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Biosorption of Metal Ions on *Arthrobacter sp.*: Biomass Characterization and Biosorption Modeling

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A culture of *Arthrobacter sp.* was tested for its ability to sorb copper, cadmium, and iron ions, and chemical modeling of experimental data was used to interpret the mechanism of biosorption. The purpose of the initial phase was to clarify the nature and concentration of active acidic sites on cell wall with the aid of potentiometric titration of an aqueous cellular suspension and IR analysis of the lyophilized biomass in solid phase. The experimental results showed that the cell wall probably has at least two weakly acidic sites. In the second part of the experimental work *Arthrobacter sp.* was suspended in the presence of copper, cadmium, and iron ion solutions at different equilibrium pH values. Adsorption isotherms were obtained by using an original procedure defined as the "subsequent additions method" (SAM). The results showed a strong influence of pH, especially over pH 5. A chemical model based on a possible set of reactions between ions in solution and the biomass has been developed. Mechanistic modeling revealed the complexity of the metal biosorption phenomenon and the need to consider different biosorption mechanisms. Up to pH 5 experimental data at different equilibrium pH values can be described using a model which takes into account only two mechanisms (ion exchange and complexation). Over pH 5 other mechanisms (probably precipitation or coprecipitation on cell wall surface) should be considered.

1. Introduction

Metal species released into the environment by technological activities tend to persist indefinitely, circulating and eventually accumulating throughout the food chain, becoming a serious threat to the environment.

Environmental pollution by toxic metals occurs globally through military, industrial, and agricultural processes and waste disposal. Fuel and power industries generate 2.4 million tons of As, Cd, Cr, Cu, Hg, Ni, Pb, Se, V, and Zn annually. The metal industry adds 0.39 million tons per year of the same metals to the environment, while agriculture contributes 1.4 million tons per year, manufacturing generates 0.24 million tons per year, and waste disposal adds 0.72 million tons per year (*1*).

Precipitation, ion exchange, electrochemical processes, and/or membrane processes are commonly applied to the treatment of industrial effluents. However, the application of such processes is often restricted because of technical or economic constraints (*2*).

Biosorption of heavy metals is one of the most promising technologies involved in the removal of toxic metals from industrial waste streams and natural waters. It is a potential alternative to conventional processes for the removal of metals, such as ion exchange processes. If the biomass employed is a waste material, then biosorption represents a cheap alternative to conventional processes, due to the use of a low cost sorbing material. Many microorganisms (algae, bacteria, fungi, and yeasts) are able to accumulate heavy metals from solutions (*3* and all references). The biosorption mechanisms can be metabolism independent and a function of the microbial cell activity (metabolism dependent) (*4*). The first mechanism (in general it is based on adsorption, ion exchange, complexation, and/or microprecipitation) appears to be the most common. In fact, cell walls, consisting mainly of polysaccharides, proteins, and lipids, offer many functional groups which can bind ions such as carboxylate, hydroxyl, sulfate, phosphate, amide, and amino groups. Metal sorption performance depends on some external factors such as pH, other ions in solution which may be in competition, organic material such as complexing agents, cell metabolic products which may cause metal precipitation, and temperature (*4*).

Experimental sorption data are usually represented by various empirical models where heavy metal adsorption is described by a simple mathematical relationship between the concentration of the heavy metal in the liquid and solid phases, at the equilibrium and at constant temperature. These adsorption isotherms are based only on empirical bases (Freundlich isotherm) or derived from isotherms originally developed for different systems (Langmuir isotherm derived for gaseous adsorption on planar surfaces). These isotherms have been widely applied since they are simple, give a good description of experimental behavior in a large range of operating conditions, and are characterized by a limited number of adjustable parameters. However, empirical models cannot predict the effect of such important factors as pH, ionic strength, and metal competition. To describe the biosorption equilibrium process mechanistic modeling may be a useful alternative. The definition of mechanistic models used here refers to all those models that represent the adsorption phenomena by the description of the main possible reactions between the ions in solution and the solid adsorbing surface. Generally ion exchange and surface complexation represent the main mechanisms involved in the metal adsorption. Mechanistic modeling is mainly used as a tool for the investigation because it allows to confirm or reject hypotheses on mechanisms, to evaluate related physical chemical parameters, and to simulate the unit operation performances in optimization studies (*5*).

Although many experimental studies have been published on metal ion biosorption, a much greater effort should be addressed in the development of mechanistic models able to predict and explain metal ion biosorption (*6*−*11*).

The aim of the present work was the development of an equilibrium model capable of identifying the chemical

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mechanisms of biosorption and to predict the effect of pH on the biosorption process.

A biomass characterization study was performed, and a chemical model was developed to describe and simulate the biosorption process. An original and reliable experimental procedure (SAM) has been here developed based on the growing need of standardizing experimental methods. This last point is of central importance because equilibrium studies are in general carried out with different experimental procedures. Very widespread experimental results (both for equilibrium and kinetic) have been found in the literature by different authors although similar nominal experimental conditions have been used (*4*). To compare different experimental data sets standardized procedures should be developed (*5*). Moreover the SAM procedure permits saving both labor and materials in equilibrium biosorption tests. Although *Arthrobacter sp*. does not have a large metal uptake capacity (*12*), in the present work this microorganism was used as a test system, and no technological aspects were considered here.

2. Materials and Methods

2.1. Microorganism. *Arthrobacter sp*. was isolated from natural waters near L'Aquila (Italy). The following medium was used for cell cultivation and maintenance: sucrose (50 g/L), NH_4NO_3 (2.5 g/L), KH_2PO_4 (1.5 g/L), $CaCl_2$ (0.1 g/L), $MgSO_4$ (1.0 g/L), and yeast extract (3.5 g/L) (*12*). Fermentation was made in a reactor at 30 °C, and the level of growth of biomass was controlled by a glucose test. The biomass produced was washed, centrifuged, and lyophilized (*13*).

2.2. Biomass Characterization. Chemical modeling of metal biosorption requires the characterization of the biomass used as biosorbent. To establish nature and concentration of active sites on the cell wall, ionic content, potentiometric titer, and IR spectrum in the solid phase of the lyophilized biomass were carried out.

2.2.1. Major Cation Content. One gram of lyophilized biomass was protonated adding 50 mL of 0.1 N HCl prepared with deionized water. The suspension was kept for 1 h in an orbital rotary shaker at 250 rpm and 30 °C, then centrifuged at 6000*g* for 15 min, and finally filtered (0.2 μm). The pellet was resuspended in HCl, and the previous treatment was repeated three times (*14*). The washing waters so obtained were analyzed to determine alkaline and alkaline earth metals originally present in the medium of cultivation (Na, K, Mg, Ca).

2.2.2. Potentiometric Titration. The protonated biomass was washed with deionized water, and then a suspension (0.5 g of lyophilized biomass in 10 mL of water) was potentiometrically titrated with 0.1 N NaOH (*13, 14*).

2.2.3. IR Spectrum. To give a qualitative and preliminary analysis of the main chemical groups present on the cell membrane an IR analysis in solid phase was performed on lyophilized biomass in a KBr disk (*15*) using a FTIR Perkin-Elmer 1760X.

2.3. Biosorption Trials. The specific uptake of Cu, Cd, and Fe at different equilibrium pH was determined by using an original experimental procedure (see Figure 1) defined as the "subsequent additions method" (SAM): (1) 0.2 g of lyophilized biomass was suspended in 100 mL of distilled water and rehydrated under agitation for 1 h; (2) the first addition of a concentrated solution (10 g/L) of the metallic ion as chloride ($FeCl_3$, $CuCl_2$ and $CdCl_2$) was added to the cellular suspension maintained in agitation and at constant pH by 0.1 N NaOH; (3) after 30 min, the system reaches equilibrium (*16*), and 1.5 mL of the suspension was taken, centrifuged, and analyzed by ICP (Varian Liberty 150) to determine the residual metal concentration; and (4) a second addition of the concentrate metal solution was made, and the previous procedure was repeated.

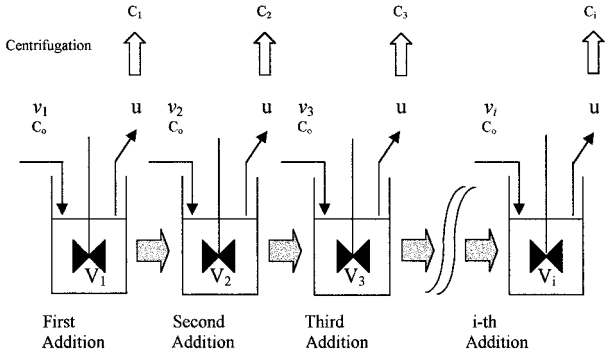

FIGURE 1. The subsequent additions method: an aliquot of a concentrate metal solution was added every 30 min to a cellular suspension; before the following addition a drawing was made to evaluate the final metal concentration.

The SAM procedure was repeated using samples without biomass and making the same additions (metal and NaOH solutions) and drawings: these blank samples are necessary to avoid confusion between the biosorption uptake and the possible metal hydroxide precipitation.

Using the SAM the whole sorption isotherm is obtained by using only one sample and saving time, reagents, and biomass.

The use of a lyophilized biomass instead of a fresh one does not change biosorption performances; furthermore with the use of a lyophilized biomass much more reliable experimental data are obtained (*13*).

The evaluation of the metal specific uptake q (mmol/g of lyophilized biomass) is obtained by using the following mass balance of the metallic ion in the system (eq 1)

$$v_i \cdot C_o + q_{i-1} \cdot X_{i-1} \cdot (V_{i-1} - u) + (V_{i-1} - u) \cdot C_{i-1} = C_i \cdot V_i + q_i \cdot X_i \cdot V_i \quad (1)$$

where the index "i" and "i-1" refer to the metal addition, C_o is the concentration of the metal solution added, v is the metal addition volume, C is the equilibrium metal concentration, V is the suspension total volume, u is the suspension drawing volume, X is the biomass concentration, and q is the metal specific uptake. No significant changes (data here not reported) in the biosorption properties have been observed comparing the biosorption tests obtained by using the SAM and the conventional procedure adopted for adsorption studies (*13*).

After every addition, the suspension volume, the equilibrium metal concentration, the added volumes of metal solution and NaOH, the metal specific uptake, and the biomass concentration change. Experimental data (here not reported) showed that the presence of alkaline and alkaline earths ions in solution, in the range of concentration investigated (0−100 mg/L), does not influence heavy metal biosorption performances.

3. Experimental Results and Discussion

3.1. Major Cation Content. The experimental results of washing waters analysis are reported in Table 1. The total ionic content resulted in 0.51 mequiv/g of lyophilized biomass; this value can be considered as an approximate measure of the ion exchange capacity of the utilized biomass (*14*).

3.2. Titration Curve and Chemical Modeling. The washed protonated biomass was potentiometrically titrated with 0.1 N NaOH. The biomass titration data are shown in Figure 2.

TABLE 1. Major Cation Content of the *Arthrobacter sp.*

no. of washing	Na^+ (mg/L)	K^+ (mg/L)	Mg^{2+} (mg/L)	Ca^{2+} (mg/L)
1	27	150	40	22.6
2	2.25	5.37	1.74	2.66
3	1.8	0.57	0.54	1.62
4	0.5	0.58	0.07	0.65
total ionic content mg/g	1.58	7.83	2.18	1.38
total ionic content mmol/g	0.07	0.20	0.09	0.03
total ionic content mequiv/g	0.07	0.20	0.18	0.06

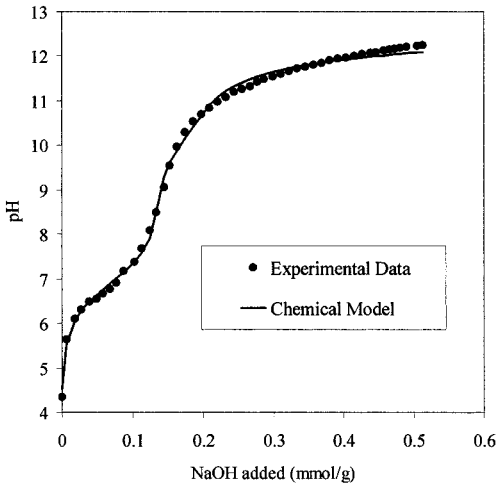

FIGURE 2. Biomass potentiometric titration: 1 g of lyophilized biomass was protonated by HCl 0.1 N, washed with water, and then potentiometrically titrated with 0.1 N NaOH. Symbols are experimental data, and the solid line is the prediction of the two sites chemical model.

It would seem that the *Arthrobacter sp.* cell wall has two main functional groups. If the only active sites are those titrated, the biosorbing abilities of *Arthrobacter sp.* are strongly influenced by pH.

In biosorption chemical modeling development, it is necessary to have a basic characterization of the adsorbent material. To evaluate nature and concentration of acidic sites, experimental data were elaborated with an original chemical model. Different sets of acid−base reactions could be postulated. Among these the hypothesis of two weakly acidic sites (S_1 and S_2) on the cell wall (reported in the follow) resulted in the simplest that gives good results

$$S_1H \overset{K_{a1}}{\rightleftharpoons} S_1^- + H^+ \quad K_{a1} = \frac{[S_1^-]\cdot[H^+]}{[S_1H]} \tag{2}$$

$$S_2H \overset{K_{a2}}{\rightleftharpoons} S_2^- + H^+ \quad K_{a2} = \frac{[S_2^-]\cdot[H^+]}{[S_2H]} \tag{3}$$

$$[S_1]_{Tot} = [S_1^-] + [S_1H] \tag{4}$$

$$[S_2]_{Tot} = [S_2^-] + [S_2H] \tag{5}$$

$$[Na^+] + [H^+] = [S_1^-] + [S_2^-] + [OH^-] \tag{6}$$

where $[S_1]_{Tot}$ and $[S_2]_{Tot}$ stand for the total concentrations of the two weakly acidic sites in the dissociated (S_1^- and S_2^-) or associated (S_1H and S_2H) forms and K_{a1} and K_{a2} are the acidic equilibrium constants.

Combining the equilibrium acidic constants (eqs 2 and 3), the sites mass balances (eqs 4 and 5), and the electrical charge balance (eq 6), the relation between the added volume of NaOH and the measured pH was obtained (eq 7)

$$C_{Na} + [H^+] = \frac{K_{a1}\cdot[S_1]_{Tot}}{[H^+] + K_{a1}} + \frac{K_{a2}\cdot[S_2]_{Tot}}{[H^+] + K_{a2}} + \frac{K_w}{[H^+]} \tag{7}$$

where K_w is the water dissociation constant, V_{NaOH} is the added volume of NaOH, V_o is the initial suspension volume, and C_{Na} (eq 8) is the Na^+ concentration in the suspension after every addition of the NaOH solution with C^o_{NaOH} as the initial concentration.

$$C_{Na} = V_{NaOH}\cdot C^o_{NaOH}/(V_o + V_{NaOH}) \tag{8}$$

The relation 7 contains four parameters $[S_1]_{Tot}$, $[S_2]_{Tot}$, K_{a1}, and K_{a2} which were evaluated by a nonlinear regression method. In Figure 2 and in Table 2 the values predicted by the chemical model are compared with the experimental data. It is possible to see the good agreement between predicted and experimental results. Even if the bacterial cell wall is a very complex biomaterial (*17*), it is noteworthy that it behaves like an inorganic traditional adsorbent with two weakly acidic sites.

The total concentration of titrated groups (0.41 mmol/g of lyophilized biomass) is close to the total ionic content determined in the protonation phase (0.51 mmol/g, Table 1) (*14*): the little difference could be caused by biomass lost during washings or by possible excess of salt from the culture medium although the biomass was washed three time to remove it.

Considering the values of the acidic constants and the general composition of the Gram-Positive bacteria cell wall (*17*), a phosphate and an amino or amide groups may be supposed.

3.3. IR Spectrum. The IR spectrum (Figure 3) is highly complex, reflecting the complex nature of the lyophilized biomass. Despite this complexity some characteristic peaks can be assigned. A phosphate and an amino or amide group were hypothesized on the bases of titration curve and Gram-positive cell wall composition. The amino group presents characteristic absorption at 3500−3000 cm^{-1} (N−H stretching) and at 1250−1000 cm^{-1} (C−N stretching). N−H stretching peak lays in a spectrum region occupied by a broad and strong band (3600−3000 cm^{-1}). This may be due to hydroxyl groups that are hydrogen bonded to various degrees. IR analysis was made without using a drier box, and then the presence of intense OH peaks in the spectrum could be due both to the great hygroscopicity of lyophilized biomass and to the real presence of hydroxylic groups in the biomass. The C−N stretching peak is also covered by another strong band (1200−950 cm^{-1}) that can be attributed to an alcoholic C−O stretching. The amide group presents characteristic absorption at 3350−3180 cm^{-1} (N−H stretching) and at 1650 cm^{-1} (C═O stretching). Again the N−H stretching peak is covered, while a peak at 1646 cm^{-1} can be assigned to C═O stretching of the acidic group. The phosphate group presents some characteristic absorption peaks (P═O stretching at 1150 cm^{-1}; P−OH stretching at 1040−910 cm^{-1}; P−O−C stretching at 1050−970 cm^{-1}), but they fall in the region of the spectrum occupied by a strong and large band. The weak absorption peaks at 916 and 772 cm^{-1} may be attributed to the glycoside bonds in the polysaccharide structure of the biomass: polysaccharide presence is also confirmed by the strong C−O band between 1200 and 950 cm^{-1}. IR spectrum analysis seem to give some qualitative ideas on the hypothesis of the presence of amide groups on the cell wall although this result may be ambiguous because by analyzing whole cells it is possible to evaluate the functional groups present in the

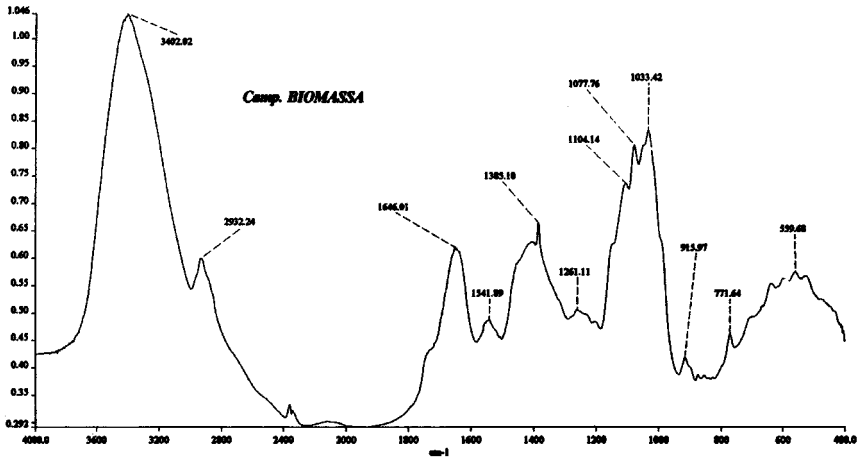

FIGURE 3. IR spectrum in solid phase of the lyophilized biomass in KBr disk.

TABLE 2. Chemical Modeling of the Potentiometric Titration Curve

parameters	estimated values	SD
$[S_1]_{Tot}$ (mmol/g)	0.272	0.004
$[S_2]_{Tot}$ (mmol/g)	0.138	0.009
K_{a1} (mol/L)	1.3×10^{-7}	0.1×10^{-7}
K_{a2} (mol/L)	7.7×10^{-11}	0.8×10^{-11}

cytoplasm too. In conclusion, the IR spectrum may give some idea on the nature of the cell membrane, but further tests are necessary to give much more experimental evidence such as the IR analysis of the cell debris obtained after cell disruption and ultracentrifugation and evaluating the change of the IR spectrum before and after the biosorpiton process. Further work is in progress to analyze these aspects, and, in any case, the results obtained at this stage were considered sufficient to give a first preliminary idea about the presence of a polysaccharide structure on the bacterial cell walls.

3.4. Sorption Isotherm of Cu, Cd, and Fe: pH Effect and Chemical Modeling. The Langmuir sorption model was used for experimental data fitting (eq 9)

$$q = \frac{q_{max} \cdot b \cdot Ceq}{1 + b \cdot Ceq} \tag{9}$$

where q_{max} (mmol/g of lyophilized biomass) is the maximum specific metal uptake and b (L/mmol) is the ratio of the adsorption/desorption rates. For experimental data fitting, the Langmuir model was linearized (by double reciprocal), and the parameters were estimated by using the least-squares method modified by Mezaky (*18*).

In Figures 4−6 are reported the experimental data of Cu, Cd, and Fe biosorption and the respective Langmuir isotherms at different equilibrium pH. Langmuir isotherm characteristic parameters are shown in Table 3. The parameters for the Fe sorption have been reported, but many more experimental points are necessary in a wider range of equilibrium concentration and for this reason these values must be qualitatively considered (see Figure 6).

At the same equilibrium pH the maximum specific uptake of Fe is higher than that of Cu, and the latter one is higher than the one for Cd. The pH increase (up to pH = 6) generally causes an increase in specific metal uptake. Fe and Cu biosorption is more sensible than Cd to pH increase. Particularly in the case of Cu, at pH > 5 there is a strong increase in the specific uptake perhaps due to the presence of different biosorption mechanisms such as superficial microprecipitation and coprecipitation.

TABLE 3. Langmuir Parameters Obtained from Cu, Cd, and Fe Biosorption Data at Different Equilibrium pH

pH	$q_{max} \pm \Delta q_{max}$ (mmol/g)	$b \pm \Delta b$ (L/mmol)
	Cu	
4	0.075 ± 0.003	3.4 ± 0.3
4.5	0.104 ± 0.009	3.2 ± 0.7
5	0.15 ± 0.01	3.4 ± 0.5
5.4	0.62 ± 0.07	4 ± 1
6	0.71 ± 0.03	14 ± 2
	Cd	
3	0.022 ± 0.001	3.9 ± 0.6
4	0.0319 ± 0.0008	10 ± 1
5	0.062 ± 0.001	8.4 ± 0.9
6	0.119 ± 0.008	3.7 ± 0.7
	Fe	
3	0.29 ± 0.01	2.7 ± 0.2
4	0.42 ± 0.02	30 ± 6
5	1.0 ± 0.1	31 ± 4

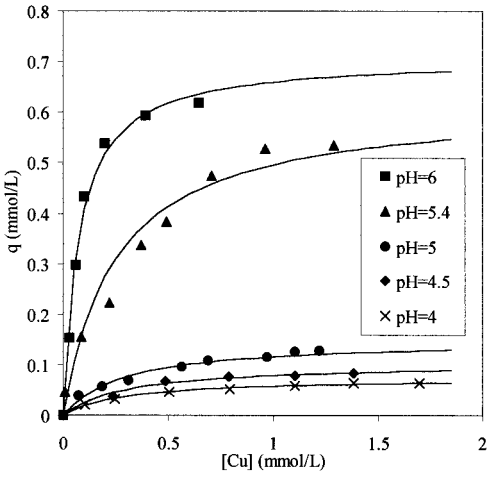

FIGURE 4. Equilibrium pH effect on copper uptake by an *Arthrobacter sp.* suspension. Biosorption data were obtained using the SAM: symbols are experimental data at different equilibrium pH, and solid lines are the respective Langmuir isotherms.

The considerations reported above lead to the development of a chemical model to understand which biosorption mechanisms may be involved in the system under study. The experimental data available for chemical modeling are titration curve and adsorption isotherms at different equilibrium pH. Different sets of possible reactions (acid dissociation, ion exchange, and complexation) can be consid-

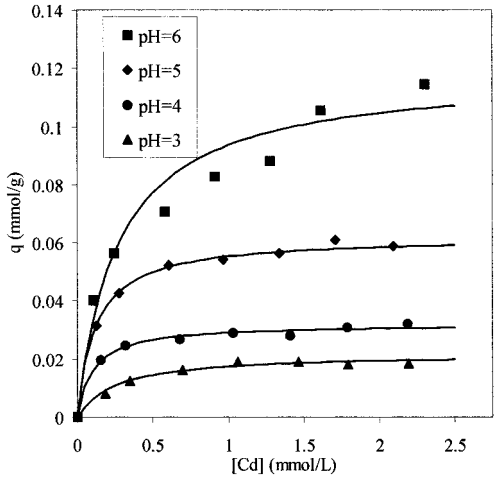

FIGURE 5. Equilibrium pH effect on copper uptake by an *Arthrobacter sp.* suspension. Biosorption data were obtained using the SAM: symbols are experimental data at different equilibrium pH, and solid lines are the respective Langmuir isotherms.

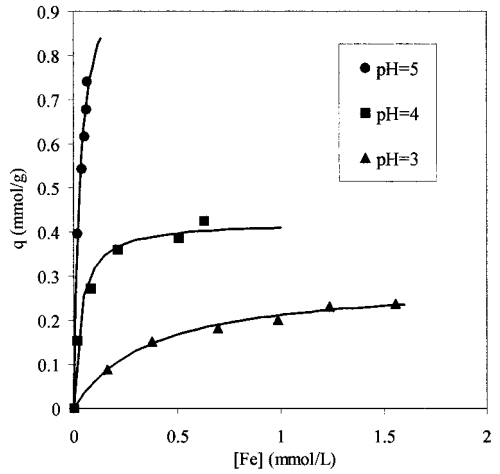

FIGURE 6. Equilibrium pH effect on iron uptake by an *Arthrobacter sp.* suspension. Biosorption data were obtained using the SAM: symbols are experimental data at different equilibrium pH, and solid lines are the respective Langmuir isotherms.

ered: supposing that every site reacts only according to one mechanism, an adequate set of reactions to describe the Cu data was (eqs 10−13)

$$S_1H \overset{K_{a1}}{\rightleftharpoons} S_1^- + H^+ \quad K_{a1} = \frac{[S_1^-]\cdot[H^+]}{[S_1H]} \tag{10}$$

$$S_2H \overset{K_{a2}}{\rightleftharpoons} S_2^- + H^+ \quad K_{a2} = \frac{[S_2^-]\cdot[H^+]}{[S_2H]} \tag{11}$$

$$S_1H + M^{2+} + OH^- \overset{K_{M1}}{\rightleftharpoons} S_1M(OH) + H^+ \quad K_{M1} = \frac{[S_1M(OH)]\cdot[H^+]}{[S_1H]\cdot[M^{2+}]\cdot[OH^-]} \tag{12}$$

$$S_2H + M^{2+} + OH^- \overset{K_{M2}}{\rightleftharpoons} S_2H\,M(OH)^+ \quad K_{M2} = \frac{[S_2HM(OH)^+]}{[S_2H]\cdot[M^{2+}]\cdot[OH^-]} \tag{13}$$

where K_{M1} and K_{M2} are the formation constants of the two hypothesized systems $S_1M(OH)$ and $S_2HM(OH)^+$.

The assumption that the metals bind as hydroxides and not as ions increases model sensibility to pH variation and

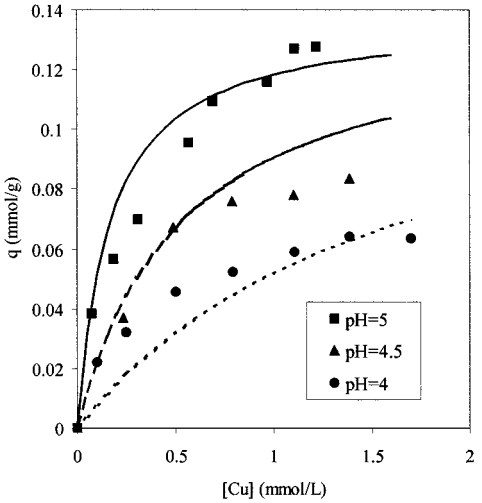

FIGURE 7. Effect of the equilibrium pH on copper uptake by an *Arthrobacter sp.* suspension. Symbols are experimental data at different pH, and lines are predicted by chemical modeling (eq 16) based on ion exchange and complexation reactions.

gives a more adherent interpretation of the experimental behavior. It was also assumed that site 2 does not undergo ion exchange because of theoretical considerations that the pK_a of the site 2 is so high that the metal is not strong enough to displace the proton.

The biosortpion tests were performed by using a lyophilized biomass which may be alive when the experiments in metal sorption are carried out. The performance and the metal binding mechanisms of the living cells could differ greatly from those of the dead ones. The active biosorption mechanism was considered of minor importance because the tests were carried out suspending the lyophilized cells in a solution containing only the heavy metals (i.e. $CuCl_2$) without any further additional nutrients. Although bioaccumulation (metabolism dependent mechanism) may take place, this phenomenon was considered negligible also considering the short experimental time in which any biosorption test was performed (*4*).

Combining the equilibrium constants (eqs 10−13) and the sites mass balances (eqs 14 and 15) reported below it is possible to obtain the relation 16 for the total specific uptake (mmol/g)

$$[S_1]_{Tot} = [S_1^-] + [S_1H] + [S_1M(OH)] \tag{14}$$

$$[S_2]_{Tot} = [S_2^-] + [S_2H] + [S_2HM(OH)^+] \tag{15}$$

$$q = \frac{[S_1]_{Tot}\cdot K_{M1}\cdot K_w\cdot[M^{2+}]}{[H^+]^2 + K_{a1}\cdot[H^+] + K_{M1}\cdot K_w\cdot[M^{2+}]} + \frac{[S_2]_{Tot}\cdot K_{M2}\cdot K_w\cdot[M^{2+}]}{[H^+] + K_{a2} + K_{M2}\cdot K_w\cdot[M^{2+}]} \tag{16}$$

where q is the metal specific uptake (mmol/g of lyophilized biomass) and $[M^{2+}]$ is the metal equilibrium concentration.

The mechanism proposed here considers the presence of only Cu^{2+} in the solution, but the presence of other ionic species such as $CuOH^+$, $Cu(OH)_2$, $Cu(OH)_3^-$, and $Cu(OH)_4^{2-}$ should be considered. However, the assumption of the only Cu^{2+} presence was considered sufficient at this stage of the work.

It is noteworthy that the q expression reported here is the sum of two Langmuir-type isotherms.

Acidic sites concentration and acidic equilibrium constants are those determined in the titration curve modeling;

the other two constants are obtained by using the least-squares method.

The proposed chemical model does not describe the experimental behavior in the whole range of pH considered (3−6).

The chemical modeling reported here is able to predict only copper biosorption data between pH 3 and 5; therefore, in this range the hypothesis of only two mechanisms (ion exchange and complexation) is not well confirmed, and further work on modeling is necessary. Presently Cd and Fe modeling were not taken into consideration. The strong effect of pH over pH = 5 on biosorption performances could be due to the presence of other physical and/or chemical mechanisms, for example, superficial microprecipitation (*4*). Moreover, *Arthrobacter sp*. application as bioprecipitating bacterial strain for metals precipitation in sand filters has been reported (*19*−*21*).

The existence of other biosorption mechanisms can be also deduced by comparing the maximum specific uptake of each metal as a function of the equilibrium pH (Table 3) with the experimental results obtained in the biomass characterization tests (titration modeling and ionic content). It is possible to see that biosorption capacities (Table 3, Figures 4−6) are higher (except the case of Cd) than those predicted by titration data (total acidic titer = 0.41 mmol/g) and ionic content (0.51 mequiv/g). In fact both Cu and Fe uptake (see Table 3) exceed the total estimated acidic sites concentration respectively starting from pH 5.4 and pH 5.

Even if the chemical model here reported is less adherent to experimental data than each different Langmuir isotherm at constant pH, the proposed model can represent biosorption data in a range of pH, while Langmuir isotherm can represent only experimental data at constant pH.

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