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Biosorption of Heavy Metals by Bacteria Isolated from Activated Sludge

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Abstract

Twelve aerobic bacteria from activated sludge were isolated and identified. These included both Gram-positive (e.g., *Bacillus*) and Gram-negative (e.g., *Pseudomonas*) bacteria. The biosorption capacity of these strains for three different heavy metals (copper, nickel, and lead) was determined at pH 5.0 and initial metal concentration of 100 mg/L. Among these 12 isolates, *Pseudomonas pseudoalcaligenes* was selected for further investigation owing to its high metal biosorption capacity. The lead and copper biosorption of this strain followed the Langmuir isotherm model quite well with maximum biosorption capacity (q_{max}) reaching 271.7 mg of Pb^{2+}/g of dry cell and 46.8 mg of Cu^{2+}/g of dry cell at pH 5.0. Study of the effect of pH on lead and copper removal indicated that the metal biosorption increased with increasing pH from 2.0 to 7.0. A mutual inhibitory effect was observed in the lead-copper system because the presence of either ion affected the sorption capacity of the other. Unequal inhibitions were observed in all the nickel binary systems. The increasing order of affinity of the three metals toward *P. pseudoalcaligenes* was Ni < Cu < Pb. The metal biosorptive potential of these isolates, especially *P. pseudoalcaligenes*, may have possible applications in the removal and recovery of metals from industrial effluents.

Index Entries: Activated sludge; biosorption; copper adsorption; lead removal; bioremediation.

Introduction

Over the past decade, the consumption of metals and chemicals in the process industries has increased dramatically. Industrial uses of metals such as metal plating and tanning, as well as industrial processes utilizing metal as catalysts, have generated large amounts of aqueous effluents that contain high levels of heavy metals. These heavy metals include cadmium,

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chromium, cobalt, copper, manganese, mercury, nickel, silver, and zinc. Metal-polluted industrial effluents discharged into sewage treatment plants could lead to high metal concentrations in the activated sludge. Microbial populations in metal-polluted environments contain microorganisms that have adapted to the toxic concentrations of heavy metals and become "metal resistant" *(1)*.

At present, metal-polluted industrial effluents are mostly treated by chemical methods, such as chemical precipitation, electrochemical treatment, and ion exchange. These methods provide only partially effective treatment and are costly to implement and use, especially when the metal concentration is low. The alternative use of microbe-based biosorbents for the removal and recovery of toxic metals from industrial effluents can be an economical and effective method for metal removal. The metal-removing ability of microorganisms including bacteria *(2,3)*, microalgae *(4,5)*, and fungi *(6)* has been studied extensively. Their capacity for heavy metal removal is apparently higher than for conventional methods and the uptake of heavy metals can be selective *(7,8)*. Microbial cells can also be supplied inexpensively as waste from industrial fermentation processes as well as biologic wastewater treatment plants *(9,10)*.

The present study was conducted to characterize the metal biosorption behavior of bacteria in an activated sludge process treating both municipal and metal-contaminated industrial wastewater. Heavy metals studied included copper, nickel, and lead. One bacterial strain, which was effective in removing metals, was selected for further investigation. The effects of initial metal concentration and pH on the metal biosorption capacity of the selected strain were studied extensively. The metal biosorption isotherms of the selected strain were also compared with activated sludge.

Materials and Methods

Isolation Procedures and Identification

Fresh activated sludge was collected from the return sludge channel at the Shatin Sewage Treatment Works in Hong Kong. The activated sludge was serially diluted with distilled and deionized water. Aliquots (0.1 mL) were spread on nutrient agar and cultivated in a Shell Lab model 2020 incubator at 30°C for 3 d. The isolated colonies were identified by Sherlock Microbial Identification System (MIDI, Newark, DE) and API 20NE as well as 20E tests (BioMerieux, France). The API tests employ a series of biochemical tests, such as amino acid decarboxylation and carbohydrates fermentation, for identifying the bacteria.

Cultivation of Biomass

The bacterial cells of each isolate were grown in 1-L conical flasks containing 200 mL of nutrient broth on an orbital shaker at 200 rpm and 30°C. The 72-h cultivated cells were harvested by centrifugation (Beckman

Model J2-21) at 9000*g* force for 30 min. After two rinses with distilled and deionized water, the cells were suspended in a designated volume of distilled and deionized water for preparing the biomass stock solution. The concentration of the biomass stock solution was determined gravimetrically by withdrawing 5 mL of the solution and oven drying it at 105°C for 24 h.

Screening Tests for Biosorption

Enough bacterial cells to create a final concentration of 1 to 2 g of cell/L was suspended in 100 mL of solution containing 100 mg/L of respective heavy metals (Cu, Ni, Pb) in Nalgene propylene bottles, which were gently agitated at 25°C. The pH of all the metal solution was adjusted to 5.0 by adding 0.1 *M* NaOH and 0.1 *M* HNO_3 just before the experiments and at 21 h during the experiments. Samples (5 mL) were taken from the solution at 3 and 24 h and were subsequently centrifuged at 9000*g* force for 10 min (Beckman Model J2-21). The concentration of each heavy metal in the supernatants was determined using a model 100 Perkin-Elmer atomic absorption spectrophotometer. The biomass concentration was determined after oven drying at 105°C for 24 h.

Kinetics of Biosorption

The kinetics of metal biosorption by a selected isolate, *Pseudomonas pseudoalcaligenes*, was investigated for two metals: lead and copper. One hundred milliliters of a metal solution containing 50 ppm of Cu or 500 ppm of Pb was mixed with about 1.0 g/L of biomass and incubated on a shaker at 200 rpm and 25°C. Samples (3 mL) were withdrawn during the 24 h of incubation at predetermined time intervals and centrifuged immediately (Sigma® 201m) at 15,000*g* force. The metal concentration of the supernatant was analyzed by atomic absorption spectrophotometry.

Adsorption Isotherms

One of the isolated bacteria, *P. pseudoalcaligenes*, had outstanding metal-biosorption ability and was selected for further studies. The selected isolate and activated sludge (1 to 2 g of cell/L) were suspended in solutions containing different heavy metal concentrations. The pH of the metal solution was adjusted to 5.0 by adding 0.1 *M* NaOH and 0.1 *M* HNO_3 just before experiments and at 21 h during experiments. After 24 h of incubation at 25°C, 5-mL samples were taken from the solutions, and the metal concentrations in the supernatants were measured by atomic absorption spectrophotometry.

Effect of pH on Biosorption

The initial metal concentration of the experiment was 100 mg/L for copper and 200 mg/L for lead. The pH of the metal and biomass suspension was adjusted to the appropriate value by adding 0.1 *M* NaOH and

0.1 *M* HNO_3 just before experiments and at 21 h during experiments. After 24 h of incubation at 25°C, 5-mL samples were taken from the solutions and centrifuged, and the metal concentrations in the supernatants were measured.

Binary Metal Biosorption System

Three metals—lead, copper, and nickel—were considered for binary biosorption studies; two of the three metals were considered each time. The initial metal concentration of metal one was changed from 0.2 to 2.0 mmol/L, and the initial concentration of metal two was held constant at 0.2, 0.5, 1.0, and 2.0 mmol/L. The other conditions were the same as in the single metal biosorption experiments.

Results and Discussion

Isolation and Identification

In nonselective isolation, a dilution of activated sludge was streaked on nutrient agar plates and 12 colonies were isolated. Isolation of microbial strains from activated sludge or other waste streams for biosorption of heavy metals has been studied extensively *(11–13)*. It is impossible to isolate all the bacteria that might be present in the sludge; only the dominant cultivable species were isolated in the nonselective isolation. Table 1 gives the identification results by the MIDI Sherlock Microbial Identification system as well as API 20 NE and 20E systems. Species A–M were nonselectively isolated bacteria.

The heterotrophic isolates from the activated sludge belonged to a wide variety of species including Gram negative and Gram positive. Most were rods and a few bacteria were filamentous or coccus. *Pseudomonas*, *Bacillus*, and *Aeromonas* species are commonly found in activated sludge *(1)*.

Biosorption of Heavy Metals

Batch biosorption experiments were conducted to investigate the metal-removing ability of each bacterium isolated. The metals chosen for tests were copper, nickel, and lead; Figure 1 presents the results.

For copper biosorption, species L and M had a Q_e (equilibrium biosorption capacity, mg of metal Cu^{2+}/g of dry biomass) >35 mg/g among the 12 isolates with an initial concentration of 100 mg/L (Fig. 1). Only three species had a Q_e >10 mg/g among all the isolates in nickel biosorption test, and no outstanding species for nickel removal were found. Under the conditions of initial concentration of 100 mg of Pb^{2+}/L and pH 5.0, the Q_e values of species H, L, and M were about 60 mg/g and about five species had a Q_e of about 50 mg/g.

Significant differences were observed in biosorption of metal ions by the various bacterial strains examined. Because metal biosorption from solution is predominantly owing to physicochemical interactions between

Table 1
Identification of Species

Species	Nonselectively isolated
A	*Bacillus pumilus* GC subgroup B
B	*Neisseria sicca*
C	*Aeromonas hydrophila*
D	*Pseudomonas* sp.
E	*Xanthomonas maltophilia*
F	*Bacillus lentimorbus*
G	*Pseudomonas putida*
H	*Bacillus subtilis*
J	*Gordona bronchialis*
K	*Kocuria varians*
L	*Pseudomonas pseudoalcaligenes*
M	*Micrococcus luteus* GC subgroup B

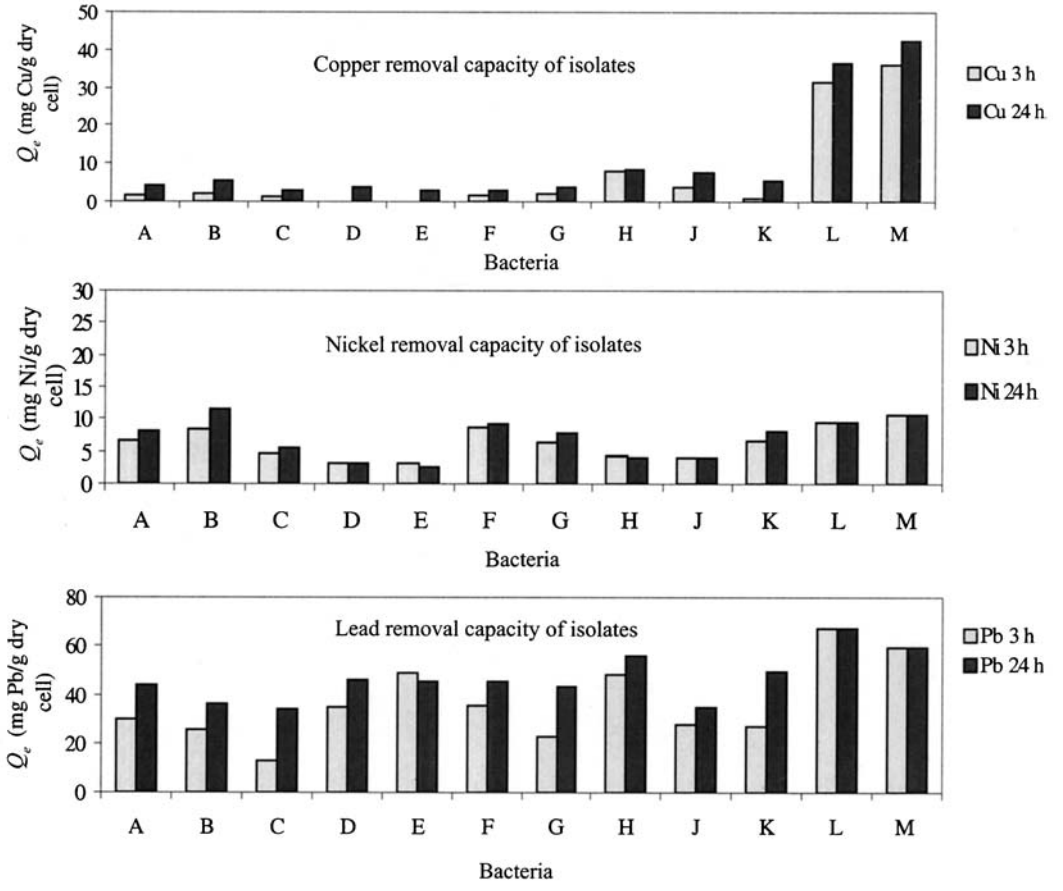

Fig. 1. Metal removal capacity of isolates with retention times of 3 and 24 h on 100 ppm initial concentration at pH 5.0.

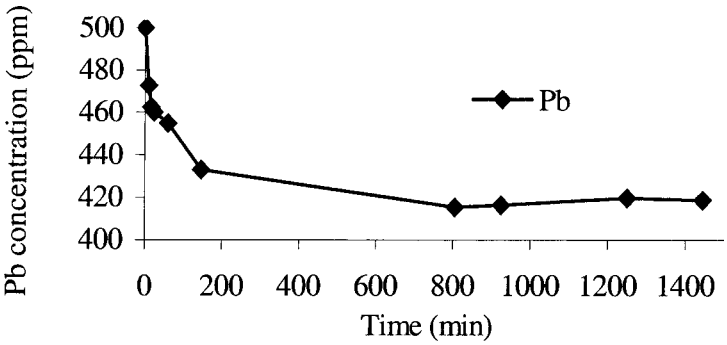

Fig. 2. Kinetics of lead sorption by *Pseudomonas pseudoalcaligenes* at pH 5.0.

the biomass and metal in solution, morphologic differences existing within the biomass can greatly influence the biosorption process. The stereochemical differences in the structures of the cell envelope can make a significant difference in the acceptance of metallic ions by these structures *(14)*. Cell wall is the most important structure that may form the cell envelope, but capsules, S-layers, and sheaths are commonly found superimposed on the wall *(15)*. The Gram-negative bacteria (e.g., *Pseudomonas*) possess cell walls that are chemically and structurally more complex than the Gram-positive bacteria (e.g., *Bacillus*), resulting in different metal biosorption capacities. Many species of bacteria isolated from activated sludge have been shown to produce extracellular polymers, which provide surface sites for adsorbing and complexing heavy metals. Increased production of extracellular polymers may enhance metal binding *(16)*.

Most studies have reported that copper and lead are removed more efficiently than many other metals, and nickel has one of the lowest removal efficiencies associated with it *(17)*. In the present study, similar results were obtained. Copper and lead were significantly removed whereas nickel was poorly removed. Among all the isolates, species L (*P. pseudoalcaligenes*) was one of the most effective bacteria in removing copper and lead from the aqueous solutions. Hence, this species was selected for further investigation on the kinetics and equilibrium of biosorption and effect of pH and competing cations.

Kinetics of Biosorption

The kinetics of metal biosorption by *P. pseudoalcaligenes* is shown in Figs. 2 and 3. Biosorption was rapid, since the initial decrease in metal concentration was very fast. Half of the total metal adsorption occurred within 10 min. For copper, the equilibrium concentration was attained at about 500 min (Fig. 3), and 800 min was required for lead adsorption to reach equilibrium (Fig. 2).

Metal Biosorption Isotherms

The Langmuir and Freundlich isotherms were used to simulate the biosorption of lead and copper by species L and activated sludge. Table 2

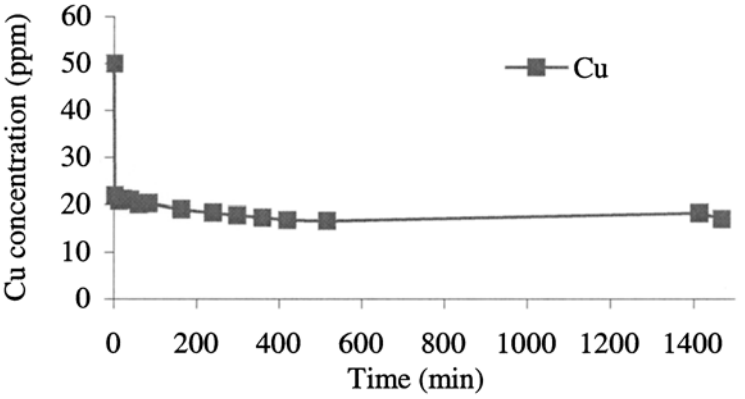

Fig. 3. Kinetics of copper sorption by *P. pseudoalcaligenes* at pH 5.0.

Table 2
Parameters for Langmuir and Freundlich Isotherms

Parameter	Definition
Q_e or Q	Equilibrium adsorption capacity (mg or mmol of metal/g of dry cell)
C_e	Equilibrium metal concentration (mg/L or mmol/L)
Q_{max}	Maximum biosorption capacity (mg of metal/g of dry cell)
b	Langmuir isotherm constant (L/mg metal)—measures effectiveness of biosorption at low metal concentrations
k	Freundlich coefficient (L/g of dry cell)—represents amount of metal adsorbed when concentration of solution in equilibrium is unity
n	Freundlich isotherm constant—measures impact on biosorption of change in residual solution concentration from unity

gives the parameters used in the Langmuir and Freundlich isotherms. Figures 4 and 5 show the copper biosorption isotherm and linearized Langmuir isotherm for both *P. pseudoalcaligenes* and activated sludge.

Figures 6 and 7 show the lead biosorption isotherms for both *P. pseudoalcaligenes* and activated sludge. Table 3 gives the calculated parameters for the Langmuir and Freundlich isotherms. The copper and lead biosorption data were well described by both the Langmuir and Freundlich isotherms with good linear relation (Table 3). Q_{max} represents the saturation level of sorbed metal at high metal concentrations. Based on the Q_{max} values, species L has higher maximum biosorption capacity for both copper and lead than activated sludge. Species L also has higher b values for both copper and lead compared to activated sludge. Since the parameter b measures the effectiveness of biosorption at low metal concentrations, species L has higher adsorption ability compared to activated sludge at low metal concentrations. In other words, species L has higher affinity than activated sludge.

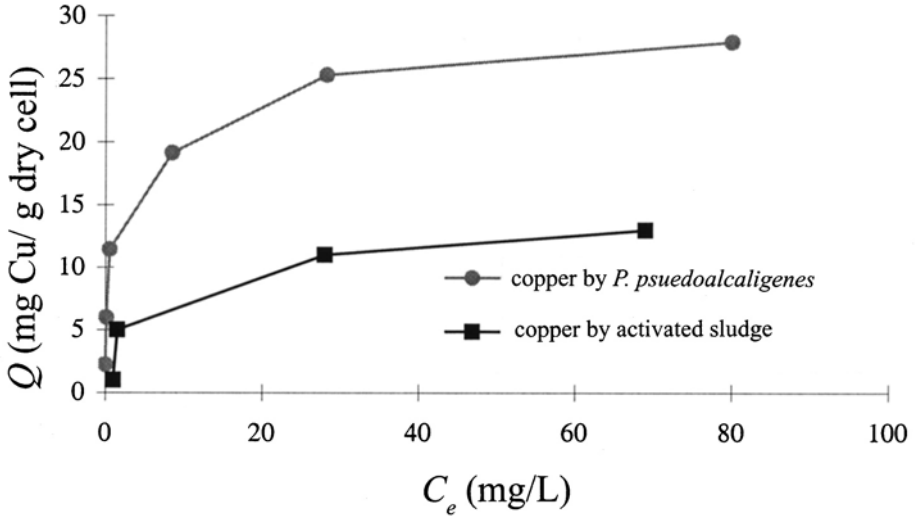

Fig. 4. Equilibrium isotherm for copper biosorption by *P. pseudoalcaligenes* and activated sludge.

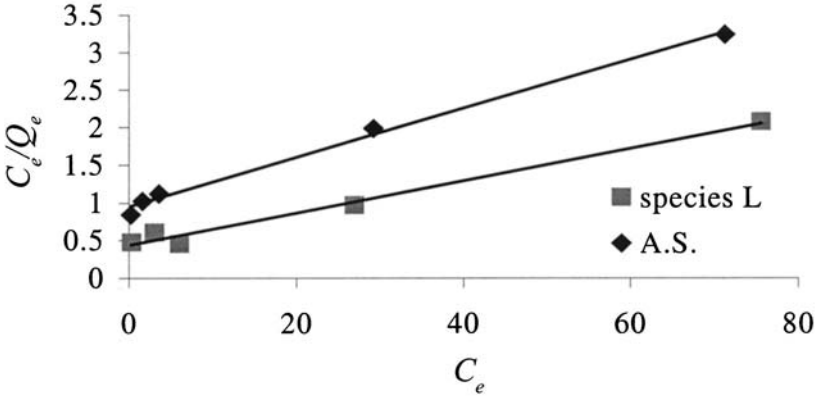

Fig. 5. Linearized Langmuir isotherm plot for copper biosorption by *P. pseudoalcaligenes* and activated sludge (A.S.).

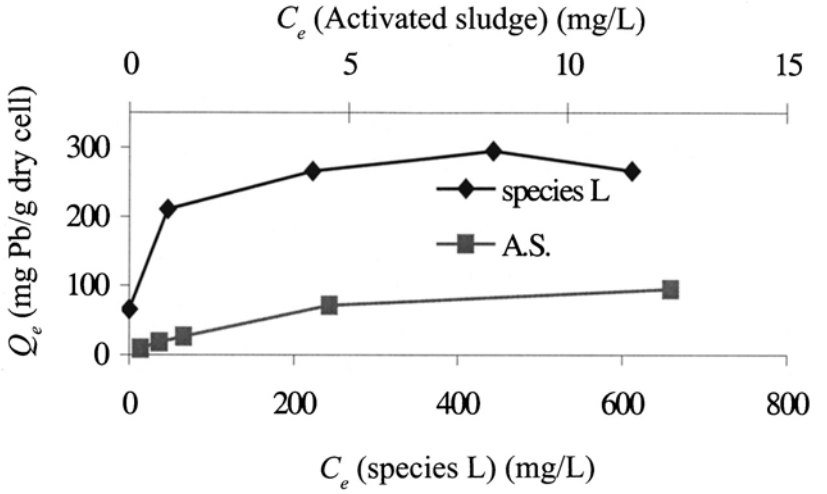

Fig. 6. Equilibrium isotherm for lead biosorption by *P. pseudoalcaligenes* and activated sludge (A.S.).

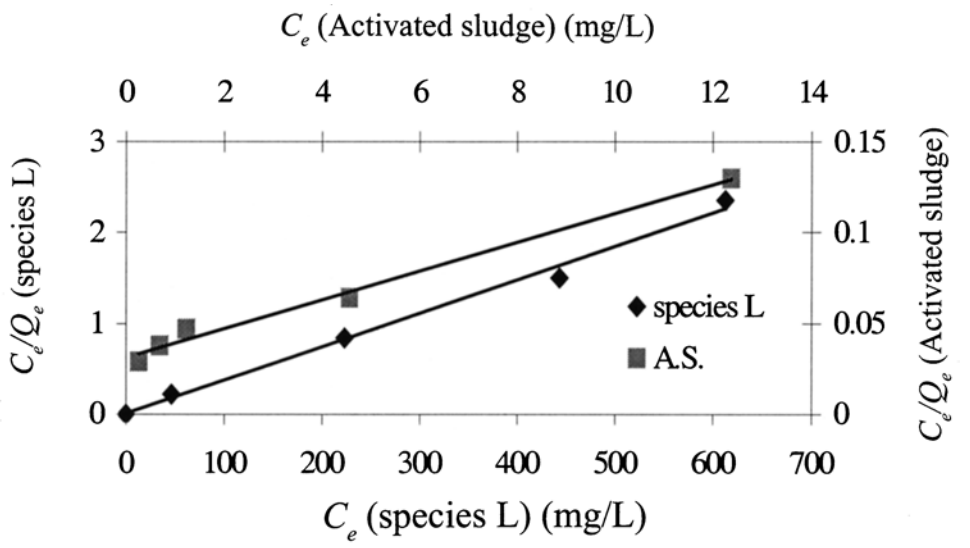

Fig. 7. Linearized Langmuir isotherm plot for lead biosorption by *P. pseudoalcaligenes* and activated sludge (A.S.).

Table 3
Langmuir and Freundlich Parameters for Copper and Lead Biosorption

	Copper		Lead	
Langmuir	Species L	Activated sludge	Species L	Activated sludge
Q_{max} (mg/g of dry cell)	46.8	30.7	271.7	126.4
b (L/mg)	0.048	0.034	0.46	0.25
r^2	0.984	0.994	0.992	0.989
Freundlich				
n	1.33	1.30	8.96	1.58
k	2.08	1.01	140.0	22.6
r^2	0.952	0.992	0.802	0.983

The Freundlich coefficient, k, represents the amount of metal adsorbed when the concentration of the solution in equilibrium is unity. The value of k for *Pseudomonas* is higher than that for activated sludge for both metals. This is consistent with the Q_{max} values in the Langmuir isotherm model. On the other hand, n measures the impact on biosorption of a change in residual solution concentration from unity. A low value of n implies a relatively large change in sorbed metal when residual concentration deviates from unity, either above or below.

Effect of pH

The lead and copper biosorption by *P. pseudoalcaligenes* was strongly affected by solution pH, as indicated in Fig. 8. Metal uptake was negligible

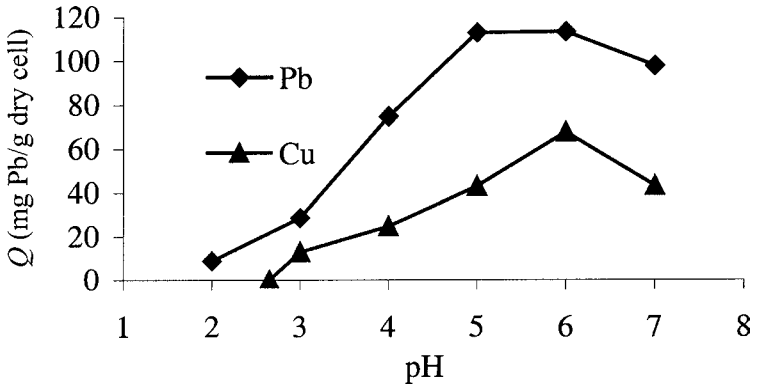

Fig. 8. Effect of pH on lead and copper biosorption by *P. pseudoalcaligenes*.

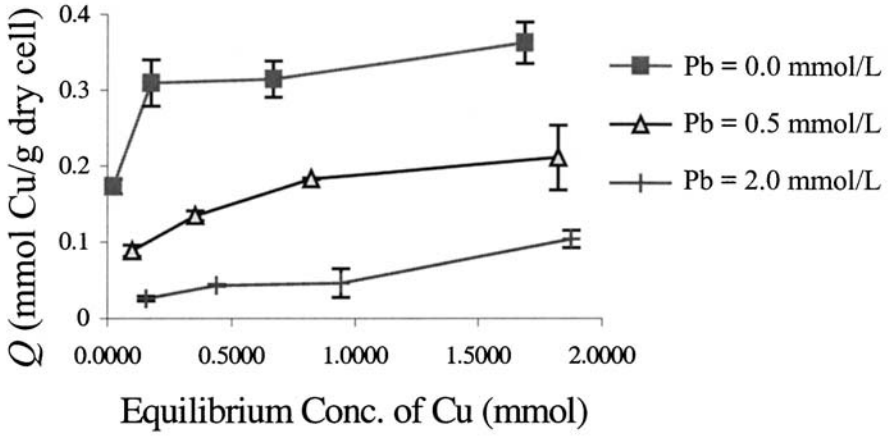

Fig. 9. Effect of lead on biosorption of copper by *P. pseudoalcaligenes* at pH 5.0.

at pH 2.0 and then increased rapidly with increasing pH. These results could be explained by the competition between hydrogen ions and metal ions for the sorption sites of cells *(18)*. At very low pH values, metal cations and protons compete for binding sites on the cell walls, which results in lower uptake of metal. As pH levels are increased, more ligands with negative charge would be exposed with a subsequent increase in attraction for positively charged metals ions.

Binary Metal Biosorption System

The adsorption isotherms of copper ions in the absence and presence of lead or nickel ions are shown in Figs. 9 and 10. The equilibrium copper uptake increased with increasing initial copper concentration up to a certain concentration at which the equilibrium uptake reached a steady level. The presence of lead ions (Fig. 9) decreased the amount of copper adsorbed at equilibrium. Figure 10 shows that the biosorption of copper is not signifi-

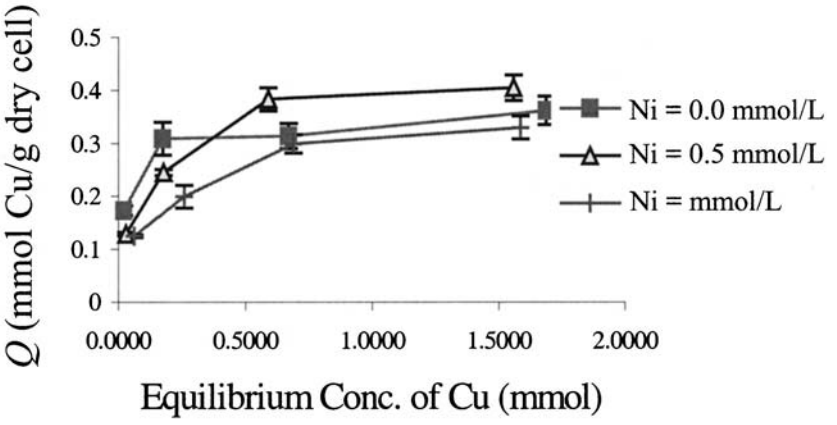

Fig. 10. Effect of nickel on biosorption of copper by *P. pseudoalcaligenes* at pH 5.0.

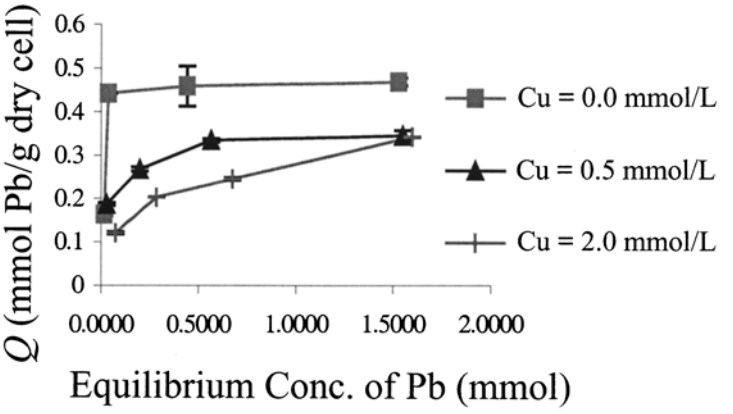

Fig. 11. Effect of copper on biosorption of lead by *P. pseudoalcaligenes* at pH 5.0.

cantly decreased by the presence of nickel. It was obvious that the effect of lead on copper sorption was greater than that of nickel.

Figures 11 and 12 depict the variations in lead uptakes at equilibrium with increasing initial concentration of copper and nickel. The presence of copper ion reduced moderately the uptake of lead (Fig. 11). The reduction was about 40% when copper was present at 2.0 mmol/L after 3 h. The presence of nickel ion also reduced lead biosorption, but to a much lesser extent (Fig. 12).

The presence of lead or copper affected significantly the nickel biosorption by *P. pseudoalcaligenes*. The nickel uptake was reduced by 80% in the presence of 2.0 mmol/L of either one of the ions (Figs. 13 and 14). Furthermore, the extent of inhibition was enhanced at increasing competing ion concentrations. This progressive interference in nickel biosorption by lead and copper ions indicated the possibility of overlap in the sorption sites. In the case of copper inhibition (Fig. 13), a decrease of about 40% uptake was observed at an initial copper concentration of 0.05 mmol/L, whereas

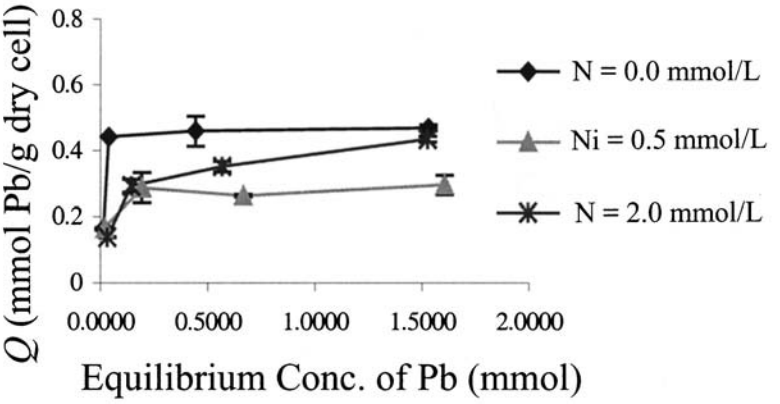

Fig. 12. Effect of nickel on biosorption of lead by *P. pseudoalcaligenes* at pH 5.0.

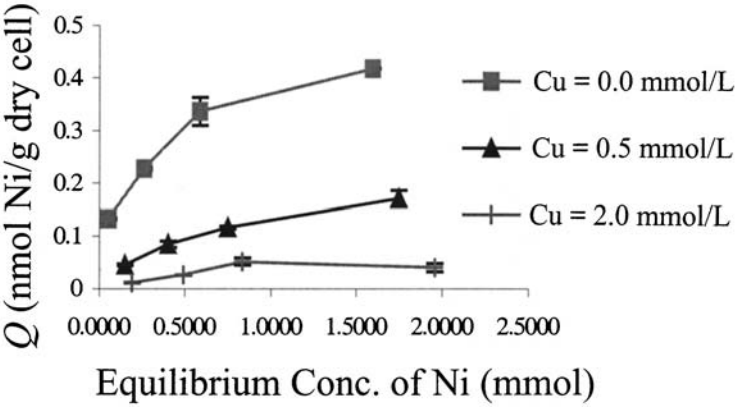

Fig. 13. Effect of copper on biosorption of nickel by *P. pseudoalcaligenes* at pH 5.0.

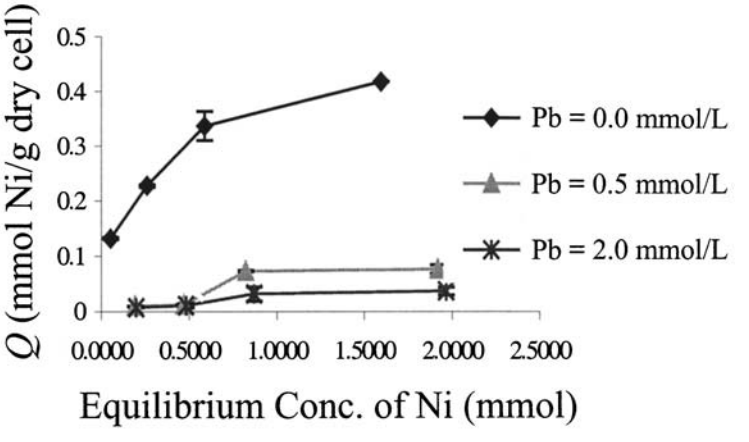

Fig. 14. Effect of lead on biosorption of nickel by *P. pseudoalcaligenes* at pH 5.0.

a decrease of about 60% was observed in lead biosorption isotherms (Fig. 14). Thus, the order of increasing inhibition for nickel biosorption was Cu < Pb.

In the present studies, a mutual inhibitory effect was observed in the lead-copper system because the presence of either one of the ions affected the biosorption capacity of the other. However, unequal inhibitions were observed in all the nickel binary systems, including the nickel-copper and nickel-lead systems. The presence of either lead or copper reduced significantly the biosorption capacities of nickel, whereas the uptakes of lead and copper were not greatly affected by nickel. Similar unequal inhibitions were reported in the zinc-cadmium biosorption system by *Chlorella vulgaris (19)*. Zhang et al. *(18)* reported that lead biosorption by *Rhizopus nigricans* was depressed in the presence of zinc or iron whereas it was not inhibited by the presence of manganese. Wong et al. *(20)* observed that the biosorption of copper by *Pseudomonas putida* II-11 was reduced in the presence of lead, whereas it was not affected by zinc and nickel. Tobin et al. *(21)* have proposed various mechanisms of metal uptake from binary metal systems. These include direct competition for the binding sites and preferential binding to different sites. In the present study, lead seems to be preferentially bound to the binding sites in addition to the capability of competing for the binding sites. The increasing order of affinity of the three metals toward *P. pseudoalcaligenes* was Ni < Cu < Pb.

Conclusion

Twelve bacteria were isolated and identified from an activated sludge process treating both metal-contaminated industrial effluents and municipal wastewaters. These isolates included both Gram-positive (e.g., *Bacillus*) and Gram-negative (e.g., *Pseudomonas*) bacteria. The biosorption capacity of these strains for three different heavy metals—copper, nickel, and lead—was determined at pH 5.0 with an initial metal concentration of 100 mg/L. Among these isolates, *P. pseudoalcaligenes* was selected for further investigation owing to its high metal biosorption capacity. Both Langmuir and Freundlich adsorption isotherms represent adequately the distribution of copper and lead for this bacterium. Study of the effect of pH on both copper and lead removal by the selected strain indicated that biosorption increased with increasing pH from 2.0 to 6.0. A mutual inhibitory effect was observed in the lead-copper system because the presence of either ion affected the biosorption capacity of the other. Unequal inhibitions were observed in all the nickel binary systems. The increasing order of affinity of the three metals toward *P. pseudoalcaligenes* was Ni < Cu < Pb. The metal biosorptive potential of these isolates, especially *P. pseudoalcaligenes*, may have possible applications in the removal and recovery of metals from industrial effluents.

Acknowledgments

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