II), and Cu(II)] on *R. arrhizus* was studied for three single-metal, three binary, and one ternary systems. In the single-ion situation, adsorption isotherms were developed at different pH values and the adsorption equilibrium data fit the noncompetitive

Freundlich model. The individual Freundlich constants were determined for each metal ion in each system. The pre-exponential coefficients and exponents of the monocomponent isotherms are also parameters of the multicomponent isotherm. The additional six parameters were determined from the competitive adsorption data and the percentage errors and correlation coefficients between experimental and theoretically predicted data were calculated. The Freundlich model for bicomponent systems satisfactorily represented the adsorption equilibrium data of Pb(II), Ni(II), and Cu(II) ions on *R. arrhizus* from binary mixtures. In the ternary system, a similar trend to the binary Pb(II)–Ni(II), Cu(II)–Ni(II), and Pb(II)–Cu(II) systems and the three single-metal systems was observed. The metal selectivity of *R. arrhizus* was of the order Pb(II) $>$ Ni(II) $>$ Cu(II) at pH 5.0.

NOMENCLATURE

a^0 or a_i^0	Freundlich constant ($\mathrm{mg}^{b^0+1}\ \mathrm{g}^{-1}\ \mathrm{L}^{-b^0}$)
a_{12}, a_{21}	competition coefficients for Freundlich model, restricted to binary mixtures
b^0 or b_i^0	Freundlich exponent (dimensionless)
b_{11}, b_{12}	competition coefficients of Freundlich model, restricted to b_{22}, b_{21} binary mixtures (dimensionless)
C_ads	adsorbed metal ion concentration in solution at equilibrium ($\mathrm{mg\ L^{-1}}$)
C_eq	unadsorbed metal ion concentration in solution at equilibrium ($\mathrm{mg\ L^{-1}}$)
C_i	initial metal ion concentration ($\mathrm{mg\ L^{-1}}$)
i	number of metal
q_eq	adsorbed metal ion quantity per unit weight of dried biomass at equilibrium ($\mathrm{mg\ g^{-1}}$)
$q_\mathrm{t,eq}$	adsorbed total metal ion quantity per unit weight of dried biomass at equilibrium ($\mathrm{mg\ g^{-1}}$)
R	correlation coefficient
t	total
Y_eq	adsorption yield at equilibrium (dimensionless)
$\%Y_\mathrm{eq}$	$\frac{C_\mathrm{ads}}{C_\mathrm{i}} \times 100$
$Y_\mathrm{t,eq}$	total adsorption yield at equilibrium (dimensionless)
$\%Y_\mathrm{t,eq}$	$\frac{C_\mathrm{ads,t}}{C_\mathrm{i,t}} \times 100$
ε	percentage error between the experimental and predicted values (%)
$\%\varepsilon$	$([\ \lvert(q_{i,\mathrm{eq}})_\mathrm{predicted} - (q_{i,\mathrm{eq}})_\mathrm{experimental}\ \rvert / (q_{i,\mathrm{eq}})_\mathrm{experimental}] \times 100)$

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